ASSESSMENT OF MOLECULAR MARKERS ASSOCIATED WITH RESISTANCE OR SUSCEPTIBILITY TO FUSARIUM WILT TR4 IN *Musa acuminata* ssp. *malaccensis*

FATIMAH BINTI KAYAT

FACULTY OF SCIENCE
UNIVERSITY OF MALAYA
KUALA LUMPUR

NOVEMBER 2009

Acknowledgements

I would like to express my sincere gratitude to Prof. Dr. Mak Chai and Dr. Asif Javed, ex-lecturer and my senior at Genetics Department, University of Malaya, for the opportunity to carry out this highly interesting work. I highly appreciate their great guidance through the project with valuable advice and creative suggestions in the experimental design and analysis, especially for Dr. Asif Javed his support for their critical works in development of mapping populations and DNA extraction.

I would also like to thank to Prof. Dr. Rofina Yasmin Othman, for accepting me as a Ph.D. student, her valuable suggestion for this dissertation and her great support in financial and administrative issues. My special thanks to Dr Robbie Waugh and the technical staff of the Genome Dynamics at Scottish Crop Research, Dundee for their excellent technical supports in my AFLP work. I am grateful to my friends at Plant Molecular Biology Laboratory for a friendly and lively atmosphere. Special thanks to Hanny and family, Niza, Nicky, Ana and friends who cheer me up while I'm alone abroad.

I would like to express my sincere gratitude to Dr. Rajinder Singh, group leader of Plant Science and Biotechnology Unit, MPOB, Puan Rahimah and Ms Grace for giving me the opportunity to use the JoinMap software. I highly appreciate their great guidance through the project with valuable advice and creative suggestions in the generating the linkage maps and analysis.

Financial support from Yayasan Pelajaran Johor for funding me during five months stay of technical training in Dundee and three years scholarships provided by MOSTI are acknowledged. Thanks also dedicated to IPPP UM for support of research grants.

I am also grateful to my mother and aunt for continued support and encouragement by cheering me up while I was in the ring. Finally, I wish to express deep appreciation to my father, family and friends for understanding me and appreciate the value of hard work.

UNIVERSITI MALAYA

ORIGINAL LITERARY WORK DECLARATION

Name of Candidate: **Fatimah binti Kayat** I.C No: **730907-01-5900**

Matric No: **SHA03008**

Name of Degree: **Doctor of Philosophy**

Title of Thesis: **Assessment of Molecular Markers Associated with Resistance or Susceptiblity to Fusarium Wilt TR4 in *Musa acuminata* ssp. *malaccensis***

Field of Study: **Plant Biotechnology**

I do solemnly and sincerely declare that:

(1) I am the sole author/writer of this Work;
(2) This Work is original;
(3) Any use of any work in which copyright exists was done by way of fair dealing and for permitted purposes and any excerpt or extract from, or reference to or reproduction of any copyright work has been disclosed expressly and sufficiently and the title of the Work and its authorship have been acknowledged in this Work;
(4) I do not have any actual knowledge nor do I ought reasonably to know that the making of this work constitutes an infringement of any copyright work;
(5) I hereby assign all and every rights in the copyright to this Work to the University of Malaya ("UM"), who henceforth shall be owner of the copyright in this Work and that any reproduction or use in any form or by any means whatsoever is prohibited without the written consent of UM having been first had and obtained;
(6) I am fully aware that if in the course of making this Work I have infringed any copyright whether intentionally or otherwise, I may be subject to legal action or any other action as may be determined by UM.

Candidate's Signature Date: 16 November 2009

Subcribed and solemnly declared before,

Witness's Signature Date: 16/11/ 2009
Name: Dr. Subha Bhassu
Designation: Senior Lecturer,
Division of Genetics & Molecular Biology
Institute of Biological Sciences,
Faculty of Science, University of Malaya.

Abstract

Banana and plantain (*Musa* spp.) are staple food crops in tropical and subtropical countries and also play a key role in the economic of many developing countries. However, the ten year review (1996 - 2005) of the production of the crop showed that the land usage for banana and plantain had increased but the yield had been reduced during the same period. In Malaysia, Panama disease or Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cubense* VCG 01213/16 also known as Tropical race 4 (TR4) is the major and the most destructive disease which causes considerable loss to the Malaysian banana export trade. Conventional banana breeding for resistance to Fusarium wilt is hampered by sterility, triploidy, long generation time and the lack of appropriate testing sites. Biotechnological advances involving *in vitro* propagation, somatic embryogenesis and molecular markers provide tools for genetic relationship studies and QTL analysis thus could enhance the development of cultivars adapted to changing environments.

The use of genes from the wild crop relatives have been recognized in breeding programs of many crops. In this study, the strategy approach is to develop new populations with traits specific to the pathogen from the indigenous wild banana *Musa acuminata* ssp. *malaccensis* (AA) which has been shown to have very high resistance to FOC TR4. Matured seeds were extracted from fruit bunches of four random open cross populations of wild banana in order to produce seed progenies raised through zygotic embryo culture. Embryo germination was achieved without any seed treatment in the dark for two weeks before being placed under light. Individual seed progenies were subjected to several *in-vitro* stages in order to gain uniform plantlets with replicates for disease screening. Different degrees of response to *Fusarium oxysporum* f. sp. *cubense* TR4 were observed in wild banana seed progenies as well as their F_1 hybrid populations

which provided a potentially useful genetic resource for the development of disease resistance markers that could be exploited in a marker-assisted selection.

A high degree of polymorphism in wild banana seed progenies shown by RAPD markers provided good early evidence for the potential usefulness of polymorphism and segregation studies using molecular markers. Analysis of RAPD, STMS and AFLP markers in this study did not show any clear differentiation between resistant and susceptible individuals. However, markers generated by those methods were included in linkage map development. A total of 18 markers had been generated from RAPD analysis while 14 SSR markers were observed from STMS analysis. A total of 4657 bands was generated from 30 *Eco*R1+3 x *Mse*I+3, one *Eco*R1+3 x *Mse*I+2 and 43 *Pst*I+2 x *Mse*I+3 primer combinations. The 639 markers consisting of 607 AFLPs, 14 SSRs and 17 RAPDs markers were determined on two mapping populations (resistance and susceptibility to FOC TR4). A total of 471 markers (286 *Pst*-markers, 155 *Eco*-markers, 17 RAPD markers and 13 STMS markers) were analyzed for the susceptible mapping population while 414 markers (249 *Pst*-markers, 143 *Eco*-markers, 9 RAPD markers and 13 STMS markers) were analyzed for the resistant mapping population. Linkage analysis of the data resulted in the generation of two sets of linkage maps consisting of 32 linkage groups for the resistant mapping population and 37 linkage groups for the susceptible mapping population. The maps have provided the basis of future mapping and marker-assisted breeding studies and strategies for this banana.

Abstrak

Pisang dan plantain (*Musa* spp.) merupakan tanaman makanan utama di negara-negara tropika dan subtropika serta penyumbang utama di dalam perkembangan ekonomi banyak negara membangun. Bagaimanapun, analisa hasil pengeluaran tanaman sedekad (1996 - 2005) menunjukkan pengurangan di dalam hasil pengeluaran walaupun jumlah penggunaan tanah untuk tanaman pisang dan plantain meningkat dalam tempoh yang sama. Di Malaysia, penyakit layu Fusarium (penyakit Panama) disebabkan oleh *Fusarium oxysporum* f. sp. *cubense* VCG 01213/16 juga dikenali sebagai `Tropical race 4' (TR4) merupakan penyakit pemusnah yang utama di mana ia menyebabkan kerugian yang besar terhadap industri pengeksportan pisang.

Pembiakbakaan pisang secara konvensional untuk kerintangan terhadap penyakit layu Fusarium terbatas disebabkan oleh ketidaksuburan biji benih, triploidi, selang generasi yang panjang dan kekurangan tapak ujikaji yang bersesuaian. Perkembangan di dalam bidang teknologi melibatkan propagasi *in vitro*, embrogenasi somatik dan penanda-penanda molecular menyediakan sarana untuk kajian terhadap hubungan genetik dan analisa QTL seterusnya dapat meningkatkan pengeluaran varieti yang dapat bertahan terhadap perubahan alam sekitar.

Penggunaan gen-gen tanaman liar telah dikenalpasti dalam banyak program pembiakbakaan tanaman. Dalam kajian ini, strategi pendekatan yang digunakan adalah menghasilkan populasi baru dengan sifat yang spesifik terhadap patogen dari pisang liar tempatan, *Musa acuminata* ssp *malaccensis* (AA) yang menunjukkan kerintangan yang tinggi terhadap FOC TR4. Biji benih yang matang diasingkan dari empat tandan buah yang masak yang terhasil secara kacukan rawak untuk menghasilkan progeni anak-anak pisang melalui kultur embrio. Percambahan embrio berjaya diperolehi tanpa rawatan biji benih di dalam simpanan gelap selama dua minggu sebelum diletakkan di bawah cahaya. Progeni-progeni individu melalui beberapa peringkat pengkulturan *in*

vitro untuk mendapatkan anak-anak pokok yang sekata dengan replikat untuk penyaringan terhadap penyakit. Kadar tindakbalas terhadap penyakit yang bervariasi terhadap *Fusarium oxysporum* f. sp. *cubense* TR4 telah direkodkan di dalam progeni-progeni liar dan juga populasi kacukan F_1 progeni berkenaan memberikan sumber genetik yang berguna untuk pembangunan penanda-penanda terhadap penyakit yang boleh dieksplotasikan di dalam pemilihan berasaskan penanda.

Sejumlah besar polimorfisma dalam progeni pisang liar ditunjukkan oleh penanda-penanda RAPD memberikan bukti awal dan potensi polimorfisma untuk kajian segregasi. Analisa penanda-penanda RAPD, STMS dan AFLP menunjukkan tiada penanda yang dapat membezakan individu-individu yang rintang dan rentan secara langsung. Bagaimanapun, penanda-penanda yang terhasil digunakan untuk pembangunan peta `linkage'. Sejumlah 18 penanda dihasilkan dari analisa RAPD manakala 14 penanda SSR diperolehi dari analisa mikrosatelit (STMS). Sejumlah 4657 jalur dihasilkan dari tiga puluh kombinasi primer *Eco*R1+3 x *Mse*I+3, satu *Eco*R1+3 x *Mse*I+2 dan 43 *Pst*I+2 x *Mse*I+3 masing-masing. Sejumlah 639 penanda yang terdiri dari 607 penanda-penanda AFLP, 14 SSR dan 8 RAPD digunakan untuk penentuan dua populasi pemetaan (rintang dan rentan terhadap FOC TR4). Sejumlah 471 penanda (286 penanda *Pst*, 155 penanda *Eco*, 17 penanda RAPD dan 13 penanda STMS) digunakan untuk analisa bagi populasi pemetaan rentan manakala 414 penanda (249 penanda *Pst*, 143 penanda *Eco*, 9 penanda RAPD dan 13 penanda STMS) telah dianalisa untuk populasi pemetaan rintang. Analisa linkasi dari data tersebut menghasilkan dua kelompok peta linkasi yang terdiri dari 32 kumpulan untuk populasi pemetaan rintang dan 37 kumpulan untuk populasi pemetaan rentan. Peta-peta ini dapat memberikan langkah awal untuk pemetaan lanjut dalam kajian pembiakbakaan pisang berasaskan penanda molekular.

TABLE OF CONTENTS

ABBREVIATIONS

ºC	degree Celsius
%	percentage
♀	female
♂	male
µg	microgram
µl	microlitre
µM	micro molar
^{33}P	radioactive isotope of phosphorus
AFLP	amplified fragment length polymorphism
$AgNO_3$	silver nitrate
APS	ammonium persulphate
bp	base pair
BAP	6-benzylaminopurine
BBTV	banana bunchy top virus
BC	backcross
BSA	bulk segregant analysis
BSV	banana streak virus
cm	Centimeters
cM	centiMorgan
$CaCl_2.2H_2O$	calcium chloride
$Ca(NO_3)_2$	calcium nitrate
CARBAP	Centre Africain de Recherches sur Bananas et Plantains
$C_2H_5NO_2$	Glycine
CIRAD-FLOHR	Centre de Coopération Internationale en Recherche Agronomique pour le Développement
CMV	cucumber mosaic virus
CP	cross population
$CoCl_2.6H_2O$	cobaltus chloride
CTAB	cetyltrimethylammonium bromide
$CuSO_4.5H_2O$	cupric sulphate
df	degree of freedom
DH	double haploid
dH_2O	distilled water
dNTPs	deoxyribonucleoside triphosphates

DNA	deoxyribonucleic acid
ECS	embryogenic cell suspension
*Eco*RI	restriction enzyme isolated from *Escherichia coli* strain R
EDTA	ethyldiaminotetraacetic acid
EMBRAPA	Empresa Brasileira de Pesquisa Agropecuária
F_1	offsprings resulted from crosses of 2 different parents
FAO	Food and Agriculture Organization
$FeCl_3$	ferrick chloride
$FeSO_4.7H_2O$	ferrous Sulphate
FHIA	Fundación Hondureña de investigaón Agrícola
FOC TR4	*Fusarium oxysporum* f. sp. *cubense* tropical race 4
g	Gram
GAS	gene assisted selection
GN	Giganewton
h	hour/hours
H_3BO_3	boric Acid
HCl	hydrochloric acid
ICTA	Imperial College of Tropical Agriculture
IITA	International Institute of Tropical Agriculture
kbp	kilo base pair
KCl	potassium chloride
KH_2PO_4	potassium dihydrogen orthophosphate
KI	potassium iodide
KNO_3	potassium nitrate
L	Litre
LD	linkage disequilibrium
LE	linkage equilibrium
LE-MAS	linkage equilibrium-marker assisted selection
LG	linkage group
LOD	logarithm of odds
LSI	leaf symptom index
m	Metre
M	Molar
M1	marker locus 1
mg	Milligramme

MAGE	metaphor agarose gel electrophoresis
MAS	marker assisted selection
$MgSO_4.7H_2O$	magnesium sulphate
$MnSO_4.4H_2O$	manganese sulphate
MS	Murashige and Skoog media
*Mse*I	restriction enzyme isolated from *E. coli* strain that carries the *MseI* gene from *Micrococcus* species (R. Morgan).
NaCl	sodium chloride
$Na_2.EDTA.2H_2O$	sodium EDTA
$Na_2MoO_4.2H_2O$	sodium molybdate
NaOH	sodium hydroxide
ng	Nanogramme
nm	Nanometer
NH_4NO_3	ammonium nitrate
OD	optical density
PAGE	polyacrylamide gel electrophoresis
PCR	polymerase chain reaction
PDA	potato dextrose agar
pH	parts of hydrogen (acidity/alkalinity index)
*Pst*I	restriction enzyme isolated from *Providencia stuartii*
Q	quantitative trait
Q1	quantitative trait locus 1
QTL	quantitative trait loci
RAPD	random amplified polymorphic DNA
RDI	root discoloration index
RISBAP	Regional Information System for Banana and Plantain
RNA	ribonucleic acid
RNase	Ribonuclease
s	Seconds
SSR	simple sequence repeats
STMS	sequence tagged microsatellite site
STS	sequence tagged site
TBE	Tris/Borate/EDTA
TE	tris-EDTA
TEMED	N,N,N',N'-tetramethylethylenediamine

TR4	tropical race 4
U	Unit
uv	ultra-violet
V	Volt
VCG	vegetative compatibility group
VNTR	variable numbers of tandem repeats
$ZnSO_4$	zinc sulphate

LIST OF TABLES

LIST OF FIGURES

1.0 INTRODUCTION

1.1 Bananas

Banana and plantain belong to the Musaceae family and the order Zingiberales. The generic name *Musa* is derived from the Arabic word *mouz*. They were known to the Arabs and appear in the Koran as the `tree of paradise', which is equivalent of the `tree of knowledge' of Christian tradition (Purseglove, 1988) and thought by both Moslems and Christians to be the forbidden fruit of paradise. The centre of origin of the wild banana stretches from India to Papua New Guinea that includes Malaysia and Indonesia (De Langhe, 1995). The family Musaceae is formed by two genera *Ensete* and *Musa* (Table 1.1). It is classified under the genus *Musa* which is divided into five sections i.e. Australimusa, Callimusa, Eumusa, Rhodochlamys and Ingentimusa (Stover & Simmonds, 1987).

Edible bananas are included in the Eumusa section making it the most important section. Wild bananas are diploid and reproduced by sexual means while cultivated bananas are polyploid (diploid, triploid, tetraploid), parthenocarpic and infertile. The principal cultivars derived from two major species *Musa acuminata* Colla (`A' Genome) and *Musa balbisiana* Colla (`B' genome) are polyploid hybrids (mainly AAA, AAB and ABB triploids), medium to highly sterile, parthenocarpic and clonally propagated. Most production is based on sterile, triploid clones and propagated vegetatively (Sasson, 1997). Generally, fruits with the `A' genome are sweeter and cultivated as dessert while fruits with the `B' genome are starchy and thus suitable for cooking.

Table 1.1: Systematic classification of the Musaceae family

Genus	Basic Chromosome Number	Section	Distribution	Number of Species
Ensete	9	-	West Africa to New Guinea	7 - 8
Musa	10	Australimusa	Queensland to the Philippines	5 - 6
		Callimusa	Indo-China and Indonesia	5 - 6
	11	Eumusa	South India to Japan and Samoa	8
		Rhodochlamys	India to Indo-China	5 - 6
	14	Ingentimusa	Papua New Guinea 1000-2000m	1

Adapted from: Stover & Simmonds (1987)

Banana and plantain are high in carbohydrates (about 35%) and fibre (6-7%), while their protein content (1-2%) is slightly lower than in potato. Banana fruits are an important source of major elements such as potassium, magnesium, phosphorus, calcium and iron as well as vitamin A, B_6 and C (Novak, 1992; Robinson, 1996; Pillay & Tripathi, 2007; Faturoti *et al*., 2007). During ripening, the starch component is gradually converted into sucrose, glucose and fructose while the water content in the pulp increases (Novak, 1992). *Musa acuminata* is a diverse species with at least nine subspecies described or suggested. The characteristics of both *Musa acuminata* and *Musa balbisiana* (Simmonds & Shepherd, 1955) are shown in Table 1.2.

1.2 Importance and Constraints of Banana Production

Banana and plantain (*Musa* spp.) are staple food crops for people living in tropical and subtropical countries. It is cultivated throughout all tropical humid areas with a total world production is around 86 million tons (FAO, 1998) increasing to 99 million tonnes in 2001 (Arias *et al*., 2003) and was estimated at 106 million tonnes in 2005 (Daniells, 2006). However, a ten year (1996 - 2005) production figure of the crops showed that land under plantain and banana production increased by 24.6% while yield reduction of 21.8% was recorded during the same period suggesting that there are constraints to the production of the fruits (Faturoti *et al.,* 2007). It has been traditionally cultivated in smallholdings as an intercrop. Fruits harvested from bananas and plantains are usually more important for local consumption thus, it plays a major role in maintaining food security in the tropical world and is also as an income provider to the farming community.

Table 1.2: Characteristics of *Musa acuminata* and *Musa balbisiana*

Characteristic	*Musa acuminata*	*Musa balbisisana*
Pseudostem colour	More or less heavily marked with brown or black blotches.	Blotches slight or absent
Petiolar canal	Margin erect or spreading, with scarious wings below, no clasping pseudostem	Margin inclosed, not winged below, clasping pseudostem
Penduncle	Usually downy or hairy	Glabrous
Pedicels	Short	Long
Ovules	Two regular row in each loculus	Four irregular rows in each loculus
Bract shoulder	Usually high x/y ratio is less than 0.28	Usually low more than 0.30
Bract curling	Bract reflex and roll back after opening	Bracts lift but do not roll
Bract shape	Lanceolate or narrowly ovate, tapering sharply from the shoulder	Broadly ovate, not tapering sharply
Bract apex	Acute	Obtuse
Bract colour	Red, dull purple or yellow outside, pink, dull purple or yellow inside	Distinctive brownish-purple outside; bright crimson inside
Colour fading	Inside bract colour fades to yellow towards the base	Inside bract colour continuous to base
Bract scars	Prominent	Scarcely prominent
Free tepal of male flower	Variable corrugated below tip	Rarely corrugated
Male flower colour	Creamy white	Variably flushed with pink
Stigma colour	Orange or rich yellow	Cream, pale yellow or pale pink

Source: Simmonds and Shepherd, 1955

In Africa, the crop provides more than 25% of the total food energy requirement for around 70 million people (Karamura, 1999). In Malaysia on the other hand, although the contribution is not as significant as in Africa, it is the second most widely cultivated fruit, covering about 26,000 ha with a total production of 530,000 metric tonnes. It possesses good potential for expansion due to the strong demand as a table fruit and also for downstream activities as well as import substitution for temperate fruits. Banana is the third largest fruit crop cultivated after durian and pineapple in Peninsular Malaysia comprising of about 10% of the total hectareage under fruit cultivation in the period of 2003 to 2008 with the third largest production output after durian and pineapple consisting the volume of 254,440 million tones in 2008 (see Table 1.3).

Bananas can be grown under a wide range of climatic extremes and different types of soil. Cultivated bananas however, are very susceptible to a range of plant pathogens including pests such as corm borer or banana weevil (*Cosmoplites sordidus*) and leaf rollers (*Erinomata thrax*), fungi (*Mycosphaerella fijiensis* and *Mycosphaerella musicola)* that causes black and yellow sigatoka, *Fusarium oxysporum* f. sp. *cubense* that causes Fusarium wilt , bacteria *Pseudomonas solanacearum* (Moko disease), virus such as *Banana Streak Virus* (BSV) and *Bunchy Top Virus* (BBTV) and nematodes (*Rhadophyllus similis*).

Panama disease or Fusarium wilt is the major and the most destructive disease in Malaysia, Philippines and Thailand. It is a soil-borne disease caused by *Fusarium oxysporum* f. sp. *cubense* (FOC) and causes considerable loss to the banana export trade (Rutherford, 1999). Some export and export-style Cavendish production areas around the world including Malaysia, Indonesia, China Taiwan, the Philippines, South Africa and parts of Australia have experienced major problems with subtropical and tropical Race 4 strains of Fusarium wilt (Molina, 2006; Daniells, 2006).

Table 1.3: Production (metric tonnes) and Planted Areas (Hectares) of Major Malaysian Fruits.

Type of fruit	2003	2004	2005	2006	2007[p]	2008[e]
Durian	303,717 (*116,271*)	399,661 (*115,675*)	378,657 (*110,615*)	292,681 (*105,388*)	311,460 *(102,390)*	323,080 *(99,410)*
Guava	20,710 *(1,788)*	24,179 *(2,248)*	23,740 *(1,934)*	16,161 *(1,739)*	18,330 (*1,810*)	20,770 *(1,880)*
Banana	**274,426 *(29,864)***	**317,104 *(29,057)***	**262,242 (*28,020*)**	**258,481 (*26,855*)**	**257,050 *(26,280)***	**254,440 *(25,710)***
Manggoes	22,072 *(9,482)*	27,075 (*9,714*)	25,043 (*9,421*)	26,247 *(10,017*)	27,270 (*9,870*)	28,290 *(9,710*)
Papaya	49,685 (*2,668)*	40,330 *(2,670)*	41,319 (*2,758*)	32,800 (*2,117*)	34,010 (*2,220*)	35,530 (*2,320*)
Pineapple	373,916 *(14,480)*	196,689 *(9,306)*	355,937 *(14,884)*	299,318 *(14,144)*	316,210 (*13,860*)	319,130 *(13,570)*
Starfruit	8,707 *(1,072)*	10,971 *(1,173)*	8,719 *(1,097)*	10,222 *(1,109)*	10,810 *(1,130)*	11,460 *(1,180)*
Water melon	105,868 *(6,803)*	115,881 *(7,393)*	147,666 *(8,691)*	148,909 *(9,214)*	157,470 *(9,990)*	166,170 *(10,780)*
Cempedak & Jackfruit	49,563 (*14,853*)	63,455 (*15,016*)	65,461 (*14,433)*	57,736 (*13,802*)	51,180 (*13,320*)	60,840 *(12,850)*
Dokong, Duku, Duku Langsat & Langsat	88,096 *(49,097)*	188,882 *(49,384)*	149,044 *(51,190)*	127,625 *(47,946*)	147,920 *(38,070*)	165,050 *(48,040*)
Dragon Fruit, Snake Fruit & Sapodilla	9,785 *(2,914)*	11,753 *(3,635)*	11,195 *(3,232)*	11,243 *(3,884)*	13,320 *(4,035)*	14,528 *(4,160)*
Rambutan, Pulasan & Manggosteen	87,478 *(33,780)*	106,227 *(34,444)*	107,687 *(34,048)*	97,504 *(33,516)*	105,520 *(32,970)*	111,880 *(32,430)*
Sweet Orange, Tangerine & Pamelo	29,026 *(9,370)*	37,862 *(8,726)*	33,482 *(7,366)*	29,922 *(7,091)*	31,570 *(6,890)*	33,220 *(6,690)*
Total	**1,423,049 (*292,442*)**	**1,540,069 (*288,441*)**	**1,610,192 (*287,688*)**	**1,408,849 (*276,822*)**	**1,482,120 (*262,835*)**	**1,544,388 (*268,730*)**

Source: Ministry of Agriculture, 2008.

Note: # production in metric tonnes, (#) planted area in hectares, ***p***–Preliminary, ***e***-Estimated

Four physiological races of FOC have been recognized based on their selective pathogenecity in different banana cultivars. In 1940, wilt disease caused by *Fusarium oxysporum* Race 1 appeared on the Gros Michel and AAB dessert cultivar such as Silk and Pome (Molina, 2006). It was followed by Race 2 attacking Bluggoe and other closely related ABB cooking banana. Race 3 however, only attacks *Heliconia* spp. (ornamental plants) thus is not considered important to the banana industry. Race 4 attacks Cavendish group cultivars (AAA) and are also virulent on Gros Michel and Bluggoe (Ploetz, 1993b; Ploetz, 2006) Races in FOC does not signify genetic relationships with the host like other pathosystems but rather represents groups of strains infecting a group of cultivars under certain field condition. Another classification system of the FOC pathogen is vegetative groups (VCG) (Molina, 2006; Puhalla, 1985). A unique population consists of VCG 01213/16 from Southeast Asian known also as Tropical race 4 (TR4) is considered the most dreaded of all identified races of the banana Fusarium wilt pathogen (Molina, 2006). TR4 is distinguished from subtropical race 4 because it is genetically distinct and specifically damages Cavendish bananas in the tropics. It is believed that FOC and its major clonal lineages have coevolved with its diverse hosts in Asia (Molina, 2006; Ploetz & Pegg, 2000).

In Fusarium wilt infected plants, the corm shows purplish vascular staining; soon followed by a yellowing of the lower outer leaves. Infected plants rarely recover, but may continue poor growth for some time. The spread of the disease is increased in actively growing roots of young plants or after damage, and by heavy rain, light soil of poor nutritional status, unbalanced nitrogenous manuring, poor drainage and hurricane damage (Purseglove, 1988). Numerous disease controls such as soil amendment with calcium or organic matter as well as fumigation with methyl bromide is practiced but it only provides temporary solutions to this problem. Therefore planting disease resistant

bananas is still regarded as the best approach as it is economical, effective and practical in the long run.

Fusarium oxysporum f. sp. *cubense* (*FOC*) is most diverse in Southeast Asia, especially where FOC and *Musa acuminata* are presumed to have coevolved (Jones, 1995). Rowe and Rosales (1993) suggested that the resistance to Race 4 appears to be under polygenic control. Resistance to race 4 in a parent in the FHIA breeding program, `Pisang Jari Buaya' (AA) was thought to be polygenic. However, studies on a segregating population of a wild *M. acuminata* ssp. *malaccensis* suggested that resistance to race 4 was due to a single recessive gene (Asif *et al*., 2004); molecular work indicated that it was homologus to the 12 gene that confers resistance in tomato (Ploetz, 2005; Escheverria *et al.*, 2004). Attempts on developing resistant clones to Fusarium wilt using conventional breeding have limited success due to the low reproductive fertility, polyploidy, highly heterozygous and complete sterility of cultivated bananas (Mak *et al.*, 2004).

Previous studies on wild banana *Musa acuminata* ssp. *malaccensis* showed a high resistance to *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (Javed *et al*., 2004). The studies also showed that seed progenies derived from *in vitro* embryo culture were segregating for resistance and susceptiblity to FOC TR4. Therefore, they would potentially be a good source for breeding and genetic strategies as they are fertile and produce a large number of seeds.

Banana micropropagation techniques were developed during the past two decades. It is simple, efficient and applicable to a wide range *Musa* genotypes (Vuylsteke, 1989; Pillay & Tripathi, 2007). Application of micropropagation has greatly improved *Musa* germplasm handling for the purposes of clonal propagation, uniform production and breeding. It has also played a key role in banana and plantain improvement programs worldwide (Rowe & Rosales, 1996b; Vuylsteke *et al*., 1997).

Embryo rescue culture increases the rate of seed germination by a factor of ten or more (Vuylsteke *et al.,* 1990). The efficient embryo germination *in vitro* facilitated the production of large segregating populations from interspecific crosses and rapid progress was made in elucidating the genetics of large number of traits in *Musa* population (Ortiz & Vuylsteke, 1996; Asif *et al.,* 2001) thus offering a rapid approach for the study of wild banana populations for *Musa* breeding programs.

Recent developments in molecular markers have provided new tools, which offered unique opportunities for the dissection of genetic relationships among breeding lines (Staub & Serquen, 1996; Saghai *et al.,* 1997). It has been used for characterization of germplasm through DNA fingerprinting and genetic diversity estimation for selection of parents for hybridization programmes (Roy *et al.,* 1992). PCR markers such as RAPDs, microsatellites and AFLPs which were cheaper, safer and produced more markers per unit of DNA provided framework maps around which the polygenes/QTL could be located (Kearsey & Farquhar, 1998). These markers segregate as single genes that are unaffected by the environment and are highly polymorphic thus providing opportunities to develop high quality linkage maps. Identification of important QTL regions could enhance plant breeding efficiency by marker-assisted selection (MAS). In addition, they are amenable to the large scale throughput demands of screening breeding populations (Crouch *et al.,* 1998c).

Michelmore *et al*., (1991) had developed a procedure termed bulk segregant analysis (BSA) to identify RAPD markers linked to a disease resistance gene in lettuce. BSA eliminates the need for near-isogenic lines because only a segregating population for a trait of interest is needed. It is an efficient procedure to detect markers linked to target loci (Gallego *et al*., 1998). A segregating population is required to discover a marker or QTL linkage. In plants, experimental populations such as F_2, backcross (BC) recombinant inbred (RI), and double haploid (DH) are easy to produce. The most

efficient designs for QTL are crosses of inbred lines because the linkage disequilibrium between marker and QTL is maximized in F_1. Crosses of outbred lines can also be used to identify QTL that can explain differences between the lines. The efficiency of this design depends on the difference between the two lines in marker allele frequency, and the difference in genetic effects between predominant QTL genotypes of the two lines.

Knowledge of the genetics of resistance in bananas to Fusarium wilt is limited. Genetic improvement of banana by means of conventional plant breeding strategies is not an easy task. It is a complicated and laborious process considering several peculiar characteristics of the plant and the fact that most important commercial bananas are completely sterile triploids make backcross and recurrent selection breeding not a straight forward task. Other issues such as low efficiency, undesired traits linked to desired characteristics, have led breeders to find other alternatives. Therefore the wild seeded bananas could be of great importance in understanding the genetics of resistance as previously suggested by Buddenhagen (1990). Seed progenies of the wild banana *M. acuminata* ssp. *malaccensis* were found to have both FOC resistant and susceptible individuals and can thus be used for breeding segregation populations to investigate the genetic basis of FOC disease resistance, their interactions with known races of FOC and their allelic relationships. Similarly these segregating populations could be used for molecular studies to look for markers linked to the resistance (Javed *et al.,* 2004). F_2 or backcross populations are most commonly used in developing a segregating population, but F_1 can be useful in highly heterozygous crops like apple (Hemmat *et al.,* 1994), olive (Baldoni *et al.,* 1999) and potato (Ritter *et al.,* 1990). In this case, the population that we are working on is also highly heterozygous. Therefore, we should be able to use F_1 to develop a segregating population.

The main objective of the project is to identify molecular markers potentially associated with resistance and/or susceptibility to *Fusarium oxysporum* Tropical Race 4 (FOC TR4).

The specific aims of the study are;

a) To develop a hybrid F_1 population of wild banana *Musa acuminata* ssp. *malaccensis* based on resistance and susceptibility to FOC TR4.
b) To use molecular markers to study the F_1 population of *Musa acuminata* ssp. *malaccensis*
c) To select quality marker data from the F_1 populations.
d) To study the potential associations between markers and genotype.
e) To construct linkage maps from the marker data.

2.0 LITERATURE REVIEW

2.1 Bananas

Bananas are an important source of food, fiber and income for millions of people throughout the world (Moore *et al.,* 1999). In 1992, the total world production of *Musa* was 76.4 million tones of which 65% was classified as bananas and 36% as plantain (Robinson, 1996) and was estimated at 99 million tones in 2001(Arias *et al.,* 2003). World banana and plantain production was estimated at 106 million tones in 2005 (Daniells, 2006), ranks fifth after cereals and it is important in the subsistence diet of millions poor people. They are a staple food for nearly 400 million people in the tropics and about one billion people eat banana and plantain regularly (Jain, 2004).

In Jamaica, banana is the second largest agricultural export and employs between 5 to 10% of the labour force. In the Windward Islands, the banana industry was once the economic backbone of the islands with the export production almost 260,000 tonnes in the period 1981 to 1992 (Shillingford & Edmunds, 2006). In some African countries, daily consumption may exceed one-and-a-half kilograms per person whereas in North America and Western Europe the consumption is on an average about one banana per week per person (Jain, 2004).

Edible bananas (with exception of Fe´i bananas) are derived from *M. acuminata* (A genome) and *Musa balbisiana* (B genome) in the section Eumusa (Sharrock, 1995). The main centre of origin of *acuminata* types is Malaysia, (Stover & Simmonds, 1987; Jones, 2000) and that of hybrid types is India (Robinson, 1996). Human intervention may have played an important role (Simmonds, 1962) in the generation of edible bananas and the history of banana varieties is closely linked to the early movement of human populations in the tropics (De Langhe, 1995). A diverse selection of *Musa* cultivars is thought to have arisen in South-East Asia along with the earliest

developments of agriculture thousands years ago. The largest numbers of primary cultivars recorded are in Papua New Guinea followed by the Philippines, Malaysia and India. Carreel (1994) has hypothesized that the genomic constituents of other *M. acuminata* subspecies were incorporated into edible banana as primitive, diploid clones which spread westwards into South-East Asia (Jones, 2000). Dessert cultivars probably arose by the integration of genetic material from *M. acuminata* ssp. *malaccensis* and ssp. *zebrine* in area of Indonesia and Malaysia where these subspecies occur naturally. Hybridization with *M. balbisiana* may have occurred in the Philippines and/or when early cultivars spread to Indo-China, northern Burma and India. The wild *Musa* species and subspecies implicated in the ancestry of the cultivated Eumusa banana cultivars are listed in Table 2.1 (Jones, 2000).

The earliest `scientific' classification of bananas by Linnaeus in 1783, named dessert bananas which are sweet when ripe and eaten fresh as *Musa sapientium* and *Musa paradisiaca* for the plantain group which are starchy and cooked (Purseglove, 1988; Robinson, 1996). However, these two apparent species both refer to closely related interspecific triploid hybrids of the AAB group and they cannot be used to differentiate between bananas and plantains (Robinson, 1996). Simmonds and Shepherd (1955) classified edible bananas based on the relative contribution of the two wild species to the constitution of the cultivar and the ploidy or chromosome number of the cultivar (Stover & Simmons, 1987; Robinson, 1996).

Crossing and natural hybridization enlarged the variability existing among the diploids thus creating the different triploid subgroups (Montcel *et al.*, 1996). Since triploids proved to be more vigorous and productive, they gained greater popularity. Bananas and plantains have achieved greater importance as cash or as subsistence crops in regions away from their primary centres of origin. *Musa* (AAA) dessert bananas are also produced commercially in subtropical and Mediterranean climates, far away from

Table 2.1 Wild *Musa* implicated in the ancestry of the Eumusa series of edible banana cultivars.

Species	Subspecies	Geographical distribution
Musa acuminata	*banksii*	New Guinea, north-east Queensland (Australia), Western Samoa
	errans	Philippines
Musa acuminata	*burmannica*	Burma
	(burmannicoides)	
	siamea	Thailand, Indo-China
Musa acuminata	*malaccensis*	Southern Thailand, West Malaysia, Sumatra
Musa acuminata	*microcarpa*	North Borneo
Musa acuminata	*zebrina*	Java
Musa balbisiana		Indo-China, northern Burma, India, Sri Lanka, Philippines, New Guinea, Malaysia, Thailand
Musa schizocarpa		New Guinea
Australimusa species		New Guinea

Adapted from: Jones, 2000

their centres of origin (Robinson, 1996). It has been estimated that about 200-500 different clones exist (Jones, 2000) and this number could increase if the bananas of Borneo and Indonesia are completely documented (Stover & Simmonds, 1987; Robinson, 1996). They are yet incompletely known, but 500 clones are thought to be exist (Purseglove, 1988). There are hundreds of duplicate names and close clonal relatives found in every region of every banana-growing country. Estimated numbers of cultivars worldwide range from 300 to more than 1000 (Ploetz *et al*., 2007) the disparity probably arose from the different local name used in each country (Jones, 2000).

This early dispersal of banana cultivars resulted in the development of distinct subgroups of varieties in different geographic locations (Daniells *et al.*, 2001). About 1,000 cultivars in 50 subgroups are recognized (Ploetz, 2005). The world's largest collection of *Musa* held at the INIBAP Transit Centre (ITC) currently contains 1183 accessions (Swennen, 2005). It is internationally accepted that all banana cultivars should be referred to by the genus *Musa* followed by a code denoting the genome group and ploidy level followed by the subgroup name (if any) and then followed by the popular name of the cultivar e.g. *Musa* AAA (Cavendish subgroup) `Grand Nain', *Musa* AAB (plantain subgroup) `Horn', *Musa* BBB `Saba' and *Musa* AB `Ney Poovan' (Robinson, 1996). The same clone may have different names (synonym) in different locations especially in Papua New Guinea, a country with 700 languages, where the names of cultivars can vary between villages. Subgroups are named after the best known synonym of the most important clone. Some well-known synonyms of AA, AB, AAA and AAB have bee described by Jones (2000).

2.2 Constraints in Banana Production

The world banana market consists mainly of trade in Cavendish type bananas which replaced the Gros Michel over 50 years ago, due to its resistance to Race 1

Fusarium wilt, widely accepted flavour, long transport life and its higher productivity. Currently, Cavendish dominates around 40% of the total world banana and plantain production and more than 98% of the world export trade equating to 43 million tones and 16 million tones respectively (Daniells, 2006). The major drawback of Cavendish cultivars is its susceptibility to pests and diseases including the currently circulating Fusarium wilt Tropical Race 4 (TR4). The production of quality-export products require frequent applications of pesticides particularly fungicides which is undesirable. Concerns have been expressed as most bananas traded worldwide are cloned and therefore ill adapted to fight new diseases (Arias *et al.,* 2003). Bananas are attacked by a range of plant pathogens including fungi, viruses, bacterium and nematodes.

The most devastating disease of modern banana production is currently the Sigatoka disease as it affects the growth and productivity of plants in the main growing regions and is the main reason the fruit is rejected by exporters. The causative fungal pathogens *Mycosphaerella fijiensis* (black sigatoka) and *M. musicola* (yellow sigatoka) decrease photosynthesis, reducing fruit size and induces a premature maturation. It attacks all types of banana and is common in most banana producing regions where yield losses may reach up to 30-50%. Annual costs of fungicide spraying control in plantation range between US$ 600 to US$ 1800 per hectare. The second major fungal disease is Panama or banana wilt caused by *Fusarium oxysporum* f. sp. *cubense*. This soil-inhibating fungus has been the cause of one of the most destructive epidemics in history as by 1960 its race 1 had destroyed approximately 40,000 ha commercial plantation. The emergence of tropical and subtropical race 4 may represent a serious new threat because it too cannot be controlled by chemicals. The fungi infect through the lateral root and block the host vascular system which results in typical wilt symptoms. Since no fungicide control is available, production can only be continued by planting new plantings in non infested soil (Sagi *et al.*, 1998).

The most serious viral disease affecting banana is bunchy top disease caused by banana bunchy top virus (BBTV) which is persistently transmitted by the aphid *Pentalonia nigronervosa* (Caruana, 1992; Purseglove, 1988). Among the more serious of diseases caused by bacteria is Bacterial Wilt or Moko disease caused by a strain of *Pseudomonas solanacearum.* It is spread by soil contact, infected pruning knives, flower-visiting insects and diseased planting material. It can be distinguished from Panama disease by the yellowish-brown staining of the vascular tissue and grayish bacterial ooze from the cut surface of the rhizome. The burrowing nematode *Radhopholus similis* is also becoming a serious pest which invades many banana and plantain varieties. It causes destruction of the roots which results in water stressing of the leaves and the tendency for the plant to be blown down. Other serious pests are the banana weevil *Cosmopolites sordidus* and banana rust thrips, *Chaetanaphotrips orchidii* (Purseglove, 1988; Stover & Simmonds, 1987).

2.3 Fusarium Wilt Disease

Fusarium wilt of banana is recognized as one of the most destructive diseases of banana worldwide (Ploetz, 1993a; Moore *et al.,* 1999). It is a major constraint in the production of `Silk' (AAB) `Apple' `Mah' and `Manzano') in Brazil, Costa Rica, Cuba, Peru, and USA (Florida), Venezuela and other countries. It is also affected `Gros Michel' (AAA), `Pome', AAB cultivars (`Prata' etc.), ` Bluggoe' (ABB) (`Burro', `Chato' etc.) and to a lesser extent `FHIA-03' (AABB), `FHIA-18' (AAAB), `FHIA-23' (AAAA), `Hua Moa' (AAB) (`Hawaiano'), ` Maqueño' (AAB), and Pisang Awak (ABB) (Ploetz, 2003). It was first reported in Australia on 1874 but became endemic in Central America (Panama) in 1890 (Ploetz, 1994; Robinson, 1996). The first instance of Fusarium wilt on Cavendish was observed in Taiwan in 1967 and later serious affected banana production in other sub-tropical banana areas, including the Canary Islands,

South Africa and Australia (Ploetz, 1994). Farmers in the central and eastern regions of Kenya have been badly affected with wilt incidence incurring 100% losses. Surveys have also revealed that many of the banana cultivars considered to be of great economic importance in East Africa are highly susceptible to Fusarium Wilt (Rutherford, 1999). It has also caused heavy losses in the lowland areas of Uganda and in some cases entire crops have been destroyed (Kangire *et al.*, 1999).

Strains of the fungus have most commonly been grouped by their ability to cause disease, described as `race' 1, 2, 3 or 4 (Davis, 2005; Smith, 2007). Race 1 was responsible for the epidemics on `Gros Michel' and also affects `I.C.2' (AAAA), `Silk', `Pome' (AAB), `Pisang Awak' (ABB) and `Maqueño' (AAB). Race 2 affects cooking banana especially those from Bluggoe subgroup (ABB). Race 3 however, only attacks *Heliconia* spp. (ornamental plants) and is thus not important to the banana industry. Race 4 attacks Cavendish groups (AAA) and are also virulent on Gros Michel and Bluggoe (Ploetz, 1993b; Lin *et al*., 2008). A recent variant, it affects cultivars that produce more of 80% of the world's bananas including the important Cavendish and plantain subgroups (Ploetz, 2005). This is the predominant Race affecting banana in Malaysia.

The race system has often caused confusion and it has been accepted that Vegetative Compatibility Group (VCG) should be used to name strains based on reproductive compatibility of different strains of the fungus (Davis, 2005). There is no distinct relationship between VCGs and races. For example, some of VCGs infecting Cavendish in certain environmental conditions are classified as Race 4 but are considered as Race 1 in another agroecological situation (Molina, 2006).

A unique population, VCG 01213/16 a Southeast Asian isolate, is known as Tropical Race 4 (TR4) and is distinguished from Subtropical Race 4 because it is genetically distinct and damages Cavendish bananas in the tropics. It was originally

identified in Taiwan and has also been found in Australia (North Territory), Indonesia (Halmahare, Irian Jaya, Sulawesi, Sumatra), Peninsular Malaysia (Bentley *et al.*, 1998; Ploetz, 2005) and Southern China (RISBAP, 2007; Grimm, 2008). In Malaysia, this highly virulent Tropical Race 4 attacked in 1992, two commercial Cavendish plantations in the southern state of Johore (Ong, 1996) and other areas in Peninsular Malaysia. It has been shown to be a real threat to the banana industry when several large plantations were forced to close and many farmers abandoned banana cultivation for other alternative crops (Jamaluddin *et al*., 1999).

In addition to VCG, other methods have been used to characterize FOC including the use of volatile production, electrophoretic karyotyping, random amplified polymorphic DNA (RAPD), restricted fragment length polymorphism (RFLP) and DNA amplification fingerprinting analysis (DAF) (Bentley *et al*., 1999). Buxton (1962) had investigated the ability of strains of fungi to form heterokaryon (vegetative compatibility) in FOC. At least 20 VCGs have been identified from a worldwide collection of isolate of FOC, Table 2.2 (Ploetz & Corell, 1988; Koenig *et al.*, 1995; Ploetz & Pegg, 2000; Ploetz, 2005). Although VCGs provide useful means of subdividing FOC into genetically isolated groups, they can be misleading in terms of true genetic relatedness among groups of isolates (Groenewald, 2006) since it does not provide sufficient information on the extent of genetic variation within each VCG, the genetic relationships between different VCGs or the relationships between VCG and race. VCGs 01213 and 01216 (both tropical race 4) was shown to produce an identical DNA fingerprint pattern thus were considered to be the same genotype (Ploetz, 1999).

The greatest diversity of *Fusarium oxysporum* f. sp. *cubense* (FOC) was identified from Indonesia, Malaysia and the Philippines which supports the hypothesis that the pathogen has co-evolved with edible bananas and their wild diploid progenitors in Asia (Bentley *et al.*, 1999). DNA fingerprinting analysis of isolates found in wild

populations of *Musa acuminata* f. sp. *malaccensis* in Malaysia provides further evidence for the co-evolution hypothesis. The co-evolution hypothesis has important implication in the selection of banana cultivars with resistance to Fusarium wilt, as resistant cultivars are most likely to be present in regions where the greatest diversity within the host and pathogen (Bentley *et al.,* 1999). The interaction between pathogen and host in Fusarium infections is complex. Resistance and susceptibility are ultimately determined by a series of chemical and physical events that occurs in the xylem (Pegg, 1985; Beckman, 1987; Ploetz, 2000). Disease development relies heavily on the interaction between pathogen and plant genotypes and appears to be strongly influenced by environmental conditions (Groenewald *et al.,* 2006).

The symptoms of Fusarium wilt are characterized by yellowing of leaves beginning along leaf margins and advancing towards the midribs. Yellowing of leaves and bulking of petiole commences with older, outer leaves to younger leaves until the entire plant dies (Ong, 1996). Internally, infestation of the fungus results in the discoloration of vascular systems in corm and pseudostem. The pathogen grows in the vascular system and produces hyphae and microconidia. Physical blockage by the fungus together with the phytotoxic effect of metabolites and pectolytic enzymes leads to formation of gels, thyloses and degradation of vascular tissue (Beckman, 1990). At the later stage, the infected foliage becomes yellowed or destroyed and the vessel and surrounding tissues of corm and pseudostem are discolored (Rutherford, 1999).

2.4 Control of Fusarium Wilt Disease

Numerous attempts including removal and *in situ* burning of infected plant parts (leaves, pseudostems, corms and roots), drenching infected sites with formalin, fumigating, liming and protecting neighbouring healthy plants with fungicide have not

Table 2.2: Vegetative compatibility among strains of *Fusarium oxysporum* f. sp. *cubense*

VCG	Genomic Group: Cultivar(s)	Origin(s)
0120-01215	***AA***: SH-3142, SH-3362 ***AAA***: Gros Michel, Highgate, P. Ambon Putih, P. Ambon, Dwarf Cavendish, Williams, Mons Mari, Grand Nain, Lacatan ***AAB***: Prata, Lady Finger, Pacovan, Hua Moa, Silk	Australia, Brazil, Costa Rica, France (Guadeloupe, Guiana), Honduras, Indonesia (Java), Jamaica, Malaysia (Sarawak), Nigeria, Portugal (Madeira), South Africa, Spain (Canary Islands), Taiwan, USA (Florida)
0121	***AAA***: Gros Michel, Cavendish	Indonesia (Sumatra), Taiwan
0122	***AAA***: Cavendish ***ABB***: Saba	Philippines
0124-0125-0128-01220	***AAA***: Williams, Grand Nain ***AAB***: Lady Finger, Maçã, Manzano, Maqueño ***ABB***: P. Awak, Ducasse, Kayinga, Zambia, Kluai Namwa, Bluggoe, Harare, Kholobowa, Dwarf Bluggoe, Mbufu, Burro, Criolla, Pelipita, Ice Cream	Thailand, Uganda, USA (Florida), Zaire
0126	***AA***: P. Berlin ***AAA***: Highgate ***AAB***: Maqueño, P. Manurung	Honduras, Indonesia (Irian jaya, Sulawesi), Papua New Guinea, Philipines
0129	***AAA***: Mons Mari ***AAB***: Lady Finger	Australia
01210	***AAA***: Gros Michel ***AAB***: Manzano	Cayman Islands, Cuba, USA (Florida)
01211	***AA***: SH-3142	Australia
01212	***AB***: Ney Poovan ***AAB***: Silk, Kisubi ***ABB***: P. Awak, Bluggoe	Tanzania
01213-01216	***AA***: P. Lilin, P. Mas ***AAA***: P. Ambon, Valery, Williams, Grand Nain, Novaria, Red, P. Udang, P. Susu, P. Nangka, P. Berangan ***AAB***: P. Raja Serah, Rastali, Rajah, Relong ***ABB***: P. Awak, Awak Legor, Saba, Kepok, Caputu, Kosta Unknown: P. Batan	Australia, Indonesia (Halmahera, Irian Jaya, Java, Sulawesi, Sumatra), Malaysia (Peninsular), Taiwan
01214	***ABB***: Harere, Mbufu	Malawi
01217	***AAB***: P. Rastali	Malaysia
01218	***AAB***: P. Rastali, P. Raja Serah *ABB*: P. Awak, Kluai Namwa, Kepok, Siam	Indonesia (Java, Sumatra) Malaysia (Peninsular), Thailand
01219	***AAA***: P. Ambon, Ambon Putih Unknown: P. Raja Garing	Indonesia (Java, Sumatra)
01221	***ABB***: Kluai Namwa	Thailand

Source: Ploetz & Pegg, 2000

been effective in the control or containment of the disease (Ong, 1996). The most effective approach is still considered to be selection of resistant varieties or clones. Although, field evaluation is considered the most reliable method for disease resistance screening, it is demanding in terms of cost, time, manpower and space requirements (Pegg *et al*., 1996). There is also the need to maintain strict quarantine control to avoid pathogen spread. The uneven distribution of the pathogen in the field can also lead to `diseases escape' while many variables that can affect infection and symptom expression cannot be controlled. The double tray method with two month old tissue culture plantlets was found to be adequate for early screening against FOC (Mohammad *et al*., 1999). Besides being a rapid method for early screening at the seedling stage, it effectively contains the disease thus eliminates cross-contamination and allows investigations on concurrent virulence testing of multiple FOC isolates against a range of test cultivars. It is readily adapted for growth-chamber studies on the effects of various environmental factors and treatments on disease expression of FOC (Mak *et al.*, 2004b).

2.5 Breeding and Propagation Systems in Banana

Over many years, various inedible diploid subspecies of *Musa acuminata* crossed naturally resulting in the production of numerous intraspecific hybrids. Some of these hybrids were parthenocarpic, female sterile and triploid in genome structure and local inhabitants discovered that such plants had edible fruits and could be vegetatively propagated by suckers. The superior edible crosses of *Musa acuminata* would have been selected, cultivated, propagated and distributed locally as a food crop (Robinson, 1996). In common practice, bananas are propagated vegetatively by peepers, sword suckers, maiden suckers, water suckers and bits of large corms. The material used for planting

varies widely in different parts of the world (Stover & Simmonds, 1987; Purseglove, 1988; Jones, 2000).

Simmonds and Sheperd (1955) indicated that wild bananas have arisen in five main stages in their evolution from *Musa acuminata* Colla and *Musa balbisiana* Colla: (1) through the evolution of parthenocarpy and sterility in diploid *Musa acuminata* Colla; (2) through outcrossing of edible diploid of this species to *Musa acuminata* Colla and *Musa balbisiana* Colla, followed by human selection; (3) through the occurrence of triploidy in *Musa acuminata* Colla which further cross with *Musa acuminata* Colla and *Musa balbisiana* Colla; (4) through the occurrence of tetraploid hybrids, and (5) through somatic mutations, as in other vegetatively reproduced crops. The genetic system is complicated by specific interhybridization, heterozygosity and polyploidy (Loh *et al.*, 2000). Although the *Musa acuminata* and *Musa balbisiana* are accepted as the progenitors of modern bananas and plantains, the exact subspecies of *Musa acuminata* involved in the process are unknown. Similarly, the progenitors of most of the cultivated *Musa acuminata* diploids are still largely unknown (Ude *et al.*, 2002). The various possible pathways leading to the development of edible bananas have been described by Valmayor (2000).

In wild bananas, pollination is essential for fruit development as their ovaries which are protected against pollination do not develop but simply swell slightly and persist for a few weeks before shriveling. Mature fruit contains a mass of hard black seed surrounded by a scanty sweetish pulp which develops from the ovary walls and septa. In contrast, edible bananas are vegetatively parthenocarpic where most of the pulp develops from the outer edge of the loculus (i.e. inner face of the skin), with the swelling septa and axis also contributes to the mature fruit. The ovules shrivel early but may be recognized in the mature fruit as minute brown flecks embedded in the edible pulp (Stover & Simmonds, 1987; Purseglove, 1988; Robinson, 1996).

Flowering is initiated when the apical meristem stops producing leaves and forms an inflorescence (Jones, 2000). At flowering time, the ovaries of the female flowers of one hand are closely packed together and lie along the surface of the axis pointing in the distal direction. As flowering starts, they rise and stand vertically from the axis and subsequent behaviour depends upon growth. Ovaries which grow whether they contain seeds or edible pulp show a negatively geotropic reaction which causes them to take up as nearly vertical a position as the posture of the bunch and available space permit. Size of inflorescences show a transition between male and female phases with is a decline in ovary length passing from female to male flowers. Male and female flowers differ as the female flower are larger, bearing a well developed ovary which much exceeds the perianth in length and a massive style but with their stamens reduced to only staminodes. The male flowers by contrast are smaller while the ovary is abortive, the style and stigma are slender and the anthers are morphologically well developed (Stover & Simmonds, 1987; Robinson, 1996; Silva *et al.*, 2001). Both male and female flowers produce abundant nectar and the pollen is sticky, thus suggesting animal pollination. Bats are probably the most important pollinating agents in bananas and in Java the most conspicuous species has been identified as *Macroglossus minimus* (Purseglove, 1988).

The morphology of the embryo of *Musa balbisiana* and *Musa acuminata* are almost similar with both having mushroom shape embryos with the length ranging from 1-2mm (Humphery, 1896; Asif, 2004). The embryo is embedded in the endosperm between the micropyle which is a plug-like structure and the calazal mass (Sharrock, 1995) which forms a cavity after drying, (Chin, 1996). Germination is hypogeal (Purseglove, 1988) the first sign is the exudation of a drop of brownish fluid from the micropyle followed by extruding of the plug and emergence of radicle and replaced by rapid growth of seminal adventitious roots (Simmonds, 1959; Stover & Simmonds,

1987). In nature, banana seed buried in the soil can remain viable for years (Simmonds, 1955) and germinate when soils are exposed especially after felling of forest trees. Fresh banana seeds with high moisture content germinate readily but become dormant after drying and remain viable for a few months to two years (Chin, 1996).

The use of banana seedlings as a research tool and the increased emphasis on banana breeding programs require improved germination rates of banana seeds (Asif *et al.*, 2001) as there is a low rate of seed production per pollination and a low rate of germination (Stover & Simmonds, 1987; Purseglove, 1988). However, extracted embryos placed on a special medium and grown to a suitable size for transplanting greatly increased germination rate (Stover & Simmonds, 1987) by a factor of 3 to 10 (Vuylsteke & Swennen, 1993). Embryo cultures were mainly used to `rescue' embryos which would not germinate under normal conditions. It has also become an important aid for classical breeding in banana (Strosse *et al.*, 2004) by increasing the germination rate up to 90% (Mak *et al.*, 2004). It has been proven of great use in banana breeding by increasing the viability and survival of seeds from pollination especially in crosses where few seeds are being produced (Stover & Simmonds, 1987). Afele and De Langhe (1991) reported that embryos of *Musa balbisiana* with their longitudinal axis laid flat and half embedded on solidified agar medium produced the highest germination and most desirable plantlet characteristics. It had been found that embryos grown in the dark produce longer shoots and roots than light grown embryos (Asif *et al.*, 2001; Mak *et al.*, 2004).

2.6 Genetic Improvement

The main focus of banana breeding programs for the industry is disease resistance and improvements of fruit quality. Searching for varieties resistant to pests and diseases has been a major drive in the history of banana breeding programs. The

extreme susceptibility of the old cultivar `Gros Michel' to *Fusarium* wilt (Panama Disease) forced breeders and producers to shift to a more resistant variety. Eruption of new races of pathogens also can endanger the existence of the entire banana industry due to the narrow genetic pool of selected clones (Khayat *et al.*, 2004). Considering the long-term survival of FOC in infested soils, the absence of effective biological, chemical and physical control measures, and susceptibility of many desirable cultivars, the development of new, resistant genotypes is of great importance (Ploetz, 2005). The edible triploid bananas in Southeast Asia were selected for vigour, fruit size and adaptability and were developed at the expense of the original diploid types which were inferior in these traits (Robinson, 1996).

Conventional breeding of *Musa* AAA Cavendish subgroup bananas is not feasible because these triploid are totally sterile and seedless. Intractable fertilization barriers such as moderate to high levels of female sterility and triploidy makes genetic improvement of parthenocarpic plants are slow and technically difficult (Asif *et al.*, 2001). However, a genetic abnormality in `Gros Michel' of unreduced triploid gametes makes the breeding process possible. The AAA cultivar `Highgate', a dwarf mutant of Gros Michel subgroups produced an average of two seeds per bunch when pollinated by a diploid parent (Rowe, 1998b; Robinson, 1996).

2.6.1 Conventional Breeding

The first banana-breeding program began in 1922 at the Imperial College of Tropical Agriculture (ICTA) in Trinidad and followed by the Jamaica Banana Board in 1924 (Stover & Buddenhagen, 1986; Silva *et al.*, 2001; Ploetz, 2005). Its primary goal was to develop a Panama disease-resistant (Ploetz, 1993a) replacement for `Gros Michel' and later for resistance to Sigatoka leaf spot (Stover & Buddenhagen, 1986; Jones, 2000; Ploetz, 2005). Both centres collected germplasm and shared interesting

accessions with much of the early work focusing on taxonomy, cytology and cytogenetics (Jones, 2000). In 1928, the IC2 clone arising from crosses between `Gros Michel' and the wild seeded *Musa acuminata* was released. It was resistant to Panama Disease and leaf spot but bunches were of poor conformation. Up to 1966, apart from IC2, only one clone from crosses between `Gros Michel' and `Pisang Lilin' have been found to be resistant to Panama Disease, leaf spot and nematodes. Unfortunately, it was too tall and therefore subject to wind damage. Attempts have been made to intercross and self tetraploids but this approach has produced inferior plants due to meiosis and segregation in which `Gros Michel' genomes have broken down (Purseglove, 1988).

New breeding programs were initiated throughout the world in the mid 1970s to combat Black Sigatoka including the Fundación Hondureña de investigaón Agrícola or FHIA in Honduras (Escalant & Jain, 2004; Ploetz, 2005). The breeding strategy first developed by FHIA and now adopted by other programs is based on the production of improved diploids that possess useful resistance characteristics introduced from wild sources in an improved genetic background (Stover & Buddenhagen, 1986; De Langhe, 1992; Rowe, 1998a; Escalant *et al.,* 2002; Ferreira *et al.,* 2004). A major contribution of the FHIA program has been the development of synthetic diploid hybrids that are used as pollen parents, SH lines (Rowe, 1998a; Ploetz, 2005). Most of them are still male or female fertile and some have a low rate of heterozygosity which ensures the heritability of their interesting characters. Their genetic variability offers a large genetic base to the breeders (Montcel *et al.,* 1996) and sources of genetic resistance to major banana diseases except for bunchy top disease have been identified among the extensive collections of diploid accessions of *Musa acuminata* (AA) (Novak, 1992). The (AA) diploids are improved by crosses of selected parents for desired traits and that present fertile male and/or fertile female gametes, therefore obtaining improved diploid hybrids (Ferreira *et al*., 2004). This long process was successful in producing many improved

Table 2.3: Diploids used as males in banana breeding between the years 1979 to 1983

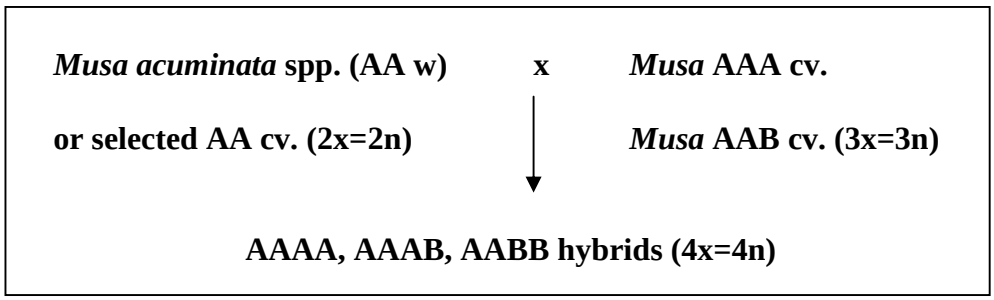
Clones and year developed	Origin and salient features
TRINIDAD-JAMAICA	
Musa acuminata subsp. *malaccensis*	Wild diploid resistant to fusarial wilt and leaf spot in Trinidad, crossed with Gros Michel, and IC2 (AAAA) selected.
Pisang Lilin (1940s)	Wild edible diploid resistant to fusarial wilt and leaf spot; crossed with Gros Michel; boodles Altafort (AAAA) selected
HONDURAS	
SH 2095 (1973)	(Sinwobogi x Tjau Lagada) x (wild *malaccensis* x Guyod) susceptible to black Sigatoka; excellent agronomic feature agronomic features; poor pollen
SH-2989 (1976)	*burmannica*-derived resistance to black Sigatoka
SH-3142 (1977)	Resistance to burrowing nematode and black Sigatoka from Pisang Jari Buaya
SH-3105 (1977)	SH-2095 x SH 2741; dwarf character from SH 2741
SH-3176 (1978)	SH-2095 x SH 2989; *burmannica*-derived resistance to black Sigatoka
SH-3248 (1979)	SH-3142 x SH-2989; slightly susceptible to black Sigatoka
SH-3249 (1979)	SH-2095 x SH-3049; dwarf
SH-3217 (1979)	SH-2095 x SH-2766; excellent agronomic features
SH-3273 (1980)	SH-3142 x SH-3176; black Sigatoka resistant
SH-3350 (1980)	SH-3142 x SH-3176; black Sigatoka resistant
SH-3351 (1980)	SH-3142 x SH3176
SH-3352 SH-3354 SH-3358 SH-3359	SH-3142 x various diploids
SH-3362 (1981)	SH-3142 x SH-3217; high level resistance to black Sigatoka
SH-3371 (1981)	SH-2095 x SH-3142; excellent agronomic features
SH-3393 (1981)	SH-3142 x SH-3217; high level resistance to black Sigatoka
SH-3397 (1981)	SH-3142 x SH-3217; high level resistance to black Sigatoka
SH-3320 (1981)	SH-3142 x SH-3180 (derived from SH-2989); immune to black Sigatoka
SH-3437 (1983)	SH-2989 x SH-3217; high level resistance to black Sigatoka

Adapted from, Stover & Buddenhagen, 1986.

diploids (Table 2.3) after many crosses between different natural diploids and diploid hybrids between the years 1979 to 1983. From 12613 diploid hybrids created, 16 improved diploids were selected with mainly yellow Sigatoka resistance but one also had FOC Race 4 resistance (Montcel *et al.,* 1996). More populations have been developed in several laboratories including CIRAD-FLHOR, CARBAP, EMBRAPA and IITA (Escalant & Panis, 2002).

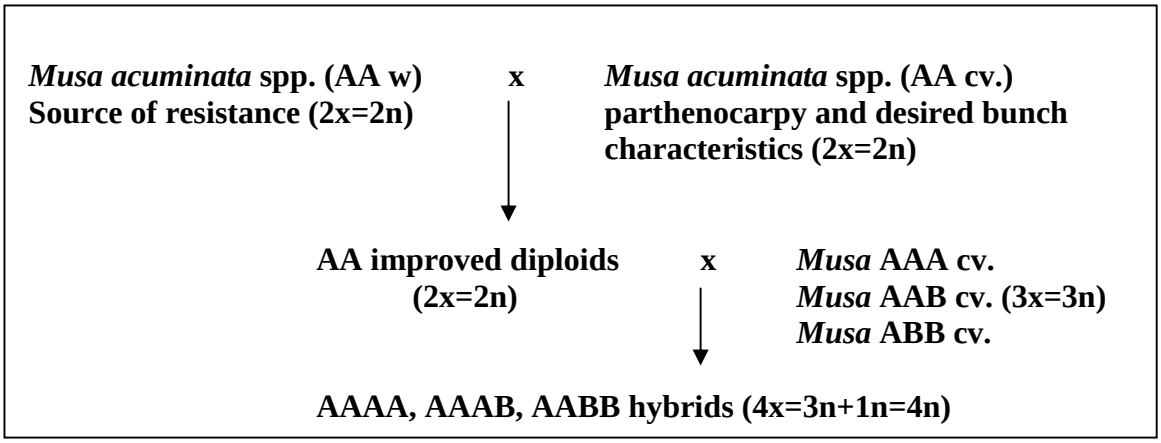
2.6.1.1 Current Strategies Used for Creation of New Hybrids

The oldest *Musa* breeding strategy use the remaining female fertility of triploid clones of interest and combine this with the high fertility of the wild diploid ancestors to obtain tetraploids hybrids (3n+1n) whose resistance to disease. Improved diploids also can be obtained which later used as parental lines.



(*Escalant et al., 2002*)

However, low yields inherent to wild diploid species caused the breeding programs to orient themselves towards the improvement of the diploid cultivars (Escalant *et al*., 2002; Escalant & Panis, 2002). Two strategies had been considered in the creation of new hybrids which is based on the development of tetraploid hybrids using improved diploids and subsequent development of secondary triploids. Efforts on producing secondary triploids had overcome constraints from low levels of fertility of desired female parents and the possibility that the residual fertility in the tetraploids (Rowe & Rosales, 1996a; Escalant *et al*., 2002).

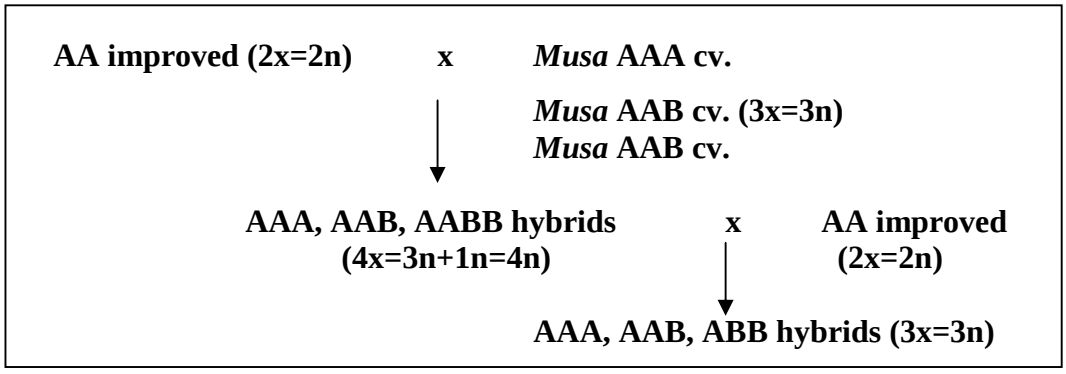


(*Escalant et al., 2002*)

In the early 1960s a diploid with superior bunch characteristics was selected from a cross of subsp. *banksii* and subspp. *malaccensis*. The Honduras program, produced a few nematode-resistant diploids obtained by crossing fertile diploids of *M. acuminata* on to `Pisang Jari Buaya' accessions. Numerous crosses and selections among diploids resulted in the first diploid with superior agronomic features, SH 2095 which derived from crosses of (`Sinwobogi' x `Tjau Lagada') x (wild *malaccensis* x `Guyod'). Several black Sigatoka-resistant diploid hybrids with advanced agronomic features were selected from a segregating population of SH 2095 x SH 2989 crosses (Stover & Simmonds, 1987).

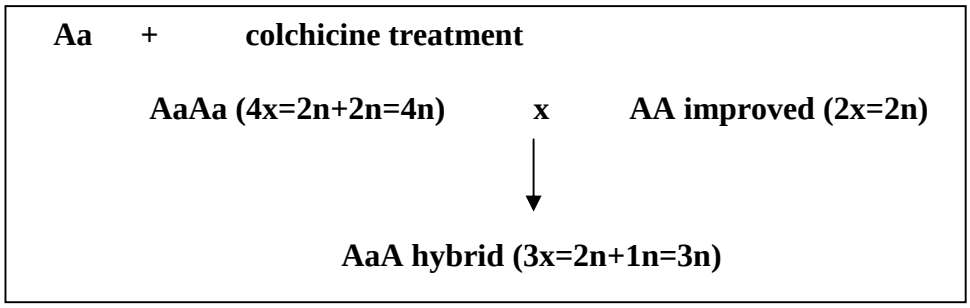
The EMBRAPA (Empresa Brasileira de Pesquisa Agropecuária) program began in 1982 emphasized the development of Panama disease-resistant AAB dessert clones to replace `Maça' (`Silk'), `Prata' and other susceptible clones (Escalant & Jain, 2004; Ploetz, 2005). It is based mainly in the improvement of (AA) diploids and subsequent crosses with AAB triploid Prata and Silk types generating AAAB tetraploids which agronomically superior and resistant to diseases (Silva *et al.*, 2001; Ferreira *et al.*, 2004).

In 1983, Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD-FLOHR) was initiated in France and Guadeloupe (Escalant *et al.*, 2002) while in 1987, the International Institute of Tropical Agriculture (IITA) in Nigeria (Vuylsteke & Swennen, 1993 ; Crouch *et al*., 1998b) and the Centre africain de recherches sur bananiers et plantains (CARBAP) in Cameroon. The initial breeding approach in IITA consisted of the production of tetraploid progenies by 3n x 2n crosses (Vuylsteke *et al*., 1993b) in which the female triploids were plantain cultivars (requiring improvement) and the diploids were wild or cultivated AA bananas with disease resistance. Twenty plantain-derived tetraploid hybrids with reduced severity of black Sigatoka, equal to higher bunch weight relative to their plantain parents and the occurance of parthenocarpy were selected from two hundred and fifty field-established progenies. High yielding and black sigatoka-resistant tetraploid plantain hybrid such as TMP x 548-9 from crosses between plantain cv Obino I'Ewai and Calcutta 4 showed potential for disease-ravaged plantain areas of tropical Africa (Vuylsteke *et al*., 1993b). Calcutta 4 has also been used in several breeding programs (De Langhe, 1992; Bakry *et al*., 2001) as a source of resistance to black sigatoka as well as in test cross designed to investigate the genetic basis of various traits (Vuylsteke *et al*., 1993a; Crouch *et al*., 1999b).



(*Escalant et al., 2002; Institution: IITA, EMBRAPA, FHIA*)

A different breeding strategy based on the use of colchicines for doubling the chromosome number of desired diploids which are then used in tetraploid x diploid crosses for producing triploids is being developed by CIRAD-FLHOR and CARBAP (Escalant *et al*., 2002).



(*Escalant et al., 2002; Institution: CARBAP, CIRAD-FLHOR*)

The experimental formation studies of tetraploids using colchine by Vakili (1965) indicate that the *Musa acuminata* tetraploids have shorter fruit length, slow producing suckers with a thick and drooping leaves and weak pseudostem compared to than the diploid `parent'. In comparison with diploids, *Musa balbisiana* tetraploids produced fewer roots with droopy and fragile leaves and produced fewer suckers that took longer to emerge, slower rationing and smaller bunches but larger fruit. The most unfavorable characteristic is that they took three months longer to produce bunch (Stover & Buddenhagen, 1986).

Conventional breeding of new triploid cultivars of bananas and plantains has not been an easy task and success has been rare (De Léon & Fauré, 1993) due to low reproductive fertility, polyploidy and complete sterility (Robinson, 1996; Escalant *et al*., 2002). Beside that, many important features are bound to be altered or losing during the breeding process such as plant size, bunch size, fruit disposition, taste, maturity, shelf life, speed of growth and others (Pinochet, 1988). The high cost of a breeding program due to large amount of space required ($6m^2$ per plant) and their long growth cycles (10 to 18 months) are additional obstacles (Crouch *et al*., 1998b; Crouch *et al*., 1999a;

Ploetz, 2005). Despite vast efforts of attempted banana breeding over more than a half century, almost all commercial varieties have been field selected. Parthenocarpy and sterility of most commercially grown genotypes do not permit breeding by hybridization. Among the banana breeding programs, the most advanced are those from FHIA in Honduras and CIRAD-FLHOR in France, IITA in Nigeria, CARBAP in Cameroon and EMBRAPA in Brazil. Most of the banana triploid hybrids which are being distributed to farmers have been released by FHIA (Escalant *et al*., 2002). The FHIA hybrid's has had a wide distribution in the world through the INIBAP-ITC with the biggest dissemination of improved hybrids (FHIA-03, FHIA-23 and FHIA-18) in Cuba (Rowe, 1998b; Morán, 2006).

2.6.2 Non-conventional Breeding

Efforts to breed cultivated banana continue to face many obstacles (slow propagation, low fertility and lack of variability), a wide array of plant tissue culture and molecular genetic techniques are also being applied as enabling and enhancing technologies for improvement of *Musa* germplasm (Vuysteke *et al*., 1993b; Crouch *et al.,* 1998b; Grimm, 2008). Somatic embryogenesis from callus or single cell cultures and by gene transfer and protoplast fusion offers a great potential than the conventional route for new hybrids (Panis *et al*., 1993; Rout *et al*., 2000). Genetic changes in tissues of explants and mutagenic action of the tissue culture media are responsible for somaclonal variation. Somaclonal variation in banana micropropagation is a common feature and several superior-quality banana clones are produced through selection for various traits including disease resistance (Asif & Othman, 2005). Seven promising Fusarium-tolerant clones of `Giant Cavendish' was produced via selection of tissue culture material tested on a disease-infected soil (Robinson, 1996). Although many resistant clones like Tai-Chiao have been obtained through somaclonal variation, a

really good substitute that out-performs the existing best Cavendish has not been obtained. Resistant clones such as GCTCV-119 carry inferior agronomic characters, while the others have poor fruit quality (Hwang, 1999). An improved variant GCTCV-215-2, selected from Tai Chiao 1 was found to be sensitive to environmental stress despite of easier to harvest and its resistance to FOC and strong wind (Hwang & Ko, 2004). Many variants are recognized in the Cavendish sub-group which differs in height, bunch and finger characteristics. Resistant clones with improvements in several other traits such as dwarfness, earliness in flowering, erect leaf, smaller fingers and higher yield were also reported (Ho *et al*., 1999). It is believed that in natural populations or in centre of diversity like Malaysia, variant forms might exist within the cultivar type, particularly with respect to disease resistance because the pathogen-host complex has co-evolved for a long time.

Breeding through mutation has been suggested as an important alternative approach for banana improvement (Ho *et al*., 1999; Heslop-Harrison & Schwarzacher, 2007). Induction of mutation by chemical or physical mutagens in a vegetatively propagated crop is sometimes able to change one or more desirable characters without altering the undesirable features (Robinson, 1996). Exposure of cultured shoot tips to gamma irradiation (GN-60 Gy/A) resulted in the release of an early-flowering mutant of AAA (Cavendish subgroup) `Grand Nain' called `Novaria' (Mak *et al.*, 2004; Robinson, 1996). However, it was found to be susceptible to fusarium wilt race 4 (Mak *et al*., 1996). Irradiation of the AAA cultivar `Dwarf Parfitt' produced the mutant `Giant Parfit' whish is taller, tolerant to race 4 FOC, tolerant to winter leaf chilling and horticulturally more acceptable (Robinson, 1996).

Since 1982, CIRAD-IRFA has developed a program of banana breeding that combines the conventional breeding methods with new techniques in genetic engineering (Bakry *et al*., 1993; Horry *et al*., 1993). In a complementary approach to

Musa breeding programs, molecular biologists have developed techniques for the isolation and insertion of genes for desirable traits. Recombinant DNA technology is of a special interest for *Musa* improvement since most of the commercially grown cultivars are sterile (Escalant *et al*., 2002). Progress in plant genetic engineering has been spectacular since the recovery of the first transformed plants in the early 1980s. Once particular genomic regions have been identified, they can be transferred from germplasm to another by conventional crossing (De Léon & Fauré, 1993). Molecular techniques have been applied to an array of species, resulting in generation of numerous transgenic plants with commercially important genes including those enabling agronomic improvement, easier processing and alternative uses (Christou, 1996). However till 2008 none have entered commercial plantings. Most recently transgenic bananas with resistance to fungal infection are entering field trials in Australia. In 2001, the International Network for the Improvement of Banana and Plantain (INIBAP) launched the Global Musa Genomic Consortium (GMGC) which aims to apply genomics to the sustainable improvement of banana and to develop freely accessible resources for *Musa* genomics using new knowledge and tools to enable both targeted conventional breeding and strategies. This strategy may allow better utilization and maintenance of *Musa* biodiversity to ensure future food and income security for millions of people in developing countries (Santos *et al.*, 2005). Recently the consortium announced successful funding for sequencing of the whole banana genome (Grimm, 2008).

Both conventional breeding and recombinant DNA strategies require detailed knowledge related to genetics and genomics of the bananas (Ganry, 1992; De Léon & Fauré, 1993). Knowledge of the extent and distribution of structural rearrangements through the combined investigations of meiotic behaviour of hybrids of interest and the search for specific genetic markers would be an invaluable asset to expedite breeding

strategies involving diploid bananas (De Léon & Fauré, 1993). Therefore the quality of the products and their availability to farmers will depend on both improvement strategy and the progress made on genetics and genomics (Escalant *et al*., 2002; Escalant & Jain, 2004). One approach making an impact on both conventional and non-conventional breeding is the uses of molecular markers.

2.7 DNA Markers in Banana Breeding

Considerable attempts have been made to distinguish and classify *Musa* accessions on the basis of morphological characteristics (Simmonds & Shepherd, 1955). However, the classification of certain accessions on this basis has been disputed (Gawel & Jarret, 1991). Biochemical and genetic techniques allow the accurate of species and cultivars, determination of evolutionary pathways between clones, identification of duplications among accession in field and tissue culture germplasm, monitoring of genetic stability in micropropagated material for commercial use and identification of key markers for breeding programs. It has also facilitated plant patents used to legally protect newly bred cultivars (Robinson, 1996). Isozymes and other biochemical markers have been used to study the diversity in *Musa* but it is not sufficiently abundant nor polymorphic to form the basis of a comprehensive marker assisted breeding system (Crouch *et al.*, 1998b). The progress made in developing additional molecular markers and the development of several segregating populations will accelerate the identification of genes of interest (Escalant *et al*., 2002). In effect, it provides a potentially indefinite number of markers that can serve as selection criteria in the manipulation of the banana genome (De Léon & Fauré, 1993).

Molecular genetic analysis has been proposed as an effective means of identifying cultivars and establishing patents to protect plant breeder rights (Melchinger *et al*., 1994; Crouch *et al*., 1998a). It has been used in many studies of fungal plant

pathogens and investigations of mating systems, gene flow, the establishment of epidemics and adaptation to host crops (Brown, 1996). It is also being used for gene tagging and identification of QTLs for qualitative and quantitative traits through marker-trait association for MAS and preparation of molecular maps (Gupta & Roy, 2002). DNA fingerprinting has helped in *Musa* germplasm conservation and documentation for breeding programs particularly in identification of genome specificity for the phylogenetic study of genus *Musa* and identification of somatic mutants (Rout *et al*., 2000). The polymerase chain reaction (PCR) has also been frequently used to develop a variety of DNA marker systems. All PCR-based molecular markers appear to detect a high level of polymorphism within a range of *Musa* breeding populations.

DNA markers, offer advantages compared to morphological characteristics and biochemical markers as it is stable and unaffected by environment (Ude *et al*., 2002). It has been used for characterization of germplasm through DNA fingerprinting and genetic diversity estimation for selection of parents for hybridization programmes (Roy *et al*., 1992; Tenkouano *et al.*, 1999). DNA markers are preferable for the assessment of genetic variability as they permit investigation of both coding and non-coding variation (Haines, 1994). The advantage of molecular techniques is their capacity to detect genetic diversity at higher level of resolution than other methods; furthermore, DNA-based assays are robust, speedy, information may be obtained from little amounts of plant material at any stage of development and it is not affected by environmental conditions. Molecular tools may contribute to the sampling management and development of `core' collections as well as the utilization of genetic diversity and might help in the recognition of the most representative populations within the `gene pool' of a landrace and the identification of the most suitable strategies for their managing and use (Lanteri & Barcaccia, 2005).

The random amplified polymorphic DNA (RAPD) technique has been successfully used to distinguish diverse *Musa* germplasm (Kaemmer *et al.*, 1992; Howell *et al*., 1994; Bhat & Jarret, 1995; Pillay *et al*., 2002). The technique involves the use of short segments of DNA (oligonucleotide primers) links to homologous sequences of the genome which generates a great number of copies of the different sections through PCR reaction. The oligonucleotide will prime amplification from a genomic template if binding sites on opposite strands of the template exist within a distance which can be traversed by the DNA polymerase. Genomic polymorphism at one or both priming sites result in the non-amplification of a band. RAPDs are thus dominant marker by the appearance of a band implies homology with the primer used while other alleles at the priming sites will be represented by absence of the band (Williams *et al*., 1990). RAPD assays are particularly useful as they require no prior knowledge of the genome and have been proven to be powerful and efficient of assisting introgression and backcrossing breeding. RAPD analysis has been used to *Musa* genome groups (Howell *et al*., 1994; Rout *et al*., 2000), more closely related germplasm (Bhat & Jarret, 1995; Pillay *et al*., 2001) and full-sib hybrids. However, RAPD analysis has several disadvantages including the dominant nature of the marker system and very sensitive to the reaction conditions (Jones *et al*., 1997; Bert *et al*., 1999; Farooq & Azam, 2000) which may limit their application in marker assisted selection (MAS).

An alternative class of PCR markers developed are sequence-tagged microsatellite sites (STMS) based on VNTR polymorphism of microsatellites (Weising *et al*., 1998). They are based on simple sequence repeats (SSR) consisting of 1-5 bp units arranged as repetitive head-to-tail tandem arrays with differences in the number of repeats, even between closely related individuals. They are highly polymorphic and have been reported to be highly abundant and randomly dispersed throughout the genomes of many species (Tautz & Renz, 1984; Crouch *et al.*, 1988a; Gupta &

Varshney, 2000; Ford *et al.,* 2002). The regions surrounding the repeats are highly conserved and can be used to design primers that will amplify across the repeat during PCR and behave in a codominant manner (Gupta & Varshney, 2000). Differences in number of repeats between alleles will appear as different size bands after electrophoresis of the PCR products thus reveal polymorphisms due to variation in the lengths of microsatellites at specific individual loci (Boluarte, 1999; Gupta & Varshney, 2000). SSR assays have the advantage of showing co-dominant inheritance and assays facilitate the handling of large number of samples as they are PCR based and problems with reproducibility are rarely encountered (Kijas *et al*., 1997; Crouch *et al*., 1998a). The variation in segregation ratios of polymorphic alleles suggests that they are in diverse chromosomal locations. The co-dominant nature of marker systems based on SSR length polymorphism is also highly informative as it allows the identification of heterozygotes (Crouch *et al*., 1998a) and estimation of allelic relationships among the genotypes (Creste *et al*., 2003).

The high variability of repeat numbers among individuals has led to the use of microsatellites for the development of genome-specific DNA fingerprints (Weising *et al.,* 1992; Sharma *et al*., 1995; Ford *et al*., 2002). Although the production of STMS markers is labour-intensive and costly, they offer advantages over other molecular marker methods of high reproducibility, unique locus-specific allelic profiles and codominance (Ford *et al*., 2002; Jarret *et al*., 1994; Lagoda *et al.*, 1995; Kaemmer *et al.*, 1997). STMS markers have become available in several individual crops due to production of genomic libraries enriched for microsatellites. Consequently, STMS markers have been used intensively not only for mapping SSR loci but also for tagging genes for a number of economic traits and study of genetic diversity in many crop plants (Gupta & Varshney, 2000; Crouch *et al.*, 1998a). It also has been shown to detect a high level of polymorphism between individuals of *Musa* breeding populations (Crouch *et*

al., 1999b). Segregation of 24 alleles donated by maternal genotype in tetraploid hybrids from crosses between triploid plantain Obino I'Ewai and diploid wild *Musa acuminata* ssp. *burmannica* (cv. Calcutta 4) demonstrate that microsatellites marker are well suited for marker assisted selection systems in *Musa* (Crouch *et al.*, 1998a). Integration of microsatellite markers into a linkage map of *Citrus* provide evidence that microsatellite markers will become an important mapping tool within plants (Kijas *et al*., 1997).

2.7.1 Amplified Fragment Length Polymorphisms (AFLPs)

Engelborghs (1998) reported the potential of amplified fragment length polymorphism (AFLP) to detect genetic variability in somaclonal variants in *Musa* species. AFLP involves the amplification of selected restriction fragments from a total genomic digest ant the electrophoretic separation of these amplicons (Cato *et al*., 1999). This PCR based method combines the strengths of different marker systems and provides new opportunities for mapping a new time-saving for generating large numbers of polymorphic bands (AFLP markers) on polyacrylamide gels. Digested DNA fragments from one or two restriction enzymes were ligated with suitable adapters and ligated fragments are selectively amplified with different primer combinations (Becker *et al*., 1995). It has been demonstrated to have a very high multiplex ratio (average number of alleles detected per assay) in a number of plant systems including potato (Van Eck *et al*., 1995), rice (Cho *et al*., 1996) and soybean (Keim *et al.*, 1997). Like RAPD, it also has the ability to identify a large number of polymorphic bands without any prior knowledge of the organism (Crouch *et al*., 1999a). It allows a retrospective analysis of the consequences of breeding and selection on the production of new cultivars and facilitated the strategic planning of new breeding approaches.

The AFLP approach provides an important practical advance for DNA profiling and plays a major role in the effective management of germplasm resources (Ellis *et al*., 1997) thus give a great value not only for germplasm characterization but also for the management of genebanks (Negi *et al*., 2004). AFLPs can detect size differences in restriction fragments caused by DNA insertions, deletion or changes in target restriction site sequences. The complex DNA fingerprinting patterns produced are reproducible and appear to show higher correlations to one another than is observed among many sets of RFLP or RAPD (Loh *et al*., 2000; Groenewald *et al*., 2006). It has been used for several purposes such as the study of genetic diversity, constructing high-density genetic maps and for finding closely linked molecular markers in combination with bulk segregant analysis (BSA) in a wide range of species (Uzun *et al.*, 2003; Cato *et al*., 1999). The genetic diversity studies in *Musa acuminata*, *Musa balbisana* and other banana using AFLP (amplified fragment length polymorphism) technique suggesting the existence of new relationships between subspecies inside the *Musa acuminata* complex (Ferreira *et al*., 2004).

Selection of a suitable marker system depends on a number of factors (Karp & Edward, 1997). Throughput and speed, equipment and skill required, the need for automation, and cost effectiveness is the important technical considerations. Other considerations relate to the technique itself is the informativeness and sensitivity of the marker system. Finally, the demands on accuracy and data analysis must be considered (Farooq & Azam, 2002). In many instances, DNA markers will be a vital link in the development of knowledge breeding schemes in banana (Crouch *et al*., 1998b). Marker identification relies on populations of host progeny that segregate for the trait investigated such as for resistance and susceptibility and is a non-ambiguous means by which the presence or absence of the marker and disease response of the progeny can be scored (Ploetz, 1993a). The use of markers to follow the inheritance of genes,

particularly those genes that cannot be readily identified and selection of a marker flanking a gene of interest, allows selection for the presence (or absence) of a gene in a new progeny.

Current breeding efforts for the improvement of bananas rely on introgressing useful genes from the wild and cultivated diploid progenitors (Ude *et al*., 2002). Crop wild relatives including the progenitors as well as others species closely related to them have been undeniably beneficial to modern agriculture. It provides plant breeders with a broad pool of potentially useful genetic resources (Hajjar & Hodgkin, 2007). The AFLP markers can be converted into a simple (sequence characterized amplified region) SCAR markers which involves characterization of the linked marker and the design locus specific primers for easy use. The conversion of a linked marker to SCAR has been applied successfully in a number of cases involving RAPD markers and AFLP markers (Negi *et al*., 2000).

2.8 Genetic Linkage Map

A saturated genetic map can be used to screen populations and detect individuals carrying traits of interest. High density molecular marker linkage maps have been constructed for more than 15 different species including *Arabidopsis thaliana*, barley, soybean, maize, tomato, wheat, rice and potatoes (De León & Fauré, 1993). Several genes have been mapped including genes coding for structural proteins and several resistance genes which are major genes or loci contributing to the expression of a continuously varying character (QTL) (Fauré *et al.,* 1993). It is a representation of the relative positions of genetic loci i.e. genes and markers on chromosomes, determined on the basis of how often the loci are inherited together (thus: linkage) or become separated by genetic recombination. Markers which lie close together show a small percent recombination and are said to be linked (Paterson *et al*., 1991). The map is linear, i.e.

one-dimensional, reflecting the linear structure of the chromosomes which distances are usually expressed in *centiMorgans,* (cM) (Stam, 1993). Linkage analysis requires sufficient understanding of molecular biology, genetics, statistics and optimization techniques (Kyazma, 2004). Segregation analysis and construction of a linkage map are stepwise processes which can be facilitated using computer programs. Recombination frequencies for all pairwise comparisons between loci estimated using maximum likelihood method and map units (cM) are calculated using a mapping function. The linkage map is deduced by the best fit to these values (Stam, 1993; Haines, 1994).

The development of PCR-based molecular markers has facilitated the construction of genetic linkage maps of the diploid banana genome for better understanding of genetics of resistance and the localization of resistance genes (Fauré *et al.,* 1993). Highly dense genetic linkage maps are potentially powerful tools for the localization and map-based cloning of genes (positional cloning) (Kriegner *et al*., 2003) and also constitute the framework for the use of genetic markers in breeding programs via marker-assisted selection (MAS) and play a prominent tool in various fields of fundamental and applied genetic research e.g. QTL analysis and map-based cloning of genes facilitate map-based cloning (Weising *et al.,* 1995; Jansen *et al*., 2001; Cervera *et al*., 2001). AFLP framework maps constitute the skeleton on which co-dominant microsatellites and STS markers can be mapped progressively to construct a saturated `species consensus map' which will be a useful tool for evaluation studies and breeding purposes (Cervera *et al.,* 2001). High density molecular linkage maps have been constructed and utilized for studying quantitative traits in rice (Rabiei *et al*., 2004). The development of genetic maps is important for crops with long generation times and complicated breeding (Kijas *et al*., (1997). Ideally, the number of linkage groups is equal to the number of chromosomes but in practice it may be smaller or larger, particularly during early developmental stage of a linkage map.

Currently, the generation of highly relevant and precise linkage map is not routine in *Musa* due to the triploid nature of the crop and lack of good breeding populations. To develop the traditional linkage maps, large diploid populations have been generated from plantain banana interploidy crosses and from crossing *M. balbisiana* accessions. This is based on diploid relatives and extrapolation of polyploidy crop. The precision of estimations of recombination frequencies or genetic distances depends on three interrelated factors: the size of the population, the types of markers to be mapped and the density of these markers (De Léon & Fauré, 1993). A number of segregating populations are needed for the production of more dense linkage. For highly heterozygous crop e.g. alfalfa (Barcaccia *et al.,* 1999), rhodegrass (Ubi *et al.,* 2004) and potato (Ritter *et al.,* 1990), F_1 can be useful because the parents has the possibility of carrying several alleles that will segregate in the F_1 progeny. Crossing between diploid of cultivated and wild banana accession has become increasingly important (Bakry *et al*., 1990; Rowe, 1987). Mapping is a means toward a better understanding of genome evolution, organization and function allowing extensive genetic manipulations while quickly enhancing and strengthening our knowledge of genome diversity and organization of their ancestral contributions thus a more rational basis for breeding practices and strategies (De Léon, & Fauré, 1993).

2.9 Quantitative Trait Loci (QTL)

Choice of parents for developing base populations is crucial in breeding of line cultivars because it largely predetermines the outcome of subsequent selection steps and affects the optimum allocation of resources in breeding programs. Efficiency of breeding programs by concentrating the efforts on the most promising crosses would be increased if the breeders could predict the prospects of crosses for line development before producing and testing in field trials (Bohn *et al*., 1999). Plant breeding relies on

quantitative variation and selection to improve plants for traits and characteristics that interest growers and consumers (Asíns, 2002). However, the problem in predicting the selection response is still unsolved due to lacking of knowledge about the genetic variance for the trait(s) of interest (Bohn *et al.*, 1999). Many characters of agronomic importance are controlled by genes at several unlinked loci defined as quantitative trait loci (QTL).

In the early twentieth century, Johannsen demonstrated that quantitative variation resulted from the combination of multiple segregating genes and environmental factors. Therefore, it is usually studied in a general term by using statistical techniques (population means, variance, covariance of relatives, heritabilities, etc.) rather than in terms of individual gene effects. However, it is possible to detect and locate the loci affecting quantitative traits (QTL) by combining the analysis of segregation of marker genotypes and phenotypic values of individuals or lines. The availability of DNA markers and powerful biometrical methods has led to considerable progress in QTL mapping in plants (Asíns, 2002). The combination of molecular marker and trait data to explore the individual genes concerned with quantitative traits (QTL analysis) has become an important tool to dissect the genetics of complex characters (Kearsey, 1998). The basic idea of mapping QTLs through co-segregation analysis has been available since 1923 (Lander & Bolstein, 1989). Statistical methods based on the normal distribution and three point mapping can locate genome regions contributing to a QTL (Haines, 1994).

QTL analysis involves selecting and hybridizing parental lines that differ in one or more quantitative traits and analyzing the segregating progeny to link the quantitative trait locus to known DNA markers. It can be employed to enhance plant breeding efforts and speed up the creation of new cultivars. It also unveils interesting wild alleles thus facilitate the introduction of beneficial genetic material from related and unrelated wild

species without the drawbacks associated with `wild genes' faced through conventional methods. The principle objective of QTL analysis is confining QTL to narrow chromosomal regions which implies the type of experimental design or segregating population, its size, number, informativeness and level of polymorphisms of DNA markers and the statistical methods to build up the linkage map and to perform QTL analysis (Asíns, 2002).

QTL analysis not only provides DNA markers for efficient selection, but also value in resolving environmental interaction and genetic effects which are common in agronomically important traits such as `days to flowering', `stay-green' or tolerance to abiotic stress. Linkage between a genetic marker and a QTL was first demonstrated by Sax (1923) who found that *Phaseolus* genotypes with different seed coat colours also differed in average seed size (Young, 1996). As genetic maps came to include more markers, it became possible to more precisely estimate the location of a QTL by studying several markers along the chromosomes. New algorithms for QTL mapping minimized the number of individuals and genetic markers needed to map QTLs (Paterson *et al.*, 1991). In banana plants, QTL mapping is generally achieved using bi-parental cross populations; a cross between two parents which have a contrasting phenotype for the trait of interest are developed. Linkage between the phenotype and markers which have already been mapped is tested in these populations in order to determine the position of the QTL. Such techniques are based on linkage and are therefore referred to as "linkage mapping".

Linkage maps have revolutionized quantitative genetics by creating the technological base necessary for mapping genes underlying quantitative traits, so called quantitative trait loci (QTL). Hypothesis tested by classic quantitative genetic methods describes the characteristics of populations but not genes. It does not lead to an understanding of the effects and location of genes underlying quantitative traits or to the

discovery of favorable allele which is essential for predicting marker-assisted selection (MAS) (Knapp *et al*., 1992).

Quantitative Trait Loci (QTL) is a polymorphic locus which contains alleles that differentially affect the expression of a continuously distributed phenotypic trait. These traits are typically affected by more than one gene and also by the environment. Thus, mapping QTL is not as simple as mapping a single gene that affects a qualitative trait such as colour of the flower. Modern molecular biology provides the ability to detect genetic variation directly at the DNA level and has provided an essential supply of markers for fine scale analysis. The availability of molecular markers RFLPs, AFLPs, RAPDs and STMS enable mapping of QTL. These markers segregate as single genes that unaffected by the environment. Furthermore, they are highly polymorphic and thus provide opportunities for developing high quality linkage maps.

A large number of experimental design and statistical methodologies have been proposed for detection of the individual genes affecting quantitative traits with the aid of genetic markers. The statistical methods used to detect QTL have generally used parameters that are based on assumptions as to the nature of distributions of the observations (Weller, 1992). More markers may be searched for in the region of interest by using BSA to locate the QTL more precisely (Cervera *et al.*, 2001). Several studies have demonstrated the relationship between molecular variants and the phenotypic expression in several animal species (Montaldo *et al*., 1998) and stimulated the idea to add the genomic to the phenotypic information to increase the selection response to the `traditional methods' via marker-assisted selection (MAS).

2.10 Marker-Assisted Selection (MAS)

The use of molecular techniques has opened the possibility of developing selection procedure(s) based on genotype rather than on phenotype which makes the

breeding process more effective, rapid and allows the identification of seedlings of the design genotype (Uzun *et al*., 2003). The molecular markers facilitate the construction of high-density DNA marker maps for a range of economically important agricultural species, thus providing the framework needed for application of marker-assisted selection (MAS). Marker-assisted selection is based on the principle of genetic linkage that recombination occurs infrequently between loci which are very close together on the chromosome. Selection is made on the basis of an easily or reliably assessed marker(s) which is tightly linked to a character of practical important but not easily assessed (Haines, 1994).

MAS is able to offer significant advantages in cases where phenotypic screening is particularly expensive, time consuming or difficult and for those involving multiple genes, recessive genes, late expression of the trait of interest and seasonal or geographical considerations (Dreher *et al.,* 2000). MAS allows plant selection at the juvenile stage from an early generation and unfavourable alleles can be eliminated or greatly reduced (Korzun, 2003), thus reducing the field maintenance cost and speeding up the time of varietal release by focusing on reduced number of mature plants in the field (Dreher *et al*., 2000). Putative genes affecting traits of interest can be detected by testing for statistical associations between marker variants and any trait of interest through the marker map. By having identified markers located beside or within genes of interest, it is possible to select identifiable marker variants (alleles) in order to select for non-identifiable variants of genes of interest.

The success of MAS is influenced by the relationship between the markers and the genes of interest. Dekkers (2004) distinguished three kinds of relationship: (i) the molecular marker (M) located within the gene of interest, (Q) which refers as gene assisted selection (GAS). It is the most favourable situation for MAS since by the following the inheritance of the M alleles, we can directly follow the inheritance of the

Q alleles but it is difficult to find these kinds of markers. (ii) the marker is in linkage disequilibrium (LD) with the gene of interest, Q throughout the population. LD is the tendency of certain combination of alleles (e.g. M1 and Q1) to be inherited together. Population-wide LD can be found when markers and genes of interest are physically very close to each other and/or when lines or breeds have been crossed in recent generations. Selection using these markers can be called LD-MAS. (iii) The marker is not in linkage disequilibrium (i.e. it is linkage equilibrium (LE)) with the gene of interest, Q throughout the population. Selection using these markers can be called LE-MAS but it is the most difficult situation for applying MAS.

Because of the universal nature of DNA, molecular markers and genes, MAS can in theory be applied to any agriculturally important species including banana. In addition, MAS can be applied to support existing conventional breeding programs by using markers to accelerate the introduction of the gene of interest. Although the efficiency and economics of MAS are often debatable, the value of mapping quantitative trait loci (QTL) to gain a deeper understanding of the genetics of complex traits has always been useful (Knapp *et al*., 1992). MAS offers potential savings compared to conventional breeding methods if it allows breeders to identify the presence of multiple alleles related to a single trait when the alleles do not exert an individually detectable influence on the expression of the trait (Melchinger, 1990).

The ability to identify the incorporation of unobservable alleles is important especially in breeding for resistance to diseases and pests because multi-genic resistance achieved through `gene-pyramiding' (Hayes *et al.,* 2000) is much more desirable than mono-genic resistance and offers a particular advantage when inoculations are difficult to control or where a pathogen is not available due to import restrictions (Haines, 1994). Crouch *et al*. (1999a) reported that molecular markers-assisted breeding had the potential to dramatically enhance the pace and efficiency of genetic improvement of

Musa. MAS is important to identify single genes and genome segments for use in transformation of cultivars and breeding materials. It is also important for development of efficient recombinant DNA techniques for isolation and introgression into *Musa* of genes covering a wide array of desirable traits.

2.11 Bulk Segregant Analysis (BSA)

Bulk segregant analysis (BSA) is a processs that has been developed for rapidly identifying markers linked to any specific gene or genomic region. It is based on the comparison between two DNA bulks, each comprising DNA from individuals exhibiting the extreme phenotypes (i.e. high and low) of a particular trait in a segregating population (Michelmore *et al*., 1991; Boluarte, 1999; Haines, 1994). By pooling DNA from resistant vs. susceptible individuals, many large effect disease resistance genes have been found because the pooling strategy is more efficient on larger allele differences (Asíns, 2002). Once markers that distinguish the bulks are identified, precise linkage distance could be determined by segregation analysis. The effort required to construct a high-resolution map can thus be reduced manifold as the number of samples from which DNA must be isolated and analyzed can be reduced by a factor of 10 or more (Churchill *et al.,* 1993).

BSA provides a method to focus on regions of interest or areas sparsely populated with markers and for analyzing the segregation of randomly selected molecular markers in single populations. It efficiently identifies markers linked to genes of interest, allowing their rapid replacement on a genetic map. It also consolidates genetic maps by identifying markers in sparsely populated regions and at the end of linkage groups (Michelmore *et al.,* 1991). A combination of bulk segregant analysis (BSA) and AFLP technique offers the advantage of analyzing large number of markers in a single experiment with a high reproducibility (Negi *et al.*, 2000).

3.0 MATERIALS & METHODS

3.1 Experimental Scheme and Approach

The study on molecular markers potentially associated with resistance and/or susceptibility to *Fusarium* wilt Tropical Race 4 (FOC TR4) involved several different stages including material collection, population development, micropropagation, screening for disease responses and molecular analysis. The schematic representation of the approach and experimental design of the research is shown in Figure 3.1.

3.2 Development of *Musa acuminata* ssp. *malaccensis* wild seed population

3.2.1 Clonal Seed Progenies

Clonal seed progenies of wild banana *Musa acuminata* ssp. *malaccensis* were developed as starting material through embryo rescue technique and micropropagation. Embryo rescue culture was carried out following Asif *et al.* (2004). Fruits collected were thoroughly washed with distilled water followed by soaking in 50% Chlorox for 30 minutes. Seeds were extracted from the fruit skin and pulp before soaking again in 50% Chlorox for 10 minutes. Quick rinsing with 70% ethanol was performed before transferring it onto sterile petri dish and air drying in laminar flow. The seed coat was broken apart with a sharp scalpel blade and forceps to expose the embryo. A sterile needle was used to remove the embryo from the seed coat before transferring it into a glass bottle containing MS media (Table 3.1). The embryo cultures were kept in the dark for a week to induce rooting and later exposed to light for shoot elongation.

Germinated plantlets were subcultured and transferred into fresh media supplemented with 6-benzylaminopurine (BAP) to induce multiple shoots. Individual plantlets were subcultured several times to generate the clonal populations. Finally, the plantlets were subcultured into rooting media to induce roots before planting into polybags and hardening in the greenhouse prior to Fusarium screening.

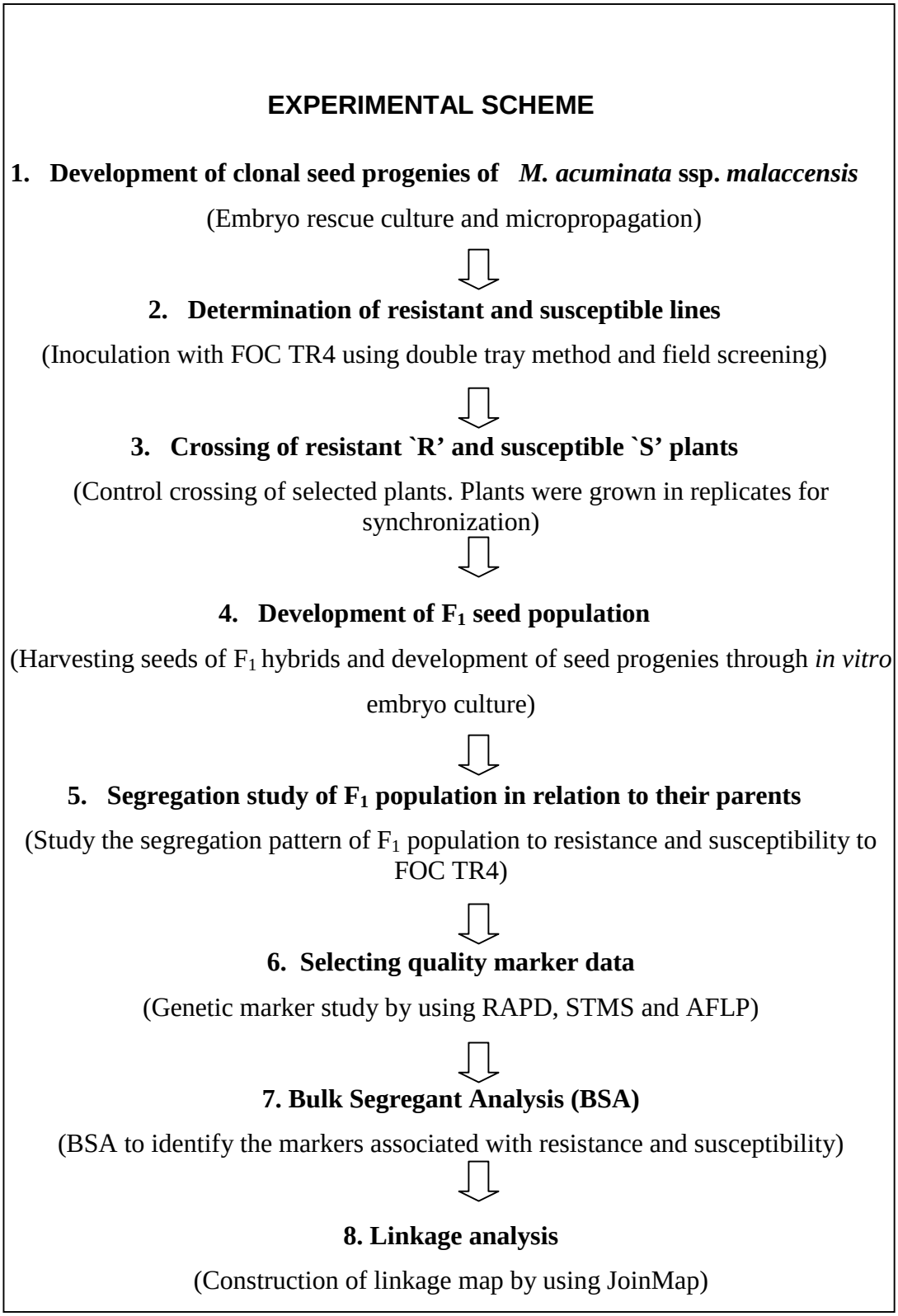


Figure 3.1: Schematic representation of the experimental scheme and approach.

Table 3.1: Murashige and Skoog media

Macro Stock Solution	**2L Stock (Use: 100ml/L**
Ammonium nitrate (NH_4NO_3)	33.0g
Potassium Nitrate (KNO_3)	38.0g
Calcium Chloride ($CaCl_2.2H_2O$)	8.8g
Magnesium Sulphate ($MgSO_4.7H_2O$)	7.4g
Potassium dihydrogen Orthophosphate (KH_2PO_4)	3.4g
Micro Stock Solution	**2L Stock (Use: 10ml/L)**
Manganese Sulphate ($MnSO_4.4H_2O$)	4.46g
Zinc Sulphate ($ZnSO_4.H_2O$)	1.72g
Potassium Iodide (KI)	0.17g
Cupric Sulphate ($CuSO_4.5H_2O$)	0.0052g
Sodium Molybdate ($Na_2MoO_4.2H_2O$)	0.05g
Cobaltus Chloride ($CoCl_2.6H_2O$)	0.0052g
Boric Acid (H_3BO_3)	1.24g
Vitamin Stock Solution	**500ml (Use: 2ml/L)**
Nicotinic Acid (Vitamin B_3)	0.125g
Thiamine HCl (Vitamin B_1)	0.25g
Pyroxine HCl (Vitamin B_6)	0.125g
Myo-Inositol	25.0g
Glycine ($C_2H_5NO_2$)	0.5g
Fe Source Stock Solution	**500ml Stock (Use: 5ml/L)**
Sodium EDTA ($Na_2.EDTA.2H_2O$)	3.75g
Ferrous Sulphate ($FeSO_4.7H_2O$)	2.78g
Others	
Sucrose	40.0g/L
Gelrite	1.75g/L
pH	5.8

EDTA: ethylediaminotetraacetic acid

3.3 Screening for Response to *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (FOC TR4)

Screening of FOC TR4 was carried out using double tray method and `Hot Spot' trial. The schematic representation of the method used to determine response of plantlets to FOC TR4 is shown in Figure 3.2**.**

3.3.1 **Preparation** of FOC TR4 **Conidial Suspension**

FOC TR4 isolated from infected Cavendish banana (Novaria, AAA). Corm tissue of infected Novaria (AAA) obtained from a maintained `Hot Spot' in Teluk Intan, Perak was cultured on 1% PDA (potato dextrose agar) media. Cultures were maintained at 28ºC with 16h light. Small pieces of PDA with FOC mycelia were further cultured in Armstrong liquid media containing sucrose 20g/L; $MgSO_4$. $7H_2O$, 400mg/L; KCl, 1.6g/L; KH_2PO_4, 1.1g/L; $Ca(NO_3)_2$, 5.9g/L; $FeCl_3$, 0.2 g/ml; $MnSO_4$, 0.2 g/ml; $ZnSO_4$, 0.2 g/ml; and dH_2O to a final volume of 1L. Cultures were incubated at room temperature and shaken twice a day for a week. Conidial suspension was prepared by filtering the suspension through two layers of cheese cloths to separate the fungal hyphae from the spores. Concentration of spore suspension was measured using a haemacytometer to obtain a final suspension of 10^6 spores/ml.

3.3.2 Double Tray and `Hot Spot' Screening of Seed Progenies

Screenings were carried out using double tray method and `Hot Spot' screening. Tissue cultured plantlets were hardened in a double tray consisting of 43 x 29 x 9cm (upper tray) and 46 x 31 x 20cm (outer tray) for 4-6 weeks to produce plants of 10cm height or more. Plantlets were uprooted gently from a flooded tray to avoid root injury. Roots were washed with distilled water and dipped into FOC TR4 spore suspension of 10^6 spores/ml for two hours. Plants were watered daily and kept in the

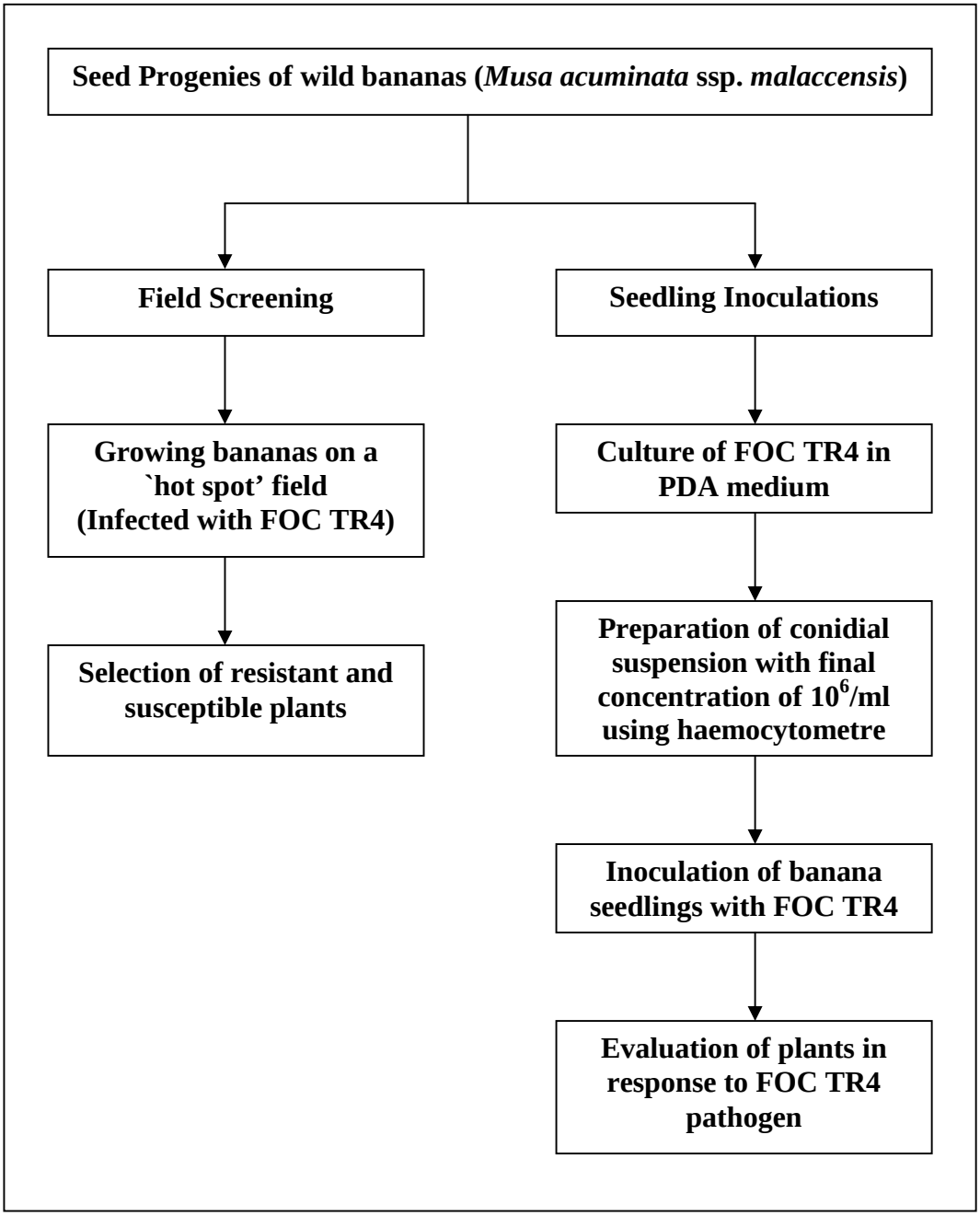


Figure 3.2: Determination of resistant and susceptible banana lines

greenhouse under natural condition. Susceptible plantlets of Novaria (AAA) had been used as a control. Evaluation of disease infection was done base on LSI (Figure 3.3) and RDI (Figure 3.4) symptoms as described by Brake *et al*. (1995) and Asif (2004).

Tissue cultured plantlets were hardened to up to 0.6m in height before transplanting to the `Hot Spot' (heavily infested plot with FOC TR4 at United Plantation, Teluk Intan, Perak). Susceptible plantlets of Novaria (AAA) had been used as a control. Plants were grown along with the infested material of FOC TR4 in a single row of 2 x 2m spacing. Symptom expression was observed for a period of one year.

3.4 Development of F_1 Hybrid Populations

Crosses were performed on the selected resistant and susceptible plants as determined through pathogenic testing against FOC race 4 (See section 3.3). Plants were planted in a clean field and green house in replicates in order to obtain synchronization. Mature pollen was rubbed onto receptive female flowers in order to fertilize and covered with a plastic bag to avoid contamination. Fruits developed were observed for seed development before harvesting. Embryo rescue culture followed by micropropagation was again performed to develop F_1 hybrid populations. Plantlets were subcultured into rooting media to induce roots before planting in polybags and hardening in the greenhouse for FOC TR4 screening. DNA of each individual was also extracted for further analysis. The schematic representation of the stages involved is shown in Figure 3.5.

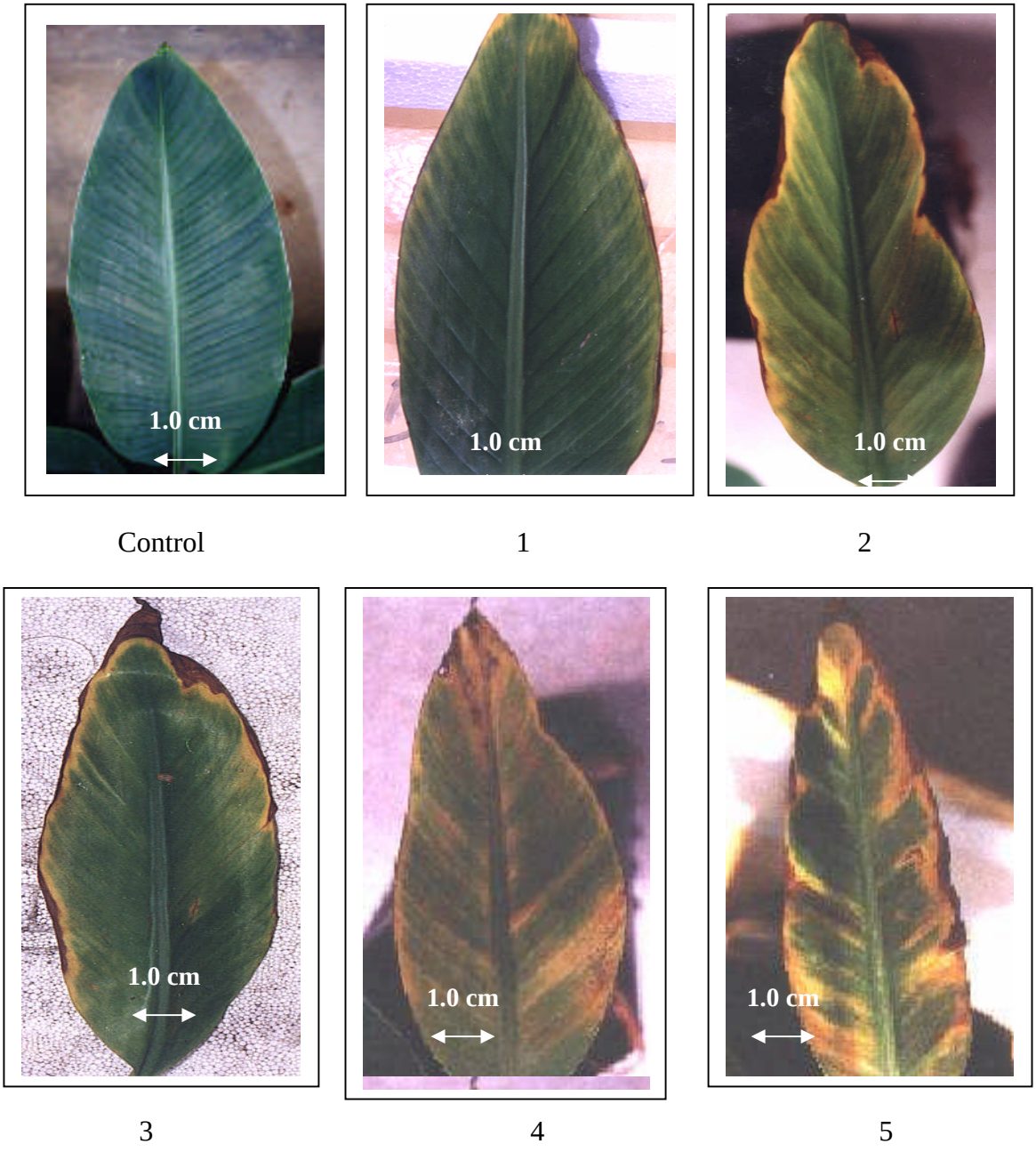


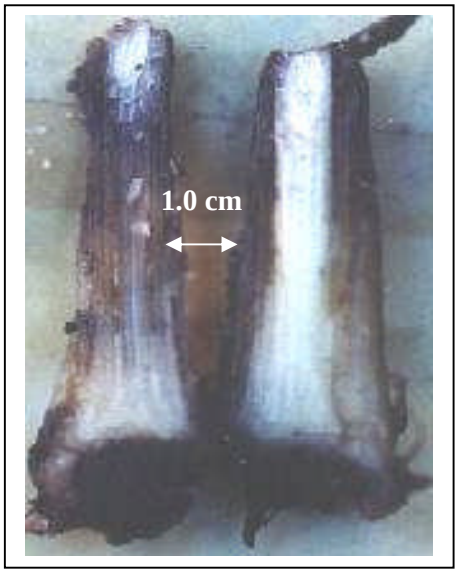
Figure 3.3: Leaf symptom index based on Brake *et al.* (1995) and Asif. (2004). All symptoms were recorded after 2-3 weeks of inoculation. All leaves shown were of *Musa acuminata* ssp. *malaccensis*

Control – showing healthy green leaf.

1-2 - Slight to less than 5% yellowish of the margins of the older leaf.

3-5 More than 5% to extensive yellowing of the older leaf.

0 1 2 3 4 5



6

Figure 3.4: Rhizome discoloration index (RDI) adapted from Brake *et al.* (1995) and Asif (2004) used for disease screening evaluation in double tray method. All rhizomes shown were of *Musa acuminata* ssp. *malaccensis*

0 : Clean rhizome and stellar region.

1 : Slight discoloration at junction of root and rhizome.

2 : Trace to 5% of stellar region discolored.

3 : 6-20% of stellar region discolored.

4 : 21 – 50% of stellar region discolored.

5 : More than 50% of the stellar region discolored.

6 : Dead plant with blackening of the entire rhizome.

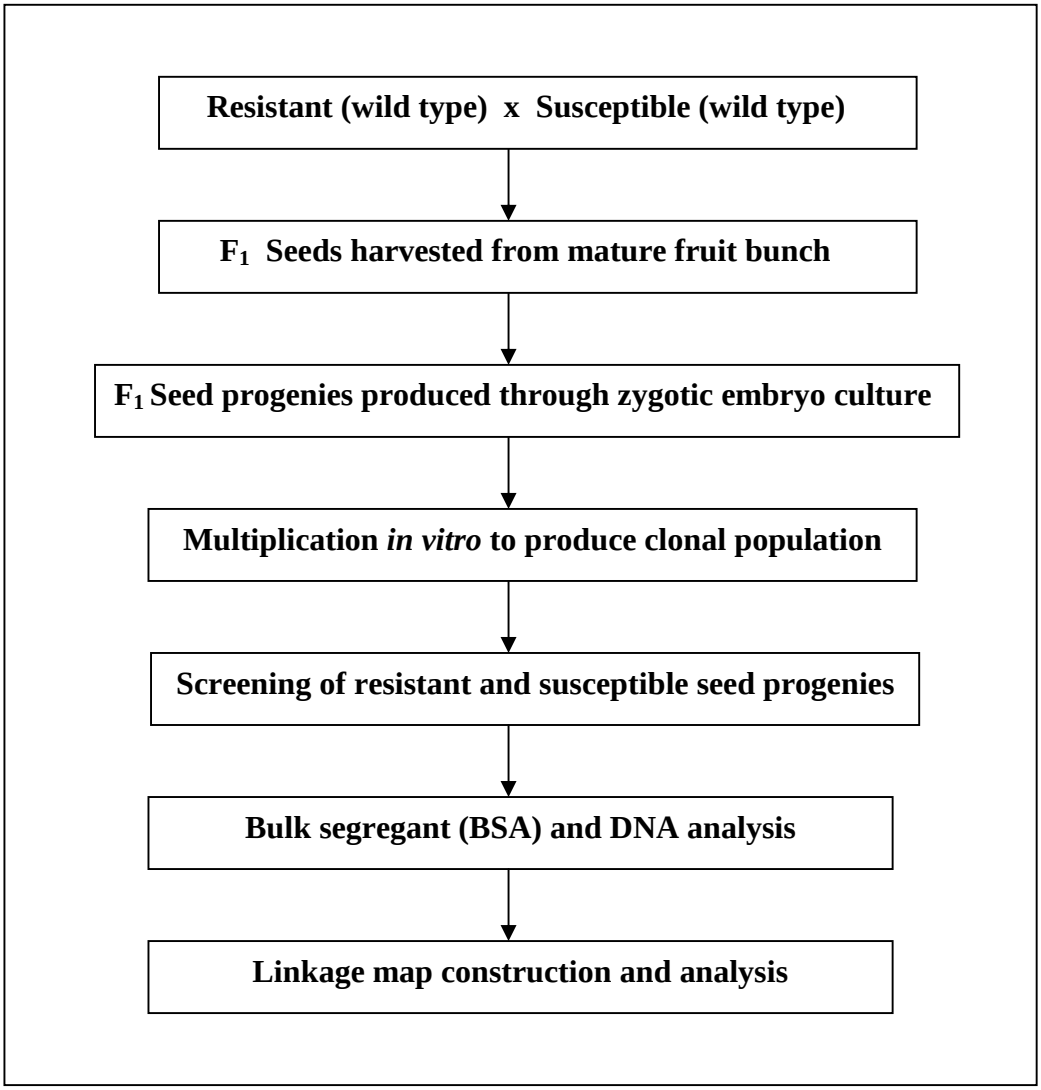


Figure 3.5: Schematic representation of the development of F_1 hybrid populations and DNA analysis

3.5. Molecular Analysis

3.5.1 DNA Extraction

DNA was extracted using a modified CTAB method based on the protocol of Doyle and Doyle (1987). Fresh leaf tissue (2-3g)) were ground into a fine powder using liquid nitrogen and a mortar and pestle. The ground powder was immediately transferred into 15 mls of pre-warmed (60ºC) extraction buffer (2% CTAB (cetyltrimethylammonium bromide); 1.4M NaCl; 20 mM EDTA; 100mM Tris-HCl, with the pH 8.0) in a capped polypropylene tube to prevent degradation of DNA by cellular enzymes. Clumps were suspended using a spatula and then incubated for 2 hours at 60ºC in a water bath.

Equal volume of Chloroform: Isomyl alcohol was added and mixed gently for 10 minutes to prevent degradation of DNA. The suspension was centrifuged for 10 minutes (10,000 rpm at 4ºC) and supernatant was transferred into a clean polypropylene tube and these steps were repeated twice. Heat treated RNase was then added to a final concentration of 100µg/ml and thoroughly mixed before incubation for 15 minutes at room temperature. The final aqueous phase was transferred to a clean centrifuge tube using a large bore pipette. Proteinase-K was added to the aqueous phase to a final concentration of 10µg/ml and thoroughly mixed before incubation for 15 minutes at room temperature. 0.6 volume of cold isopropanol was added and mixed gently but thoroughly by inverting the tube several times.

The fibrous network of precipitated DNA/CTAB complex was lifted from the solution using a pasture pipette and transferred into the washing solution for washing by agitating the pellet gently for a few minutes. Then, tubes were inverted and drained on a paper towel for about one hour. Care was taken that pellets should neither contain residual ethanol nor are too dry. Pellet was allowed to dissolve overnight (4°C) without agitation in TE buffer with high salt concentration. Dissolved DNA was extracted using

1 volume phenol and centrifuged at 5000x g for 10 minutes at 4ºC. Aqueous phase was removed with a wide bore pipette and re-extracted again with 1 volume Chloform:Isomylalcohol (24:1) ratio with gentle mixing for emulsification of the phase. Samples were then centrifuged at 5000x g for 10 minutes at 4ºC.

The aqueous phase was removed and 0.5 volume of 7.5M ammonium acetate solution was added, mixed and chilled on ice for 15 minutes. The samples were centrifuged for 30 minutes (10,000 rpm at 4ºC). The supernatant was transferred to a new tube followed by 2 volumes of cold 96% ethanol and mixed by inversion before keeping at -20C for an hour. Samples were centrifuged for 10 minutes (5000x g at 4ºC) and the pellets were washed in 70% cold ethanol. Finally the pellets were drained and dried at room temperature. The dried pellets were dissolved in an appropriate volume of distilled water.

3.5.2 Determination of Quality and Quantity of DNA

3.5.2.1 Quantification of DNA

The concentration of the DNA in solution was measured using an ultraviolet spectrophotometer (model DU 7500I, Beckman, U.S.A.). A 1 ml portion of an approximately diluted sample was measured at 260 and 280. The 260nm reading is indicative of DNA concentration and the 280nm reading indicates the protein contamination. The 260/280 ratio of pure DNA should read approximately 1.8 but any sample with a reading in the range 1.6- 2.0 was considered to be of sufficient purity. A sample reading of 1.5 assumed to be contaminated with protein and was extracted with phenol to further remove the protein. The readings were taken against a blank of 1ml of distilled water at 260nm. The 260nm reading for sample was multiplied by the dilution factor and the concentration calculated by proportion as follows;

e.g: O.D 260 = 0.25 (using 1/200 dilution)

1.0 = 50µg/ml

0.25 = 50 x 0.25 x 200

=2500µg/ml

= 2.5µg/ml

3.5.2.2 Determination of DNA Quality

The degree of DNA degradation was determined by electrophoresis of an aliquot of sample in 1% agarose gel. Large molecular weight DNA appeared as a sharp band while partially degraded DNA forms a long smear from large to small fragments. Contaminated DNA with other substances may absorb uv irradiation and impeded accurate analysis. Similarly, samples with RNA contamination may appear as fast running band near the end of the gel. Those samples were again treated with RNase. DNA samples was added with 1/10 volume of loading buffer and mixed gently. Samples were loaded with care to avoid spilling over into adjacent wells. The gel was run at 80V for 2 hours and viewed under ultra violet.

3.5.3 RAPD Analysis

PCR was performed following William *et al*. (1990) with a modification as described by Weising *et al*. (1995). PCR was performed on an Eppendorf thermal cycler applying 94ºC for 4 minutes for initial denaturing and 45 cycles of [15s 94ºC denaturing, 45s at 36ºC annealing, 90s at 72ºC extension] and a final extension at 72ºC for 4 minutes. Final concentration of the reaction solution was 1.5mM $MgCl_2$, 100µM dNTPs, 100mM PCR buffer, 0.5µM primer and 0.2U/10 l of Taq DNA polymerase (Promega). A DNA concentration of 100ng with a final volume of 25µl was used for each reaction. 4 sets of 10-mer primers (Table 3.2) were used to screen both the parent populations and the F_1 populations. All primers used were synthesized by Promega. PCR products were run in 1% agarose gel with 1:1 ratio of loading buffer. A 100bp

Table 3.2: Nucleotide sequences of four arbitrary 10-mers primers used for screening adapted from William *et al.* (1990).

Primer code	5' Sequence 3'
OPA –3	**AGTCAGCCAC**
Primer-21	**CGCTGTCCTT**
Primer -25	**GACAGACAGA**
Primer-27	**CTCTCCGCCA**

All primers were synthesized by Promega.

ladder was used as a molecular marker. Gels were run at 80V for 2 hours and visualized on a gel documentation (AlphaImager^{TM} 2200, Siber Hegner) Amplified DNA markers were scored as present (1) and absent (0) bands.

3.5.4 STMS (Sequence Tagged Microsatellite Site) Analysis

STMS was performed following Kaemmer *et al*. (1997). All the primers used are as listed in Table 3.3. PCR were performed on an Eppendorf thermal cycler using 94ºC for 4 minutes for initial denaturing and 35 cycles of [30s at 94ºC for denaturing, 30s at annealing temperature (depending on primer pair), 30s at 72ºC for extension] and a final extension at 72ºC for 10 minutes. Final concentration of the reaction solution was 1.5mM $MgCl_2$, 100µM dNTPs, 100mM PCR buffer, 0.5µM primer and 0.2U/10 µl of Taq DNA polymerase. All PCR reagents and primers were synthesized by Promega. A DNA concentration of 100ng with a final volume of 25µl was used for each reaction. All primer set used required optimization due to unexpected negative results from all PCR reactions under standard condition. The annealing temperature was set at a gradient between 55ºC to 70ºC. All amplification products were kept at -20ºC prior to analysis.

PCR products were pre-analyzed on 1% agarose or 4% Metaphor gels containing 0.1 µg/ml ethidium bromide (run at 80V for 2 hours) before analysis with polyacrylamide gels. 3 different concentrations of 8M urea-polyacrylamide gels (6%, 7% and 8%) were used to analyze the PCR products. Urea was dissolved in 10X TBE and the solution was filtered through a filter paper into a conical flask. 165µl of ammonium persulphate (APS) and 16µl of TEMED were added into the solution and mixed gently. The mixture was dispensed into the chamber by using a 5ml micropipette. The comb was inserted into the top of the chamber and the gel was allowed to solidify for 30 – 45 minutes. Then, the bottom spacer and comb was removed and the gel was mounted into the electrophoresis apparatus connected to a thermal circulator at a

Table 3.3: Sequence and annealing temperature of *Musa* STMS primers

Template	5'-primer sequence-3'	Tested Annealling Temperature ºC	Expected Product Length
AGM193	AACAACTAGGATGGTAATGTGTGGAA	50	128 bp
AGM19	GATCTGAGGATGGTTCTGTTGGAGTG		
AGMI101	TGCAGTTGACAAACCCCACACA	52	189 bp
AGMI 102	TTGGGAAGGAAAATAAGAAGATAGA		
AGMI 103	ACAGAATCGCTAACCCTAATCCTCA	55	181 bp
AGMI 10	CCCTTTGCGTGCCCCTAA		
AGMI 59	AATCGAAATCGAGTCAACAAGG	52	309 bp
AGMI 60	TTTTGTGGATGGTTGGTTCC		
AGMI 12	TTTGATGTCACAATGGTGTTCC	55	280 bp
AGMI 125	TTAAAGGTGGGTTAGCATTAGG		
AGMI 35	TGACCCACGAGAAAAGAAGC	55	106 bp
AGMI 36	CTCCTCCATAGCCTGACTGC		
AGMI 95	ACTTATTCCCCCGCACTCAA	55	200 bp
AGMI 96	ACTCTCGCCCATCTTCATCC		
AGMI 33	AGTTTCACCGATTGGTTCAT	55	151 bp
AGMI 3	TAACAAGGACTAATCATGGGT		
AGMI 105	TCCCAACCCCTGCAACCACT	53	267 bp
AGMI 108	ATGACCTGTCGAACATCCTTT		
AGMI 125	TCCCATAAGTGTAATCCTCAGTT	53	339 bp
AGMI 126	CTCCATCCCCAAGTCATAAAG		
AGMI 127	AAGTTAGGTCAAGATAGTGGGATTT	50	397 bp
AGMI 128	CTTTTGCACCAGTTGTTAGGG		
AGMI 129	GGAGGCCCAACATAGGAAGAGGAAT	53	221 bp
AGMI 130	CATAAACGACAGTAGAAATAGCAAC		
STMS1FP	TGAGGCGGGGAATCGGTA	67	126 bp
STMS1RP	GGCGGGAGACAGATGGAGTT		
STMS7FP	AAGAAGGCACGAGGGTAG	55	212 bp
STMS7RP	CGAACCAAGTGAAATAGCG		
STMS8FP	GGAAAACGCGAATGTGTG	55	250 bp
STMS8RP	AGCCATATACCGAGCACTTG		
STMS9FP	ATGTCGCTTCGGACCAGA	55	162 bp
STMS9RP	GCAGGACGAAGAACTTACC		
STMS10FP	ATGATCATGAGAGGAATATCT	55	112 bp
STMS10RP	TCGCTCTAATCGGATTATCTC		
STMS11FP	GGTTGGAACGGAGGTATACTAA	55	270 bp
STMS11RP	TCCAAGCTTATCGATCTACG		
STMS12FP	TGTCGAAGCATCCTACATC	55	262 bp
STMS12RP	CTTGGAAACATGAGAAACATAC		
STMS13FP	TTGAAGTGAATCCCAAGTTTG	50	131 bp
STMS13RP	AAAACACATGTCCCCATCTC		
STMS15FP	TGCTCTTCCACATCTCAAGAAC	50	270 bp
STMS15RP	GATTGCACGGAGATTCAACA		
STMS22FP	GGTGCTCTTCGGAGGA	58	158 bp
STMS22RP	CGCTTTATATCCATTCCCA		
STMS2FR	GAGCCCATTAAGCTGAACA	55	172bp
STMS2RP	CCGACAGTCAACATACAATACA		

Source: Kaemmer et al, 1997

constant temperature of 55ºC. 10X TBE buffer was filled at the upper and lower tanks to submerge the upper slots.

Before gel analysis, a 1:1 volume `stop mix' containing 95% of formamide, 0.05% xylene cyanole, 0.05% bromophenol blue, 12.5% sucrose and 10mM NaOH was added to the PCR product and denatured at 95ºC for 1 minute. The gels were pre-run for 15 minutes at 55ºC and 220V before loading in the samples. A 100bp DNA ladder (Promega) was used as a molecular weight marker. Gels were silver stained using a modified protocol as described by Kaemmer *et al*. (1997) and Creste *et al*. (2001). Fixing was carried out by soaking the gels in 10% ethanol and 5% acetic acid for 20 minutes followed by rinsing with distilled water. Staining was done by soaking the gel into 0.2% $AgNO_3$ solution followed by rinsing with distilled water and soaking into 0.6M NaOH and 0.06% formaldehyde. Finally, development was stopped by soaking the gel into 10% ethanol and 5% acetic acid for 10 to 15 minutes followed by quick rinsing with distilled water. Gels were photographed using a gel documentation system (AlphaImager^{TM}, Siber Hegner).

Optimization of annealing temperatures of these remaining primers was carried out by varying the temperature at the annealing stage between 50°C to 65°C while maintaining the concentration of other factors ($MgCl_2$, primers, buffer and DNA). Different allelic loci were scored based on banding patterns. Homozygous alleles were scored with the presence of fast or slow moving single band while heterozygous alleles showed two bands. Data of the allelic frequencies thus accumulated were tested for equilibrium to Hardy Weinberg equation by using a Chi Square test.

3.5.5 AFLP Analysis

AFLP assays were performed using a modified version of the protocol as described by Vos *et al*. (1995). Genomic DNA (500 ng) was subjected to digestion for 2

hours at 37ºC with 10Uof *Pst*I and 10U of *Mse*I in a 50µl reaction (10 mM Tris.HAc pH 7.5, 10 mM MgAc, 50 mM KAc, 5 mM DTT, 50 ng/µl BSA). 10µl of a ligation mix (10 mM Tris.HAc pH 7.5, 10 mM MgAc, 50 mM KAc, 5 mM DTT; 50 ng/µl BSA) containing 5 pmol *Pst*I adaptors, 50 pmol *Mse*I adaptors, 1U T4 DNA ligase (Gibco) and 12 pmol ATP was added to each digest and incubated at 37 ºC for 4 h. Pre-amplification of the adaptor ligated template DNA using non-selective AFLP primers P00 and M00 (listed in Table 3.4) consisted of 3.75 µl of adaptor ligated DNA in a 25 µl volume containing 75 ng of both P00 and M00 primers, 0.2mM dNTPs 1µl PCR buffer and 1U of Perkin Elmer Amplitaq LD. PCR was carried out on a PE 9600 thermal cycler (94 ºC denaturing for 30 s; 60 ºC annealing for 30 s, 72ºC extension for 1 min) for 30 cycles, then 55 µl of 10 mMTris pH 8.0, 0.1mM EDTA was added to each reaction.

Primer labeling reactions were performed in a total volume of 50µl and contained 33.5 ng of *Pst*I primer, 1µl (Gibco) Forward Reaction Buffer, 12.5 U T4 Kinase (Gibco) and 50 µCi ^{33}P-ATP. Selective amplifications were performed on the pre-amplified DNA using (P+2/M+3) primer combinations (Table 3.4) and for all the cases only the *Pst*I primer was labelled. 20µl selective PCRs were performed with 2µl of template DNA, 6.7 ng (1µl from labelling reaction) ^{33}P-labelled *Pst*I primer, 25 ng of unlabelled *Pst*I primer, 30ng of *Mse*I primer, 0.2mM dNTPs, 1µl Amplitaq PCR buffer, and 0.5U Amplitaq DNA polymerase. All primers, PCR reactions and adaptors were synthesized by Gibco. PCR was carried out on a PE 9600 thermal cycler as described by Vos *et al.* (1995).

Another set of digestion and amplification with *Eco*RI/*Mse*I primers were also carried out using the same procedure. The PCR products were mixed with 1ul of loading dye (95% of formamide, 0.05% xylene cyanole, 0.05% bromophenol blue, 12.5% sucrose and 10mM EDTA) and denatured at 94ºC for 5 minutes and place on ice

Table 3.4: Sequence of adaptors, universal and selective primers used for AFLP analysis. All primers and adaptors were synthesized by Gibco.

Primer name	5' sequence 3'
*Eco*RI adaptors (Forward)	CTCGTAGACTGCGTACC
(Reverse)	AATTGGTACGCAGTC
*Eco*RI universal primer (E+0)	GACTGCGTACCAATTC
*Eco*RI selective primers (E+3)	GACTGCGTACCAATTCNNN
*Pst*I adaptors (Forward)	CTCGTAGACTGCGTACATGCA
(Reverse)	TGTACGCAGTCTAC
*Pst*I universal primer (P+0)	GACTGCGTACATGCAG
*Pst*I selective primers (P+2)	GACTGCGTACATGCAGNN
*Mse*I adaptors (Forward)	GACGATGAGTCCTGAG
(Reverse)	TACTCAGGACTCAT
*Mse*I universal primer (M+0)	GATGAGTCCTGAGTAA
*Mse*I selective primers (M+2)	GATGAGTCCTGAGTAANN
*Mse*I selective primers (M+3)	GATGAGTCCTGAGTAANNN

N means the single nucleotide could be either A, C, G or T
All primers and adaptors were synthesized by Gibco.

before loading onto 6% acrylamide gel. Gels were run at 80W for 2 hours, dried and exposed to X-ray film (Kodax Biomax film) for 48-72 hours.

3.5.6 Bulk Segregant Analysis and F_1 screening

Bulk segregant analysis was carried out following Michelmore *et al.* (1991). Selected DNA from individual plants of parent population and individuals of F_1 population from crosses between selected susceptible male and selected resistant female were pooled into four groups and labeled as below;

a) Pr: selected resistant individuals (parents population)
b) Ps: selected susceptible individuals (parents population)
c) Fr: selected resistant individuals (F_1)
d) Fs: selected susceptible individuals (F_1)

Each pool (bulk) consisted of a mixture of 6 DNA samples which were selected based on response to FOC TR4 (resistance or susceptibility, respectively). They were screened with AFLP primers together with DNA of susceptible male (S_1) and resistant female (R_2). Initial analyses looked for the presence of bands (Y) in the resistant pool which were absent (N) in susceptible pool and vice versa (YNYNY or NYNYN in a sequence of Pr, Ps, R_2, S_1, Fr and Fs, or NYNY and YNYN in a sequence of R_2, S_1, Fr and Fs respectively). BSA on 177 primer combinations, 61 potential markers from 42 primer combinations was recorded.

Verification of potential markers was carried out by dispersing the pool and screening of individuals in both pools to find band presence in resistant bulks which was absent in susceptible bulks or vice versa. 14 *Eco*RI/*Mse*I primer combinations and 39 PstI/*Mse*I primer combinations were screened across the two bulks and the entire 53 individual samples of F_1 hybrids were genotyped to generate a localized linkage map.

3.5.7 Data Scoring and Linkage Analysis

Autoradiographs of ^{33}P AFLP patterns were scored for the presence (1) or absence (0) by using Cross checker before transferring into an Excel file prior to conversion into JoinMap format. Markers ambiguous in few genotypes were treated as missing data for map construction. Markers that were polymorphic for the offspring population were chosen on the basis of their presence in one parent and absence in the other, or presence in both parents and scored markers were divided into three groups depending on the presence or absence within each parent. Each AFLP marker was identified by the primer pair combination and a band number or letter as suffix. Linkage analysis was performed by using JoinMap version 3.0 under the CP (cross population) algorithm and the Kosambi mapping function was used to convert recombination frequencies into map distances.

4.0 RESULTS

4.1 Development of *Musa acuminata* ssp. *malaccensis* Wild Seed Population

Four populations of *Musa acuminata* ssp. *malaccensis* wild seed population were developed from samples collected from four different lowland locations of central and southern Peninsular Malaysia. Developed seeds from ripe banana fruits were peeled out from its pulps and matured embryos were removed aseptically and cultured in MS media by using embryo rescue techniques (See Section 3.1.1). The embryo went through different transition stages before the final seedlings growth. The first changes observed were the yellowing appearance and swelling of the embryos as observed by Afele and De Langhe (1991) and Asif (2004). After 5 to 6 days, swelling of both meristematic and haustorium ends were observed as the embryo appeared as a dumb-bell shaped structure. Shoot primordial appeared from the lateral tissue of the meristematic end followed by the emergence of root primordial from the apical tissue of the meristematic end. The primary roots emerged from irregularly swollen hypocotyls on which adventitious roots and the aerial shoots were differentiated.

After 2 – 3 weeks, plant-like structures appeared consisting of a prominent shoot, which bears an adventitious root system at its base (Figure 4.1). The root systems of the young seedlings are composed of slender branching of the adventitious roots. At about one month of age, the juvenile root system was swiftly replaced by thick, long and less branching roots which later become the basis for the formation of a mature root system. *In vitro* zygotic culture demonstrated a high germination rate with a percentage of 66.67% from the IPTJ population (University of Malaya), 60.0% from the GH population (Genting Highland), 48.0% from the BARI population (Johol, Negeri Sembilan) and 49.3% from the MIKU population (Negeri Sembilan), See Table 4.1

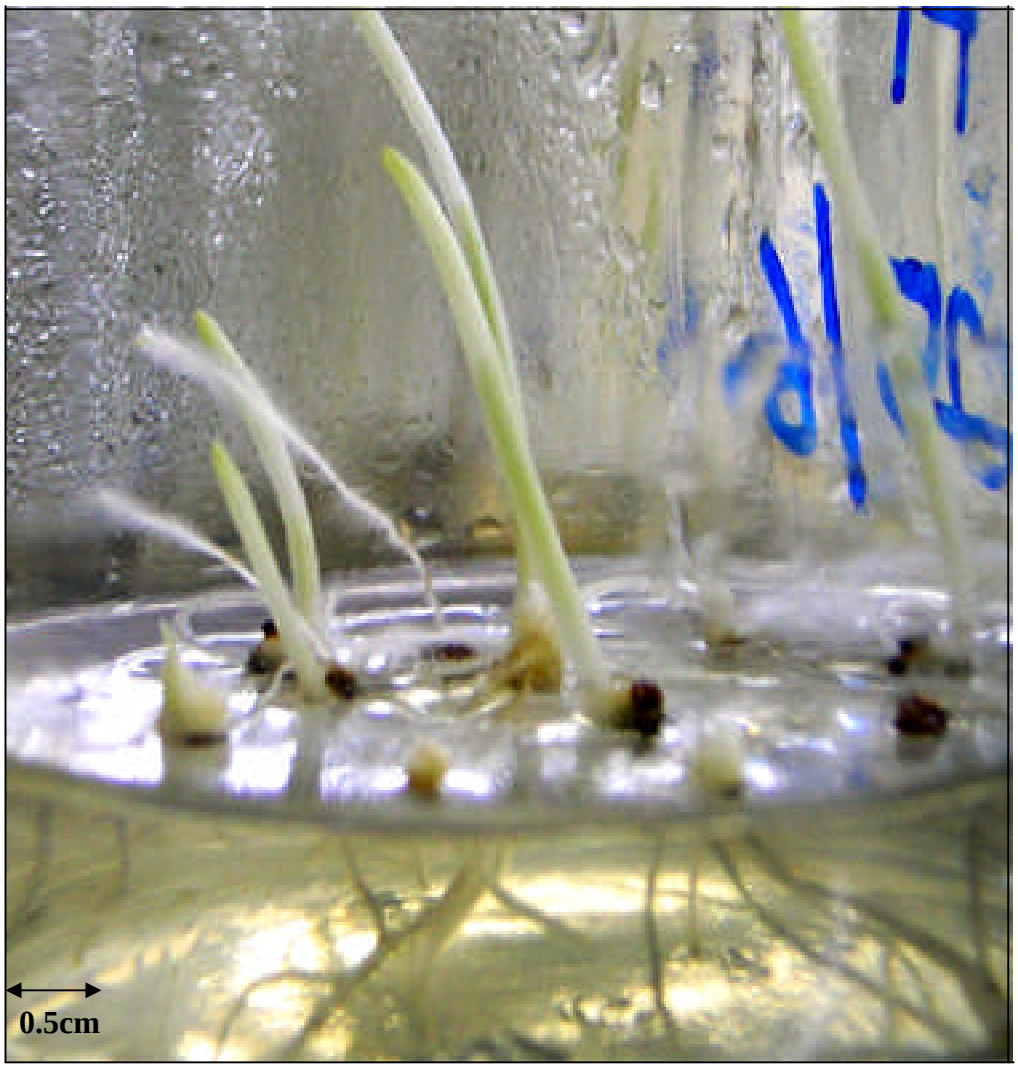


Figure 4.1: Germinated embryos in MS media of *Musa acuminata* ssp. *malaccensis* showing a plant like structure consisting of a prominent shoot with an adventitious root system at its base.

Table 4.1: Embryo germination of four open pollinated populations of wild banana *Musa acuminata* ssp. *malaccensis.*

Population	No. of Embryo Cultured	No. of embryo germinated (after 7 days)	No. of embryo discarded due to contamination	No. of non-germinated embryo	% Germinated
IPTJ	315	210	13	92	66.67%
GH	200	114	16	70	60.0%
BARI	300	144	28	128	48.0%
MIKU	280	138	34	108	49.3%

compared to less than 30% by sowing seeds in a soil bed under greenhouse conditions. Plantlets produced from the *in vitro* culture also grew healthier than those from *in vivo* cultures. Additionally, they provided the potential to get replicates from subculturing of the multiple shoots generated from each embryo. Comparatively the time taken for germination of the seeds grown in the greenhouse was also much longer (40-50 days on average) compared to about one week for the *in vitro* cultured embryos. The multiple clumps of shoots generated from the individual seed progenies through the *in vitro* system offers the advantage of generating clonal seedlings with several replicates compared to *in vivo* germination in soil. Clonal seed progenies were developed by subculturing individual plantlets (separating clumps of shoots and cultured into fresh MS media) several times to develop clonal populations (Figure 4.2). For further applications in this study, only the IPTJ populations with the highest germination rate (66.67%) were selected for crosses and analysis.

4.2 Screening for Response to FOC TR4

4.2.1 Double Tray Screening

Individual clonal seedlings were tagged and planted in the double tray container for hardening. The double tray compartment which consisted of a tray measuring 43cm x 29cm x 9cm which fits into another set of larger outer tray measuring 46cm x 31cm x 20cm. The upper tray was filled with sterilized sand while the bottom tray acts as collector for the excessive water contaminated with FOC TR4 inoculum. Seedlings were acclimatized under shade and high humidity under greenhouse conditions. Subsequently, 40 – 45 day old seedlings with a height of more than 10cm were used for inoculations with FOC TR4. The fungus was originally isolated from infected tissue of Novaria (AAA), see section 3.3.1. Plants were uprooted carefully and soaked into the suspension containing 1 x 10^6 spores/ml for about 3 hours while the control plants were

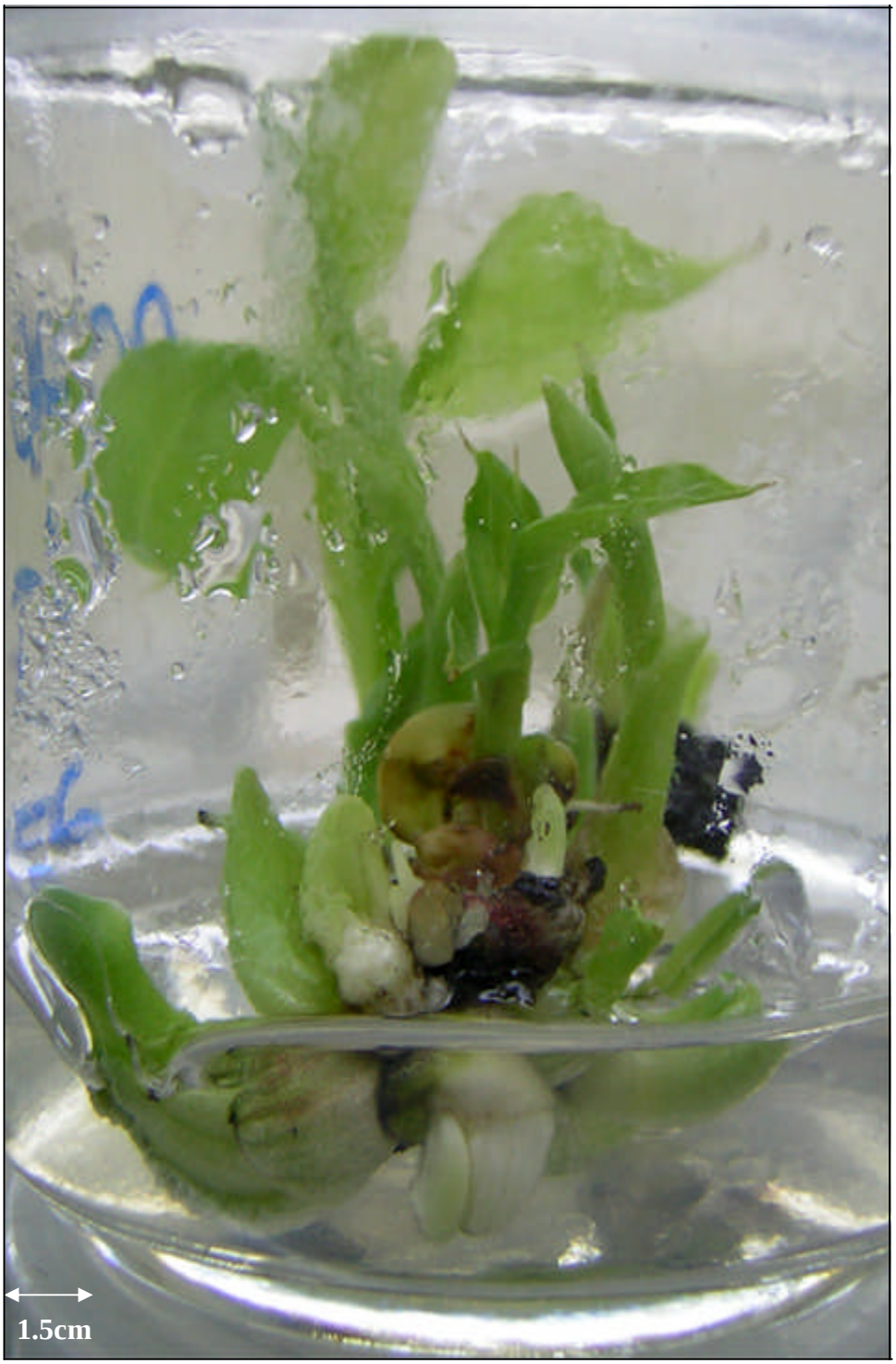


Figure 4.2: Shoots of *Musa acuminata* ssp. *malaccensis* proliferated after subculturing in MS media.

immersed in sterile distilled water. Inoculated plants were replanted upon treatment in the `double tray' set up for the disease monitoring for up to 40 to 50 days. Each container was planted with fifteen to twenty clonal seedlings with five Novaria (AAA) plantlets randomly planted in each tray as susceptible controls. A total of one hundred thirty seven wild banana plantlets were screened along with twenty Novaria (AAA) plantlets (as positive controls) and ten non-inoculated wild banana plantlets as negative controls. Resistant and susceptible seedlings were characterized based on the leaf symptom index (LSI) and root discoloration index (RDI) as described earlier (See section 3.3.2).

Results of the double tray screening showed differential degree of responses to FOC TR4 inoculations. All positive control plants died and showed typical FOC TR4 symptoms. Re-isolation of pathogen from the corm tissue of inoculated seedlings (which showing symptoms and without showing any symptoms) showed the presence of FOC TR4 (Figure 4.3). Seedlings showing normal green colour or less than 5% yellowing of the older leaves were grouped as resistant compared to susceptible seedlings with symptoms of more than 5% or complete yellowing at the older leaves. Seedlings with clear rhizome or with slight discoloration of root and rhizome were considered as resistant (RDI scale of 0 and 1) while seedlings with more than 5% stellar region discoloured or with complete blackening were characterized as susceptible (RDI scale 2 to 6, See Figure 3.4 in section 3.3.2).

The first disease symptoms were observed on the older leaves as shown in Figure 4.4(a) which later progressed to the younger leaves. Yellowing of the older leaves of infected plants was first observed along the margin of the leaves and advanced towards the midrib. As expected the leaves gradually collapsed to form a `skirt' of dead leaves around the pseudostem and the plants eventual died. In contrast, the control plants (non-infected) have a normal dark-green leaf colour as shown in Figure 4.4 (b).

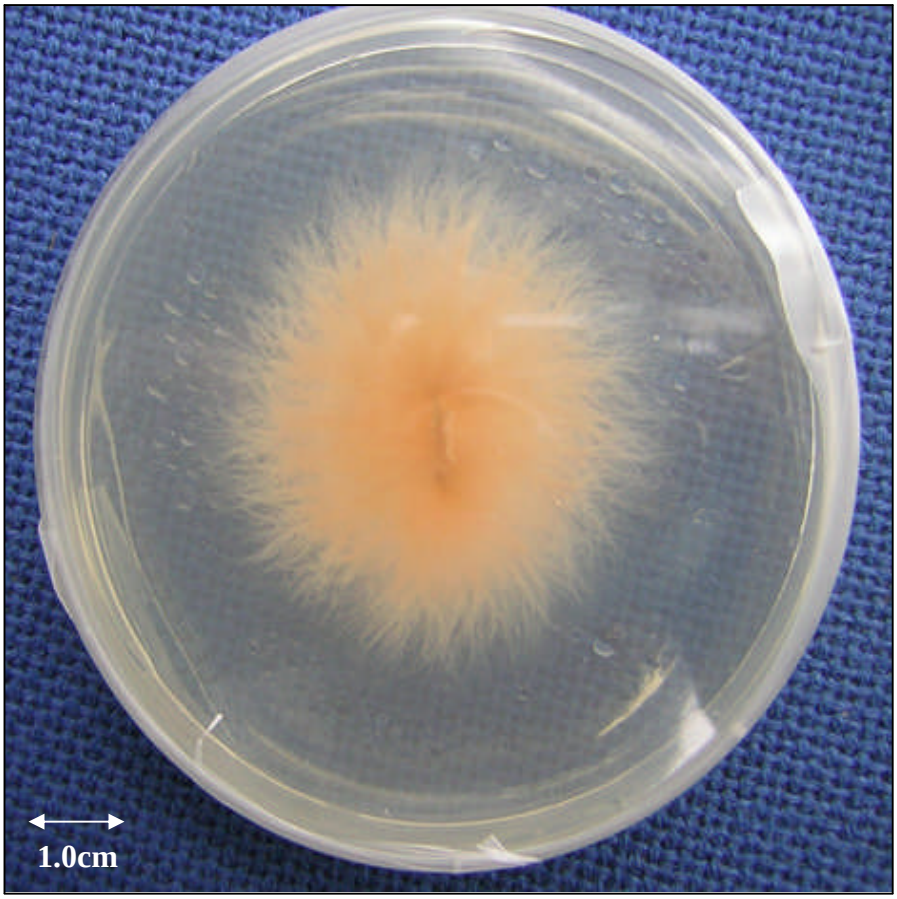


Figure 4.3: Isolation of *Fusarium oxysporum* f. sp. *cubense* (FOC TR4) on PDA media from inoculated seedling showing mycelial growth after 3-4 days incubation at 28°C.

(a) **(b)**

Figure 4.4: Disease evaluation observed after 3 weeks inoculation with FOC TR4.

(a) Gradual yellowing of leaf margin observed after two weeks of inoculation with FOC TR4 as compared to the control (b) with no symptom of yellowing.

A complete blackening of the internal vascular tissues was also observed in infected plantlets compared to control plants. Plants having extensive foliage yellowing showed extensive blackening of the corms and those which showed slight or no foliar symptoms showed slight or no blackening of the corm. Roots of the susceptible plantlets showed blackening compared to the control plants which appeared white and healthy with the scale of 0, See Figure 4.5 (a). Results showed differential degrees of response towards FOC TR4 with the RDI scale varying from 0 to 6 (Figure 4.5). 36 plants (26.3%) showed responses at scale 0 and 1 and were classified as resistant, 61 plants (44.5%) were moderately susceptible with slight discoloration of rhizome and stellar region (scale 2-4) while 40 (29.2%) were severely susceptible to FOC TR4 at the scale of 5 and 6 (Table 4.2). The Chi-square value (Table 4.3) observed for double tray method suggests that population tested had data that fit to a monogenic ratio.

4.2.2 Hot Spot Screening

A second set of the clonal plants were tested in a FOC TR4 hotspot to confirm the double tray analysis. Clonal seedlings were acclimatized under shade and high humidity in the greenhouse for 4 to 5 weeks before exposure into direct sunlight. 60 plants were planted in rows with distance of 2m x 2m along with 15 Novaria (AAA) plants which acted as controls in the Fusarium `Hot Spot' at an oil palm estate located in Teluk Intan, Perak (United Plantation Berhad). The `Hot Spot' is a managed field plot which is heavily infested with FOC TR4. During the planting, FOC TR4 infected corm tissue was also placed in each planting hole to assure no disease escapes. Results were recorded after a year of screening in the `Hot Spot'. Out of 60 plants tested, 54 plants (90.0%) were resistant to FOC TR4 whereas another 6 plants (10.0%) and all the control plants succumbed to FOC TR4 within 4 to 5 months (Table 4.4). Dissection of rhizome revealed no disease symptoms in resistant wild banana seed progenies tested while

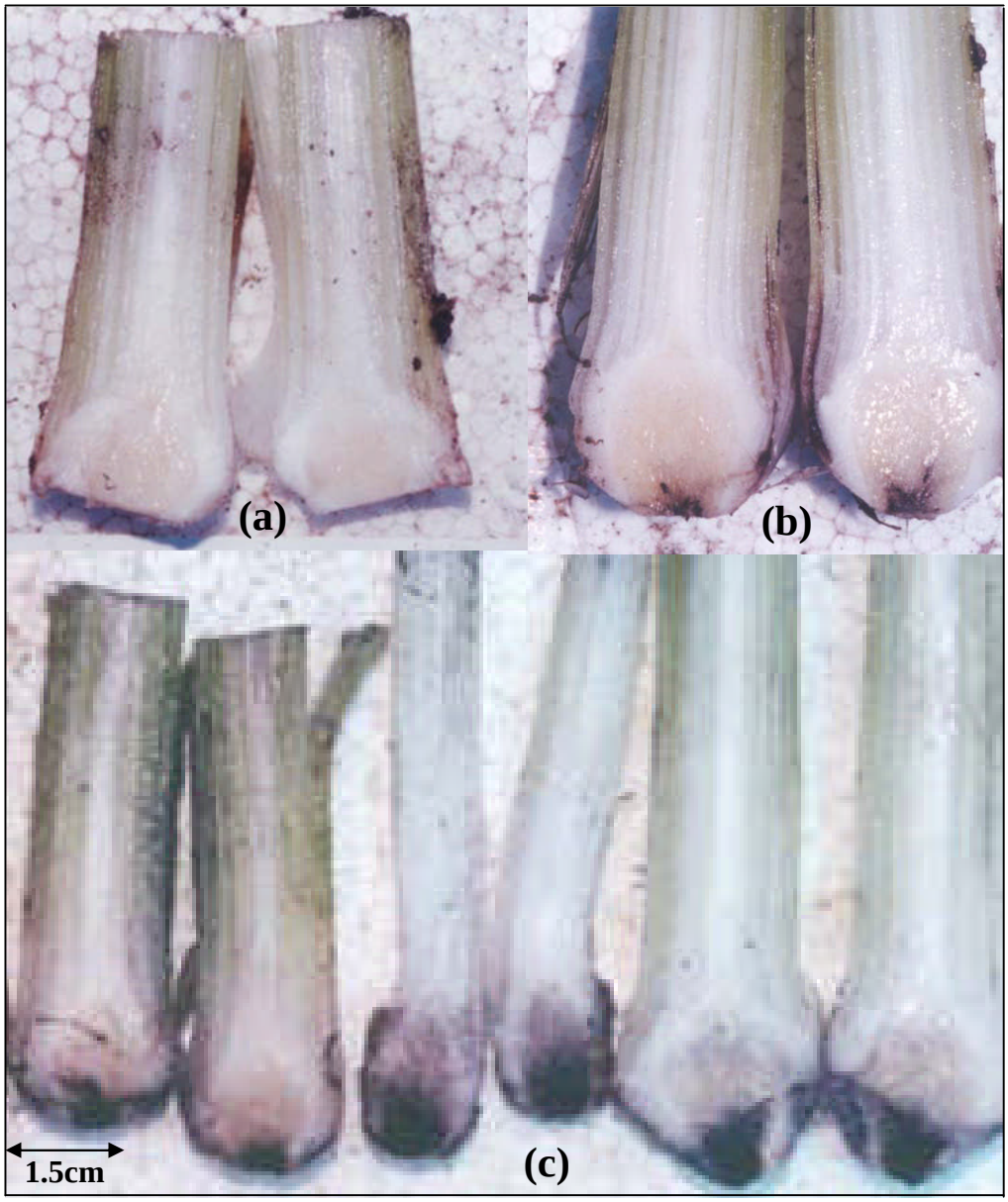


Figure 4.5: A selection of inoculated plants showing different responses towards FOC TR4 with RDI scales ranging from 0 - 5 based on Brake *et al.* (1995) and Asif (2004)

(a) Plant with clean rhizome and stellar region (scale 0), (b) Slight discoloration (Scale 1) and (c) Plants showing different response with a scale of 3, 5 and 4 respectively.

Table 4.2: Different degrees of response towards FOC TR4 among the plants tested using double tray method

	Type of plants tested using double tray method			
	IPTJ population (wild banana)	**Novaria, AAA (positive control)**	**Wild banana (negative control)**	**Novaria (negative control)**
No of plants inoculated	137	20	10	10
Resistant (Scale 0 – 1)	36	0	10	10
Moderately Susceptible (Scale 2 -4)	61	0	0	0
Severely Susceptible (Scale 5 – 6)	40	20	0	0
Percentage of susceptible (%)	73.7	100	0	0

Table 4.3: χ^2 analysis of response towards FOC TR4 among the plants tested using double tray method

No of plants inoculated	**Observed**	**Expected**	**Deviation**	**$(O-E)^2$**	**χ^2**
Resistant (Scale 0 – 1)	36	34.25	1.75	3.0625	0.2738
Susceptible (Scale 2 – 6)	101	102.75	-1.75	3.0625	0.0913
Total	137	137	0		0.3651

Note: Chi-square value was calculated based on the assumption that the resistant trait is controlled by a single recessive gene as suggested by Javed *et al.* (2004).

dead plants showed typical symptoms of FOC TR4 (Figure 4.6). However, results of the `Hot Spot Trial' did not reflect the same ratio as a highly significant chi-square value (135.2) was observed (Table 4.5) compared to 0.3651 obtained with the double tray assay (Table 4.3). Difference in the percentage of susceptibility between the two methods (double tray technique and `Hot Spot' trial) could be due to the number of plants tested, soil and inoculum variables and/or environmental factors (epigenetic factors) that may influence the susceptibility of the plants to infection and its subsequent disease expression. However, it was observed that clonal progenies showing resistance in the double tray method all survived after a year of planting in the `Hot Spot' (Figure 4.7) suggesting that there were no "escapes" using this assay.

4.3 Development of *Musa acuminata* ssp. *malaccensis* F_1 Hybrid Population

Cross pollination is considered as a better approach for generating diversity but self pollination or vegetative reproduction is more effective for "fixing" and reliably reproducing a desired genotype (Pillay, 2005). In this study three controlled crosses was performed between selected clonal seed progenies of the wild banana IPTJ population. The first control crosses were carried out between selected male resistant and susceptible female progenies (according to their response to FOC TR4, see section 4.2) while the other two crosses were between selected male resistant and female resistant respectively (Table 4.6). During the crossing, mature pollen (Figure 4.8) were rubbed onto the receptive female flowers in the green house (Figure 4.9) and properly bagged to avoid cross-contamination with surrounding pollen of wild bananas.

All the three crosses performed successfully produced fruits with developed seeds (Figure 4.10). Ripe fruits with developed seeds were harvested about six months after crossing (Figure 4.11). Results showed that there were no significant differences in the length and weight of fruits harvested within the three crosses ($P<0.005$) (Table 4.7).

Figure 4.6: Infected plants showing discoloration of pseudostem as a result of infection of FOC TR4.

Table 4.4: Response towards FOC TR4 among plants in the `Hot Spot' trial

	IPTJ population (wild banana)	Novaria, AAA (positive control)
No of plants inoculated	60	10
Resistant	54	0
Susceptible	6	10
Percentage of susceptible (%)	10.0	100

Table 4.5: χ^2 analysis of response towards FOC TR4 among plants in the `Hot Spot' trial

No of plants inoculated	Observed	Expected	Deviation	$(O-E)^2$	χ^2
Resistant	54	15	39	1521	101.4
Susceptible	6	45	-39	1521	33.8
Total	60	60	0		135.2

Note: Chi-square value based on the assumption that the resistant trait is controlled by a single recessive gene as suggested by Javed *et al.* (2004).

Figure 4.7: Seed progenies planted in Fusarium `Hot Spot' showing resistance to FOC TR4 with normal growth after a year of planting.

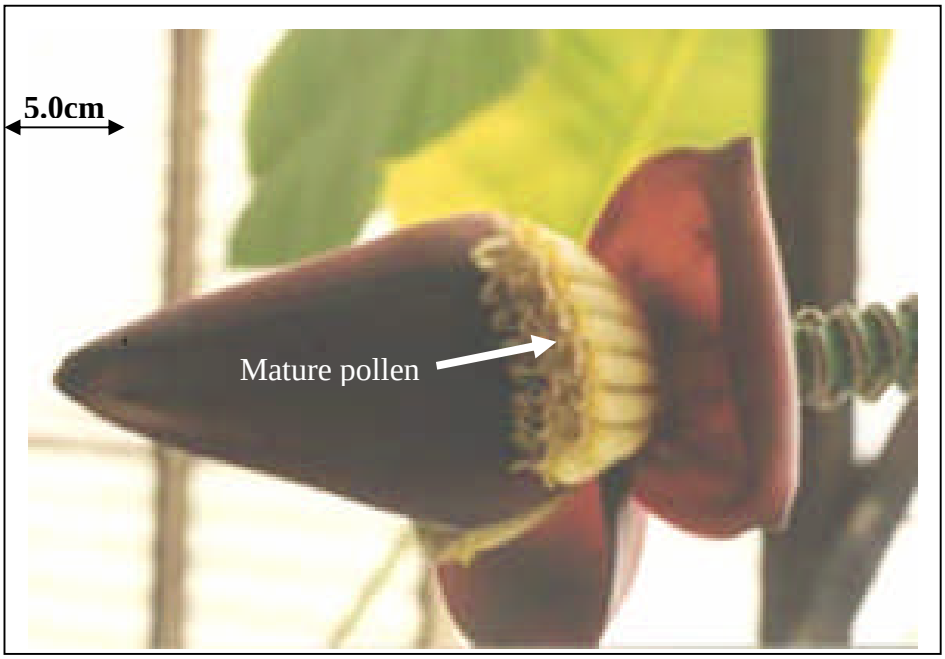


Figure 4.8: Male Inflorescence of *Musa acuminata* ssp. *malaccensis* with mature pollen used for crossing.

Figure 4.9: Female flowers of *Musa acuminata* ssp. *malaccensis* after completion of pollination.

a

b

Figure 4.10: (a) Immature fruits resulting from successful fertilization protected with plastic bags (b) Mature seeded fruits of the crossed plants.

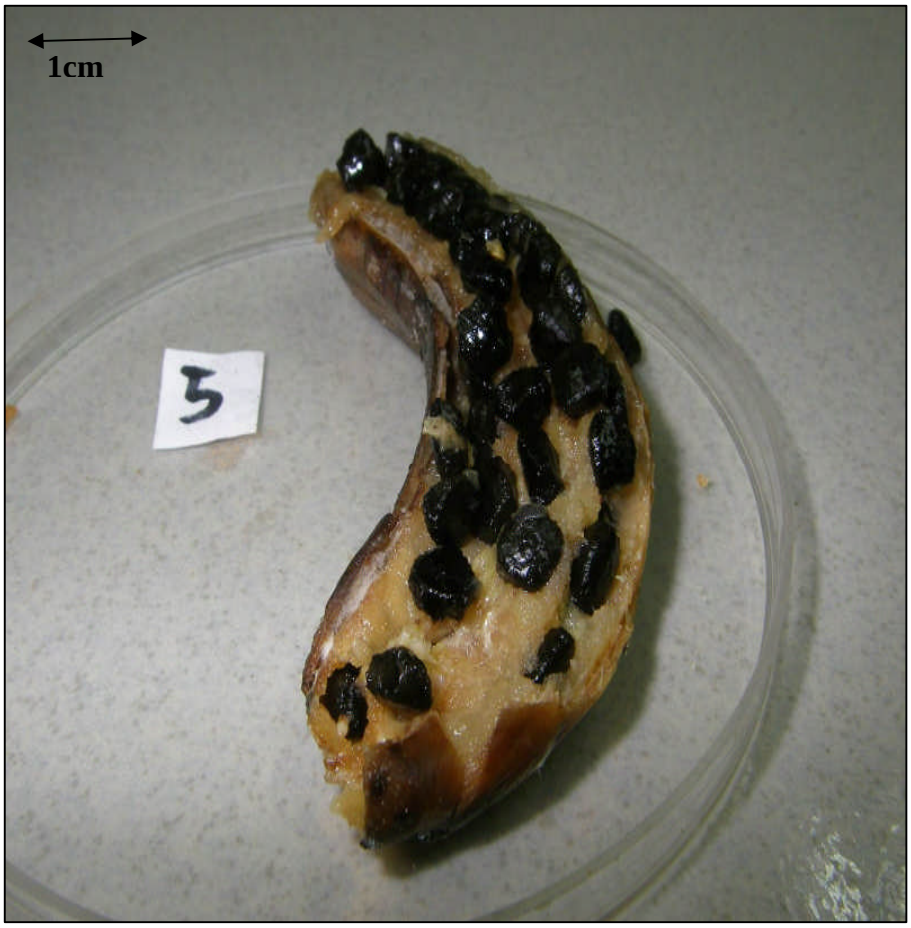


Figure 4.11: Developed seeds from the RS population observed in a fully ripe banana harvested about six months after crossing.

Table 4.6: Type of crosses performed within selected IPTJ clonal seed progenies to develop F_1 hybrid populations of *Musa acuminata* ssp. *malaccensis*

No	Name of population	♂ parent	♀ parent
1	RS	Rest. A	Suscept A
2	SCD	Rest. A	Rest. B
3	TRD	Rest. A	Rest. C

Table 4.7: Data of fruits and seeds of F_1 hybrid populations from 3 crosses between selected *Musa acuminata* ssp. *malaccensis*

Name of population	Type of Crosses		Average length (cm)	Average weight (g)
	♂	♀		
RS	Rest. A	Suscept A	8.7 ± 0.82	24.1 ± 5.26
SCD	Rest. A	Rest. B	9.6 ± 1.23	25.9 ± 5.88
TRD	Rest. A	Rest. C	8.6 ± 0.98	26.3 ± 3.97

The average length of fruit harvested ranged from 8.6cm to 9.6cm while the average fruit weight was ranging from 24.1g to 26.3g. Seeds were then peeled out from the pulp of the ripe fruit harvested and separated from undeveloped seeds by removal of floating seeds in water. A total of 752 seeds (55.7%) developed seeds (Table 4.8) were collected from 1350 seeds of RS population (crosses between selected male resistant and female susceptible line). No absolute data was recorded for number of developed seeds collected from the SCD and TRD populations (both crosses between selected resistant clones).

Developed seeds harvested were subjected to embryo rescue and cultured *in vitro* to develop the F_1 hybrid populations. The germination rates of the hybrid populations was one 146 plants (45.06%) out of 324 embryos for the RS population, 89 plants (35.6%) from 250 embryos for the SCD population and 78 plants (38.1%) from 205 embryos for the TRD population (Table 4.9) obtained through the embryo rescue technique. The stage of maturity at harvesting time and embryo rescue efficiency appears to have influenced the successfulness of the seed germination. The germination efficiency of the embryos after introduction into *in vitro* culture was observed to decrease corresponding to the length of storage after harvesting. For embryos successfully geminated, multiple clumps of shoots from the individual seed progenies were subcultured several times to develop clonal populations. For marker analysis and development of a potential linkage map, further investigation focused only on the RS population which showed the highest number of individuals and rate of germination (Table 4.9). The progeny were first subjected to FOC susceptibility screening. Individual clonal seedlings were tagged and harderned in the double tray container for 40 – 45 days (when the seedlings reached a height of more than 10 cm). Inoculations with FOC TR4 isolated from infected tissue of Novaria (AAA) were carried out as mentioned earlier (see section 4.2.1).

Table 4.8: Number of fruits and developed seeds of F_1 hybrid populations harvested from crosses between selected male resistant and female susceptible seed progenies

No. of fruits	Total seeds	No of developed seeds	Percentage of developed seeds (%)
33	1350	752	55.70

Table 4.9: Rate of germination of three F_1 hybrid populations from 3 crosses between selected *Musa acuminata* ssp. *malaccensis*

Name of population	No of Seeds Cultured	Embryo Discarded due to Contamination		Non-germinated Embryo		Germinated Embryo	
		No	%	No	%	No	%
RS	324	98	30.25	80	24.69	146	45.06
SCD	250	51	20.40	110	44.00	89	35.60
TRD	205	30	14.63	97	47.32	78	38.05

A total of 108 wild banana plantlets from the RS population were screened along with 20 Novaria (AAA) plantlets as a positive control and ten non-inoculated plantlets as a negative control. Several seedlings died during the first week of inoculations without any external symptoms. However, extensive internal discoloration of the corm observed suggested that they were highly susceptible to FOC TR4. The remaining seedlings showed extensive yellowing of the older leaves and extensive blackening of the corm and xylem vessels. Seedlings that showed slight yellowing of the older leaves with a slight blackening of the corm tissue (less than 5%) and surviving two months after inoculation were considered resistant. Results showed about 75.78% of the RS seed progenies were susceptible to FOC TR4 (Table 4.10). The data collected showed a non-significant chi-square value (Table 4.11) thus suggesting that a single recessive gene is associated with resistance to FOC TR4.

4.4 Molecular Analysis

4.4.1 DNA Extraction

High molecular weight genomic DNA was isolated from leaf material of the parent population (IPTJ population) and F_1 hybrid seed progenies (RS population) following the modified method of DNA extraction by Doyle & Doyle (1987). Both sets of DNA extracted were quantified by using a spectrophotometer (DU-7500, Beckman). Extracted DNA with good quality (Table 4.12 and Table 4.13) for IPTJ and RS population respectively and showing $A_{260/280}$ values of 1.7 to 2.0 which is considered to be of sufficient purity for further analyses were chosen. A working solution of each sample was diluted to 50ng/ul for further use.

Table 4.10: Different degree of response towards FOC TR4 among the RS population plantlets using the double tray method

	RS population (wild banana)	Novaria, AAA (positive control)	Wild banana and Novaria (negative control)
No of plants inoculated	128	20	10
Resistant	31	0	10
Susceptible	97	20	0
Percentage of susceptible (%)	75.78	100	0

Table 4.11: χ^2 analysis of response towards FOC TR4 among RS population plantlets tested using double tray method

No of plants inoculated	Observed	Expected	Deviation	$(O-E)^2$	χ^2
Resistant (Scale 0 – 1)	31	32	-1	1	0.0313
Susceptible (Scale 2 – 6)	97	96	1	1	0.0104
Total	128	128	0		0.0104

Note: Chi-square value based on the assumption that the resistant trait is controlled by a single recessive gene as suggested by Javed *et al.* (2004).

Table 4.12: DNA quantification from extraction of leaf materials of IPTJ population

Samples No.	DNA Concentration (µg/µl)	A_{260}/A_{280}	Samples No.	DNA Concentration (µg/µl)	A_{260}/A_{280}
IPTJ -3	0.750	1.80	**IPTJ - 61**	0.566	1.79
IPTJ - 9	1.188	2.16*	**IPTJ -64**	0.927	1.96
IPTJ - 11	3.449	1.91	**IPTJ - 68**	0.635	1.72
IPTJ - 12	3.125	1.93	**IPTJ - 69**	1.530	2.31*
IPTJ - 15	0.756	1.90	**IPTJ - 70**	1.068	1.85
IPTJ - 17	1.211	2.23*	**IPTJ - 75**	1.858	1.98
IPTJ - 19	1.036	1.91	**IPTJ - 76**	0.871	1.78
IPTJ - 21	3.809	1.88	**IPTJ - 77**	0.965	2.01
IPTJ - 23	0.396	1.83	**IPTJ - 78**	2.540	1.99
IPTJ - 24	0.901	1.81	**IPTJ - 79**	1.907	1.99
IPTJ - 30	1.051	1.90	**IPTJ - 82**	3.539	1.75
IPTJ - 32	0.435	1.93	**IPTJ - 83**	2.346	1.86
IPTJ - 33	1.097	1.89	**IPTJ - 84**	1.367	1.95
IPTJ - 34	2.378	1.77	**IPTJ - 85**	2.728	1.78
IPTJ – 36	0.655	1.87	**IPTJ - 86**	4.596	1.98
IPTJ - 37	0.255	1.93	**IPTJ - 88**	0.905	2.01
IPTJ - 41	1.868	2.01	**IPTJ - 90**	1.725	1.85
IPTJ - 42	1.540	1.78	**IPTJ - 94**	0.936	2.09
IPTJ - 43	1.801	1.84	**IPTJ - 97**	1.558	1.92
IPTJ - 44	5.359	1.97	**S1**	2.702	2.09
IPTJ - 45	1.541	1.80	**S2**	0.802	2.03
IPTJ - 46	0.436	1.54*	**R1**	1.725	1.85
IPTJ - 49	3.458	2.09	**R2**	0.928	2.33
IPTJ - 56	0.817	2.35*	**R3**	6.400	2.05
IPTJ - 57	1.466	1.76	**R4**	5.670	2.05
IPTJ - 58	0.824	1.92			

***** *DNA with of A_{260}/A_{280} of lower than 1.7 and exceed 2.10 were excluded from further analysis*

Table 4.13: DNA quantification from extraction of leaf materials of RS population

Samples No.	DNA Concentration (µg/µl)	A_{260}/A_{280}	Samples No.	DNA Concentration (µg/µl)	A_{260}/A_{280}
RS-1	0.293	2.06	**RS-46**	0.423	1.92
RS-2	1.602	1.89	**RS-47**	0.312	1.72
RS-3	0.271	1.84	**RS-48**	0.439	1.80
RS-4	0.363	1.86	**RS-49**	0.206	1.94
RS-5	0.647	2.00	**RS-55**	0.515	1.85
RS-6	0.243	1.92	**RS-56**	0.544	1.79
RS-7	0.378	1.99	**RS-57**	0.298	1.86
RS-8	0.264	1.81	**RS-58**	0.359	1.77
RS-9	0.407	2.02	**RS-59**	0.251	1.99
RS-10	0.128	2.00	**RS-60**	0.244	1.80
RS-11	0.445	1.90	**RS-61**	0.123	1.73
RS-12	0.248	1.76	**RS-62**	0.049	1.76
RS-13	0.624	1.79	**RS-63**	0.249	1.79
RS-14	0.342	1.82	**RS-64**	0.175	1.88
RS-17	0.685	2.04	**RS-65**	0.228	1.84
RS-18	0.328	1.88	**RS-66**	0.221	1.73
RS-20	0.459	1.80	**RS-67**	0.273	1.98
RS-23	0.630	1.92	**RS-68**	0.846	1.72
RS-26	0.346	1.88	**RS-70**	0.308	1.77
RS-34	0.205	1.92	**RS-71**	0.342	1.78
RS-35	0.307	1.72	**RS-73**	0.321	1.72
RS-36	0.148	1.90	**RS-74**	0.366	1.73
RS-37	0.373	1.76	**RS-77**	0.431	2.01
RS-41	0.099	1.89	**RS-78**	0.403	1.72
RS-42	0.518	1.84	**RS-79**	0.448	1.72
RS-44	0.194	2.03	**RS-80**	0.314	1.70
RS-45	0.714	1.74			

4.4.2 RAPD Analysis

Four primers Primer 27, Primer 24, Primer 21 and OPA-3 (See section 3.4.3) originally adapted from Howell *et al.* (1994) were used to screen the wild banana seed progenies of parent's population (IPTJ population) and F_1 hybrid seed progenies (RS population). A standard PCR reaction that has been shown to produce prominent defined scorable band in wild banana as optimized condition by Asif *et al.* (2004) was performed on both IPTJ and RS populations.

4.4.2.1 RAPD of IPTJ Population

All primers showed high degree of polymorphism among the seed progenies of IPTJ populations. The number of scorable RAPD bands varied from 4-9 with the average of seven markers per primer. All four primers showed polymorphism among the seed progenies of IPTJ populations. In general, amplified fragments ranged between 200-1500bp. Fragments that were consistently present in most of the samples (more than 65%) were considered as monomorphic while others were considered polymorphic. A total of twenty eight fragments were observed with twenty considered as polymorphic while another eight were monomorphic was recorded from the four 10-mers primers used (Table 4.14). RAPD profiles generated by Primer-27 showed two bands (350bp and 680bp) that were consistently present in most of the plants (Figure 4.12) while the remaining six were polymorphic. OPA-3 showed three monomorphic bands (960bp, 670bp and 360bp) which were shared by most of the plants (Figure 4.13) while the remaining six were polymorphic. Three monomorphic bands were scored for Primer-21 (1.1kbp, 680bp and 360bp)along with six polymorphic bands (Figure 4.14) while in Primer-25, four monomorphic bands of 850bp, 380bp, 330bp and 290bp was observed along with three showing polymorphism (Figure 4.15).

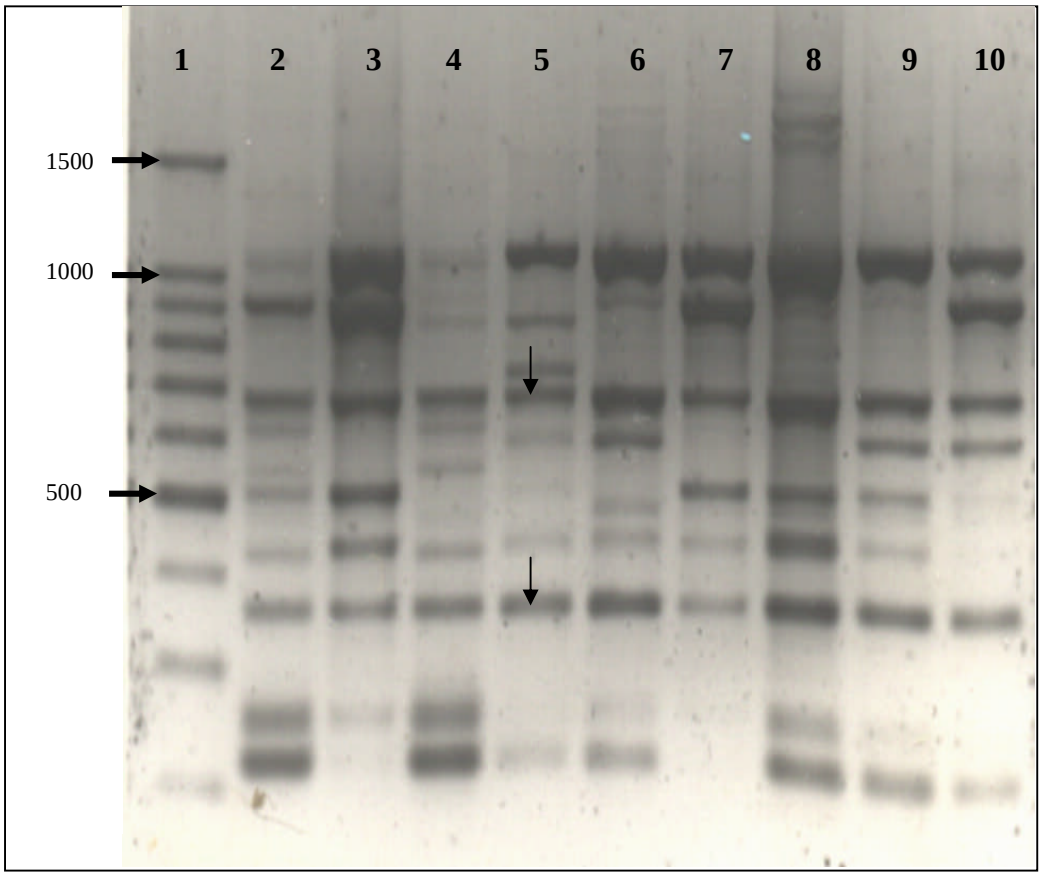


Figure 4.12: RAPD profiles of IPTJ population obtained by Primer-27 producing an average of nine major scorable bands ranging from 200bp to 1500bp.

Two major bands (370bp and 680bp) were observed in most of the plants of IPTJ population (IPTJ 8, 13, 14, 20, 35, 37, 45, 57, 58 shown in lane 2 - 10 respectively) while the remaining six were polymorphic. A 100bp molecular weight marker (Promega) was used as a ladder (lane 1).

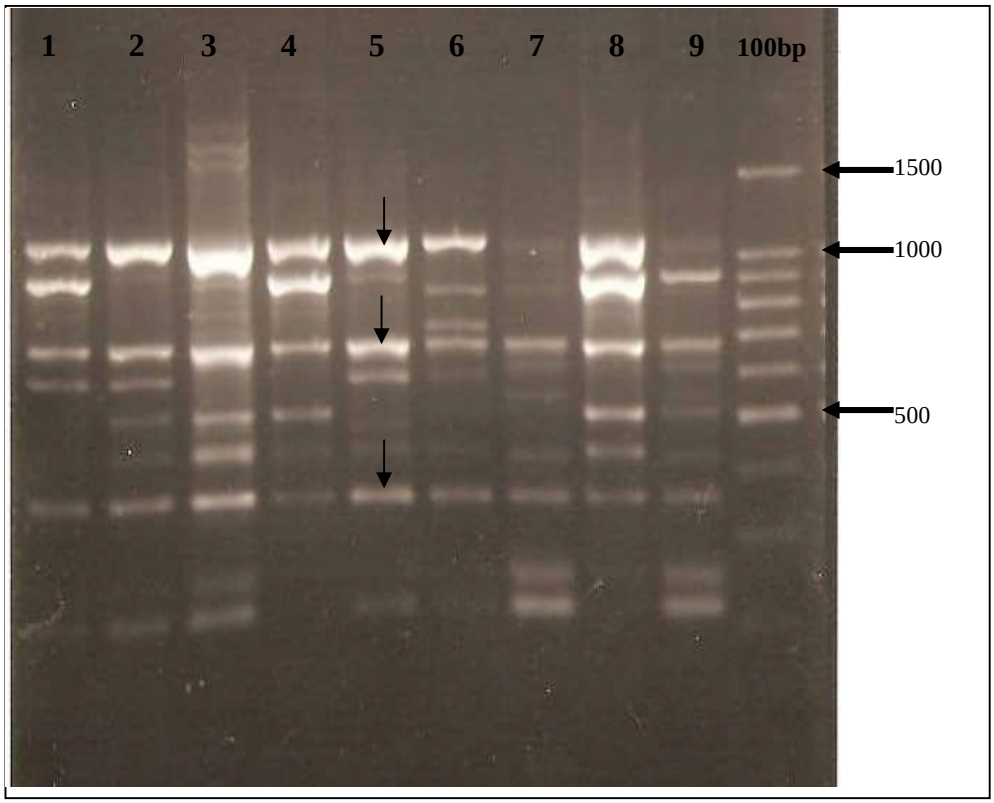


Figure 4.13: Primer-21 screening of IPTJ population producing an average of nine major scorable bands ranging from 200bp to 1500bp.

Three major bands (360bp, 680bp and 1010bp) were observed with Primer-21 in most of the plants of IPTJ the population (IPTJ 3, 9, 11, 12, 15, 17, 19, 21, 23, shown in lane 1-9 respectively) while the remaining six were polymorphic. A 100bp molecular weight marker (Promega) was used as a ladder in lane 10.

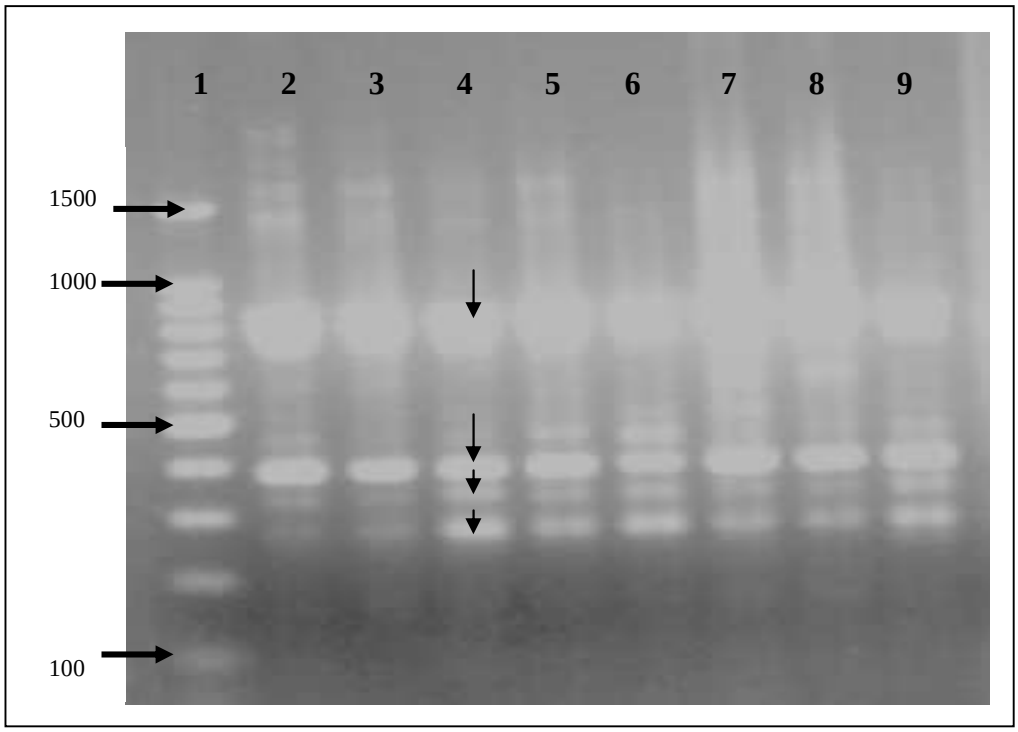


Figure 4.14: Primer-25 screening of IPTJ population producing an average of seven major scorable bands ranging from 200bp to 1500bp.

Four major bands (290bp, 330bp, 380bp and 850bp) were observed with Primer-25 in most of the plants of IPTJ population (IPTJ 3, 9, 11, 12, 15, 17, 19, and 21 shown in lanes 2 - 9 respectively) while the remaining three showed polymorphism. A 100bp molecular weight marker (Promega) was used as a ladder in lane 1.

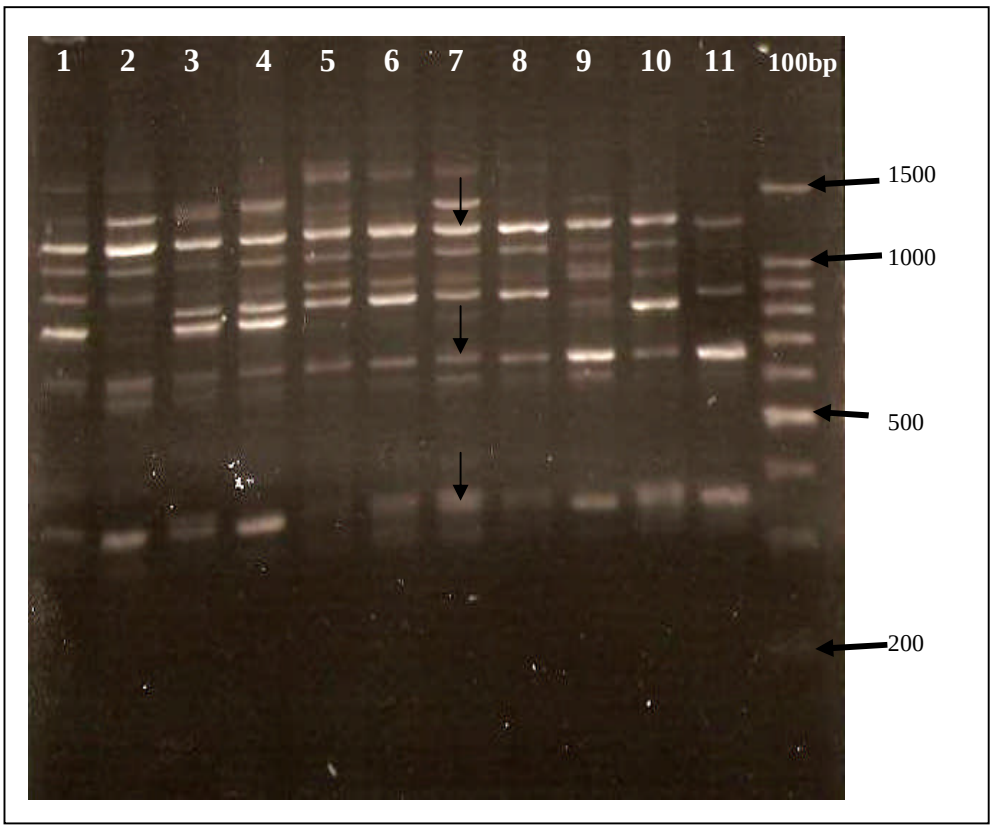


Figure 4.15: OPA-03 screening of IPTJ population producing an average of nine major scorable bands ranging from 200bp to 1500bp.

Three major bands (360bp, 670bp, and 960bp) were observed in most of the plants of IPTJ population (IPTJ 10, 11, 12, 13, 14, 17, 18, 20, 23, 26, 34 shown in lane 1-11 respectively) while the other six showed polymorphism.

Table 4.14: Number of scorable fragments and polymorphic markers observed among the wild banana seed progenies of IPTJ population and F_1 hybrid of RS population.

Code of Primer	5' Sequence 3'	No of scorable bands		Monomorpic bands		Polymorphic bands	
		IPTJ	RS	IPTJ	RS	IPTJ	RS
OPA –3	AGTCAGCCAC	9	8	3	2	6	6
Primer-21	CGCTGTCCTT	9	7	3	1	6	6
Primer -25	GACAGACAGA	7	5	4	3	3	2
Primer-27	CTCTCCGCCA	9	9	3	3	6	6
Total		**34**	**29**	**13**	**9**	**21**	**20**

4.4.2.2 RAPD of RS Population

The number of fragments generated by RAPD in the RS hybrid populations was however lower compared to the parent IPTJ populations (Table 4.14) as the number of bands in the selected plants was lower compared to other individuals in the IPTJ population thus suggesting that the bands were segregating in relation to their parents. Primer-21 showed that the number of bands was reduced to seven with a single monomorphic band of 680bp and six polymorphic bands (Figure 4.16). A total of 14 out of 53 RS plants tested were observed sharing a 250bp band present in the susceptible parent but absent in the resistant parent. However individual phenotypic expressions of the disease symptom of these hybrids did not match that of their susceptible parent. The number of bands generated by Primer-25 had decreased to five with only three monomorphic bands sized 850bp, 380bp and 290bp while the remaining two were polymorphic (Figure 4.17). Seven plants were observed sharing a 250bp band present in the resistant mother plant but absent in the susceptible parent. However, correlation of this marker with the individual phenotypic expressions of the disease symptom also did not match the characteristics of their resistant parent. Although RAPD markers generated in this study did not show any putative marker associated with resistance or susceptibility to FOC TR4, it did provide good early evidence for the potential usefulness of polymorphism and segregation studies. The RAPD markers were utilized in the construction of the linkage map (see Section 4.4.8).

4.4.3 Sequence Tagged Microsatellite Sites (STMS) Analysis

Analysis of the wild banana seed progenies was also made using STMS markers. Primer sets were selected based on published sequences (see section 3.4.4). Primers were tested against DNA extracted from selected plants which were resistant or susceptible to FOC TR4 of wild banana seed progenies (IPTJ population). The internal

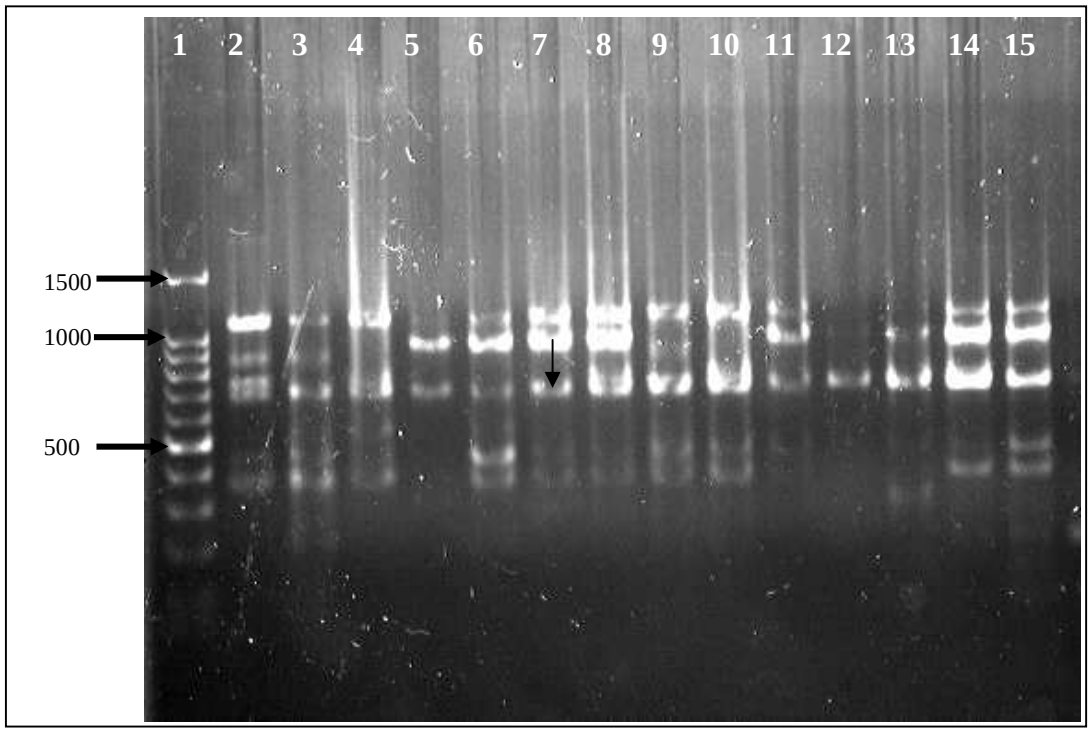


Figure 4.16: Segregation pattern observed among the RS hybrid populations with primer-21.

(Lane 2 – lane 15) with primer-21 showing a reduction in number of bands compared to the parents population in Figure 4.13.

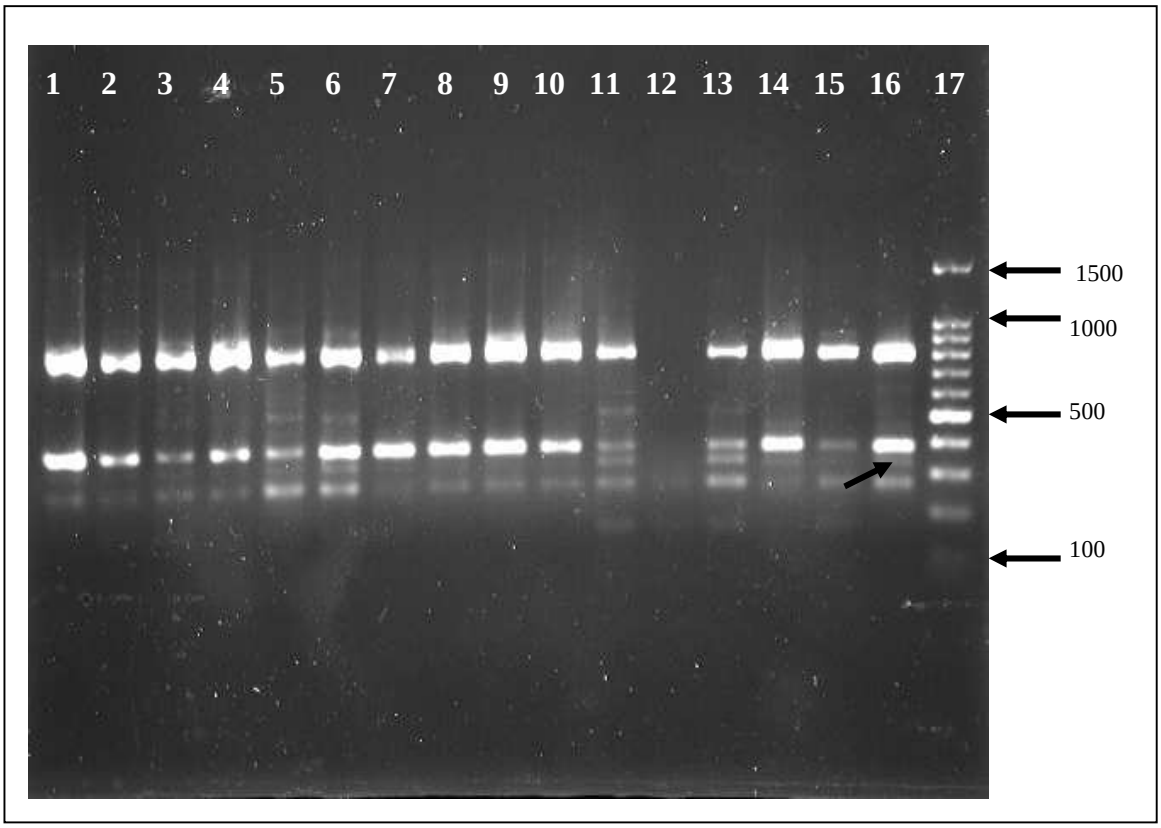
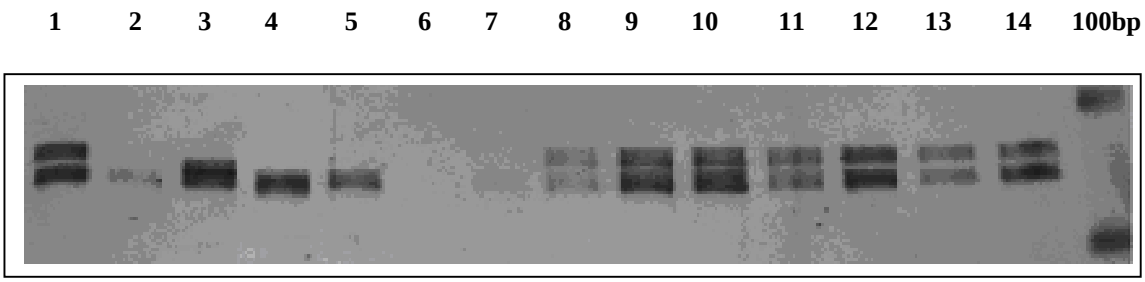


Figure 4.17: Analysis of segregation by using Primer-25 in RS population.

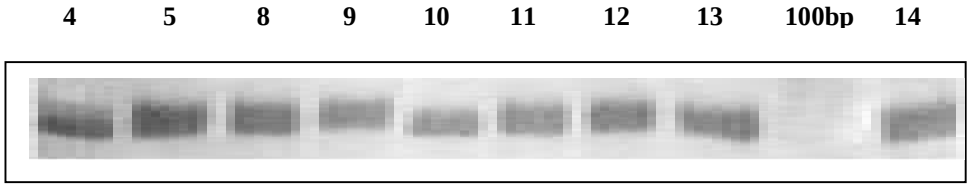
A 250bp band was observed with Primer-25 which was present in the resistant mother plant (Lane 16) but absent in the susceptible parent (lane 15). A 100bp molecular weight marker (Promega) was used as a ladder in lane 1. Segregation of the band was observed in the hybrid RS population (lanes 1-14).

positive control used throughout was the highly susceptible cultivar Novaria (AAA, Cavendish type banana). A comparison of the use between 8M urea-polyacrylamide Gel Electrophoresis (PAGE) and 4% Metaphor Agarose Gel Electrophoresis (MAGE) was also carried out to determine the media for best resolution of the PCR products. Analysis was made based on a sample set of DNA from the parent population (IPTJ population) with AGMI 105/108 primer sets. The PCR products run on silver stained 7% PAGE gels at 220V for 2 hours showed better resolution with bands separated clearly and could be scored easily compared to 4% MAGE containing 0.1 μg/ml ethidium bromide, run at 80V for 2 hours (Figure 4.18) and was chosen as the media for further analysis.

A total of 23 sets of STMS primers were tested (see Table 3.3, Section 3.5.4) to amplify specific products in PCR reactions of F_1 hybrid of *Musa acuminata* ssp. *malaccensis* seed progenies (RS populations, see Section 4.4). Out of the 23 primer sets tested, only 7 primers had amplified products resulting in discrete and repeatable polymorphic bands using the tested annealing temperatures as shown in Table 3.3 (see Section 3.4.3) while the remaining 16 primers had amplified non-specific products. Optimization of annealing temperatures of these remaining primers was carried out and the best optimized annealing temperature was later chosen to replace the initial annealing temperature for further use. Only 4 primers (AGMI 9-93, AGMI 105-108, STMS 13FP-RP and STMS 15FP-RP) that successfully gave good reproducible bands after undergoing the optimization trial were chosen. Finally, only a total of 11 primers were used in the analysis of the F_1 hybrid populations (Table 4.15).



a



b

Figure 4.18: PCR analysis resolved by silver stained of 7% PAGE gel and 4% MAGE gel.

(a) silver stained of 7% PAGE gel at 220V for two hours showing separated bands differentiating clearly into homozygous and heterozygous alleles while (b) 4% Metaphor agarose gel electrophoresis at 80V for two hours showing less clear differentiation between homozygous and heterozygous alleles.

Table 4.15: Sequence and annealing temperature of *Musa* STMS primers

Primer Set	5'-primer sequence-3'	Product Length	Annealing Temperature (ºC)
1. AGMI 2-25	**TTTGATGTCACAATGGTGTTCC**	128 bp	55
	TTAAAGGTGGGTTAGCATTAGG		
2. AGMI 9-93	**GATCTGAGGATGGTTCTGTTGGAGTG**	189 bp	50
	AACAACTAGGATGGTAATGTGTGGAA		***55****
3. AGMI 10-103	**CCCTTTGCGTGCCCCTAA**	181 bp	55
	ACAGAATCGCTAACCCTAATCCTCA		
4. AGMI 35-36	**TGACCCACGAGAAAAGAAGC**	106 bp	55
	CTCCTCCATAGCCTGACTGC		
5. AGMI 95-96	**ACTTATTCCCCCGCACTCAA**	200 bp	55
	ACTCTCGCCCATCTTCATCC		
6. AGMI 101-102	**TGCAGTTGACAAACCCCACACA**	189 bp	52
	TTGGGAAGGAAAATAAGAAGATAGA		
7. AGMI 105-108	**TCCCAACCCCTGCAACCACT**	267 bp	53
	ATGACCTGTCGAACATCCTTT		***55****
8. STMS 2FP-RP	**GAGCCCATTAAGCTGAACA**	172 bp	55
	CCGACAGTCAACATACAATACA		
9. STMS 8FP-RP	**GGAAAACGCGAATGTGTG**	250 bp	55
	AGCCATATACCGAGCACTTG		
10. STMS 13FP-RP	**TTGAAGTGAATCCCAAGTTTG**	131 bp	50
	AAAACACATGTCCCCATCTC		***52****
11. STMS 15FP-RP	**TGCTCTTCCACATCTCAAGAAC**	270 bp	50
	GATTGCACGGAGATTCAACA		**55***

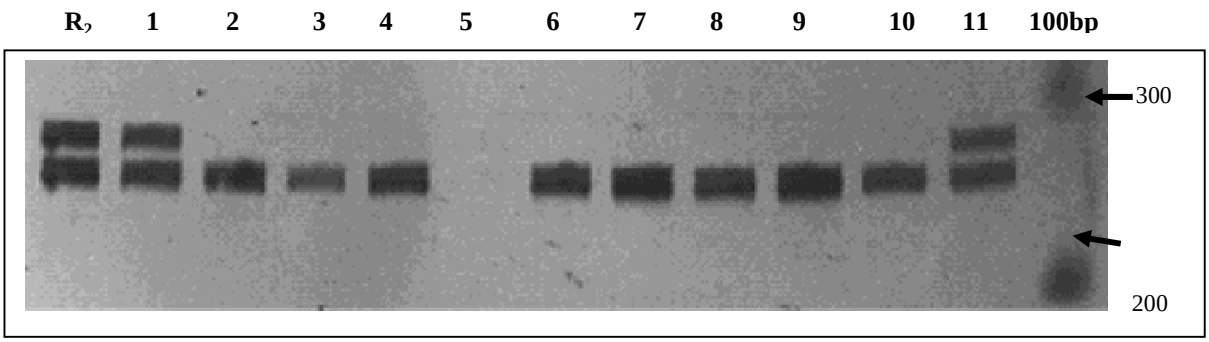
*Note: * new annealing temperature prior to optimization*

4.4.4 Selection of Quality Marker Data

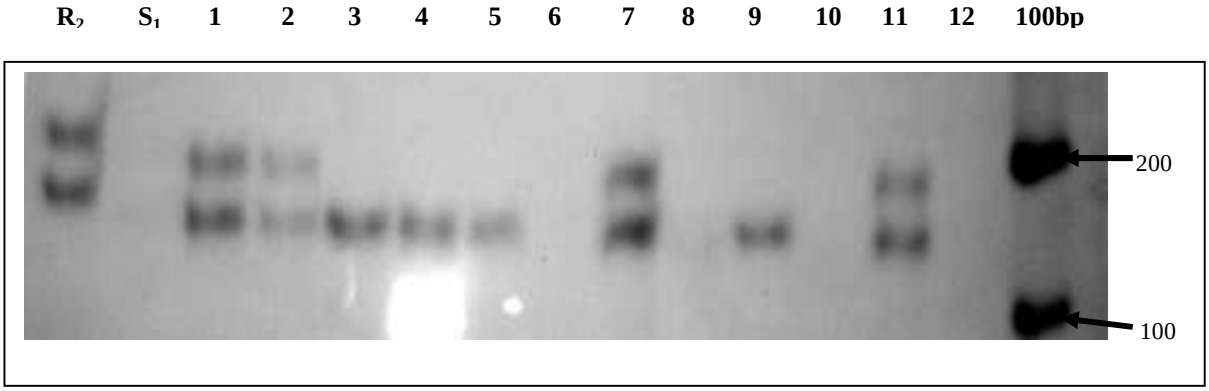
Screening for STMS markers was carried out on selected resistant and susceptible individuals of the F_1 hybrid RS population. Screening was focused on the individuals of RS population for segregation studies. Alleles were scored based on the banding patterns generated by selected primers and assessed on silver stained 8M urea-PAGE gel. Homozygous alleles were scored by the presence of fast or slow moving single bands while heterozygous alleles showed two bands (Figure 4.19 and Figure 4.20). Eleven sets of primers, which detected two alleles each were used to screen individual plants of F_1 population and the diversity of alleles were studied. Accumulated allelic frequencies were tested for equilibrium to Hardy Weinberg equation by evaluating their Chi Square value (Table 4.16). Four of the primers (AGMI 2-25, AGMI 10-103, AGMI 35-36 and AGMI 95-96) showed a significant χ^2 value thus distorted from the Mendellian pattern of segregation in hybrid population. However, this analysis did not show any clear differentiation between resistant and susceptible individuals in the individuals tested and no further analysis was carried out. The STMS markers generated were included in linkage map development (See section 4.4.8).

4.4.5 Amplified Fragment Length Polymorphism (AFLP)

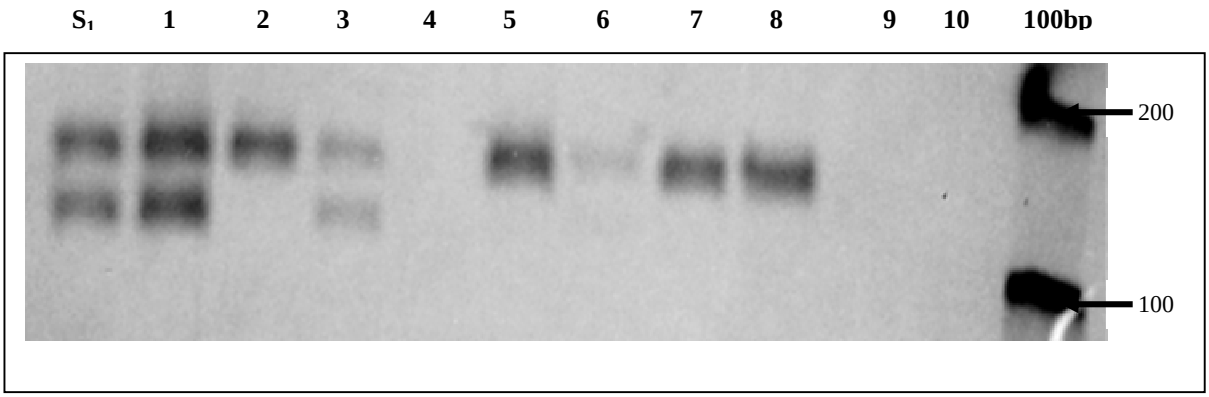
A third marker approach was used to analyze the F_1 populations generated in the study. The rationale of choosing this system is that the level of polymorphism analyzed by AFLP in *Musa* has been shown to be high and it may provide the most effective technique for genetic analysis (Wong *et al.*, 2002). The initial step in the process was the selection of the primer combinations which generated the highest number of polymorphisms using *Pst*I+2/*Mse*I+3 primer combinations as a test system.



a



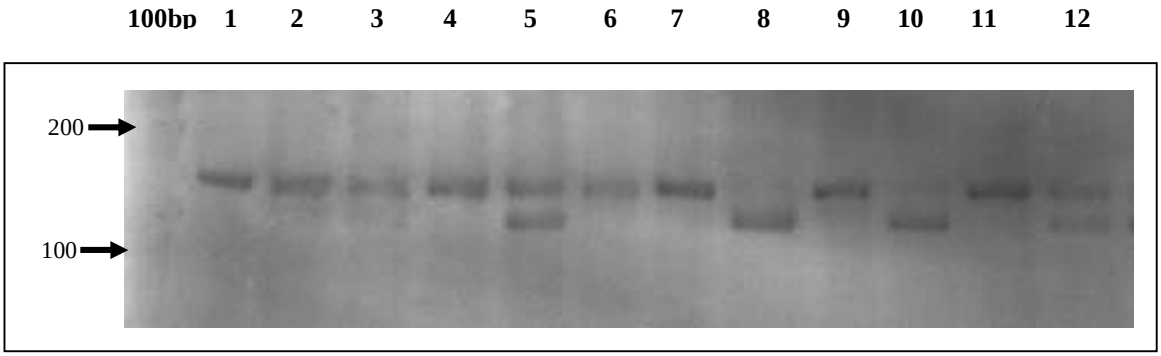
b



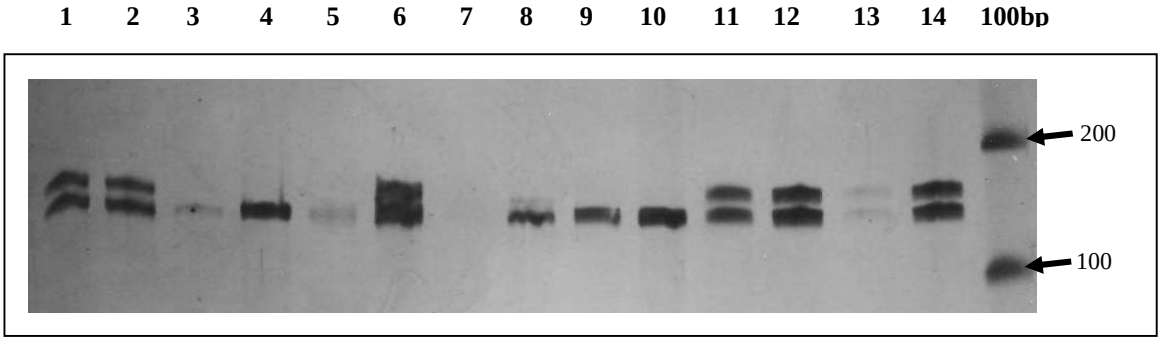
c

Figure 4.19: Polymorphism of STMS markers generated from F_1 hybrid population from selected crosses of wild banana seed progenies at three different loci

(a) AGM 105-108 (b) STMS 13FP-RP (c) AGM 9-93 on 7% PAGE at 220V for two hours stained by silver staining.



a



b

Figure 4.20: Polymorphism of STMS markers generated from F_1 hybrid population from selected crosses of wild banana seed progenies at two different loci.

(a) AGMI 10-103 (b) AGMI 2-25 on 7% PAGE at 220V for two hours stained by silver staining.

Table 4.16: STMS data of F_1 hybrid population of *Musa acuminata* ssp. *malaccensis*

Primer Set	A/A	A/a	a/a	χ^2 value
1. AGMI 2/25	0	23	16	***6.96
2. AGMI 9/93	29	21	3	^{ns} 0.12
3. AGMI 10/103	13	30	4	**5.00
4. AGMI 35/36	3	36	5	****17.99
5. AGMI 95/96	3	36	11	****11.12
6. AGMI 101/2	10	32	10	* 2.77
7. AGMI 105/108	8	26	10	^{ns}1.50
8. STMS 2FP/RP	27	20	6	^{ns} 0.59
9. STMS 8FP/RP	10	23	12	^{ns} 0.03
10. STMS 13FP/RP	14	16	7	^{ns} 0.41
11. STMS 15FP/RP	8	27	10	^{ns} 1.84

Note:
χ^2 value significantly different at 0.05(*), 0.01-0.05(**), 0.001-0.01(***) and <.001 (****), respectively
ns: non-significantly different

Prescreening of 12 *Pst*I+2/*Mse*I+3 primer combinations (see section 3.4.5) on F_1 hybrid of RS populations generated in general a relatively high degree of polymorphism for all the combinations tested (Table 4.17). A total of 864 reproducible, easily scored amplifications were generated with and average of 72 bands per primer combinations, of which 131 (15.16%) were polymorphic. The number of selected polymorphic bands varied from 6 to 16 with an average of 11 bands generated per primer combination. The percentage of polymorphism ranged from 6.18% with the primer combination *Pst*I+AA x *Mse*I+AAT to 27.27 % with primer combination *Pst*I+AC x *Mse*I+CAC.

4.4.6 Bulk Segregant Analysis (BSA)

To facilitate futher analysis, bulk segregant nalysis was used for the AFLP analysis. Bulk segregant analysis is a process whereby DNA from selected individual plants of the parent population and individuals of the F_1 hybrid of RS population (crosses between selected susceptible male and selected resistant female) are pooled into groups (see Section 3.4.6). Markers that are polymorphic between the pools will be genetically linked to the loci determining the trait used to construct the pools. In this study, bulk segregant analysis (BSA) was used to focus on regions of interest or areas sparsely populated with markers. It is also a method of rapidly locating genes that do not segregate in a population to generate the genetic map (Michelmore *et al*., 1991).

A total of 177 different primer combinations were used to screen parents and bulks of resistant and susceptible plants from the IPTJ and RS population to identify molecular markers potentially linked to resistance and susceptibility to FOC TR4 and in the process, to initiate generation of a linkage map for the hybrid F_1 (RS population). Each primer combination produced approximately 60 to 70 bands ranging in size from

Table 4.17: AFLP data generated from preliminary screening with twelve primer combinations in the F_1 population

*Pst*I+2	*Mse*I+3	No. of bands	No. of Polymorphic bands	% of polymorphic bands
AA	AAC	81	14	17.28
AA	AAT	97	6	6.18
AA	AGG	70	11	15.71
AC	AGA	64	16	25.00
AC	CAC	33	9	27.27
AC	CCA	68	10	14.70
AG	AAC	71	15	21.12
AG	AAT	101	14	13.86
AG	ACC	52	14	26.92
AT	AAT	86	7	8.13
AT	ACT	72	9	12.50
CA	CAC	69	6	8.69
Total		**864**	**131**	-
Average		***72***	***10.92***	

Note:

*Pst*I adaptor: 5`- CTCGTAGACTGCGTACATGCA-3’
3`-TGTACGCAGTCTAC-5’
*Mse*I adaptor: 5` -GACGATGAGTCCTGAG-3’
3` -TACTCAGGACTCAT-5’
*Pst*I universal primer (P+0) : 5`-GACTGCGTACATGCAG-3’
*Mse*I universal primer (M+0): 5`-GATGAGTCCTGAGTAA-3’

* The DNA used in the test was F_1 hybrid population from RS population (see Section 4.4)

50 to 500 bases. 61 potential markers with bands presence (Y) in resistant pool but absent (N) in the susceptible pool and vice versa in a pattern of (YNYNY or NYNYN) in a sequence of Pr (pool of selected resistant from IPTJ population), Ps (pool of selected susceptible from IPTJ population), resistant male parent (R_2), susceptible female parent (S_1), Fr (pool of selected resistant from RS population) and Fs (pool of selected susceptible from RS population) respectively or (NYNY and YNYN) in a sequence of resistant male parent (R_2), susceptible female parent (S_1), Fr and Fs respectively were identified using *Pst*I+2 x *Mse*I+3 and *Eco*RI+3 x *Mse*I+3 as described in Section 3.4.6 (Figure 4.21).

Verification of potential markers was carried out by dispersing the pool and screening of individuals in both pools to find band presence in resistant bulks and absence in susceptible bulks or vice versa. However, none of these potential markers could distinguish clearly any marker linked to resistance or susceptibility when tested in the individual plants (Figure 4.22). 14 *Eco*RI x *Mse*I and 39 *Pst*I x *Mse*I primer combinations were later screened across the two bulks of RS population and the remaining of 43 individual samples of F_1 hybrids (RS populations) and further genotyped to generate a localized linkage map (See Section 4.4.8).

4.4.7 AFLP Analysis and Markers Genotyping

AFLP analysis on individuals in the F_1 hybrid of RS populations was then carried out to generate markers and genotyped for the construction of linkage maps (See Section 4.4.8). A total of 3538 bands was generated from 13 *Eco*R1+3 x *Mse*I+3, 1 *Eco*R1+3 x *Mse*I+2 and 39 *Pst*I+2 x *Mse*I+3 primer combinations (Table 4.18). The total number of DNA fragments detected by the individual primer pairs ranged from 33 for *Pst*1+AC x *Mse*I+CAC to 109 in *Eco*R1+AAC x *Mse*I+CA (Table 4.18). 747 bands (12.39%) were found to be polymorphic with a range of 4 in *Pst*I+CA x *Mse*I+ACT to

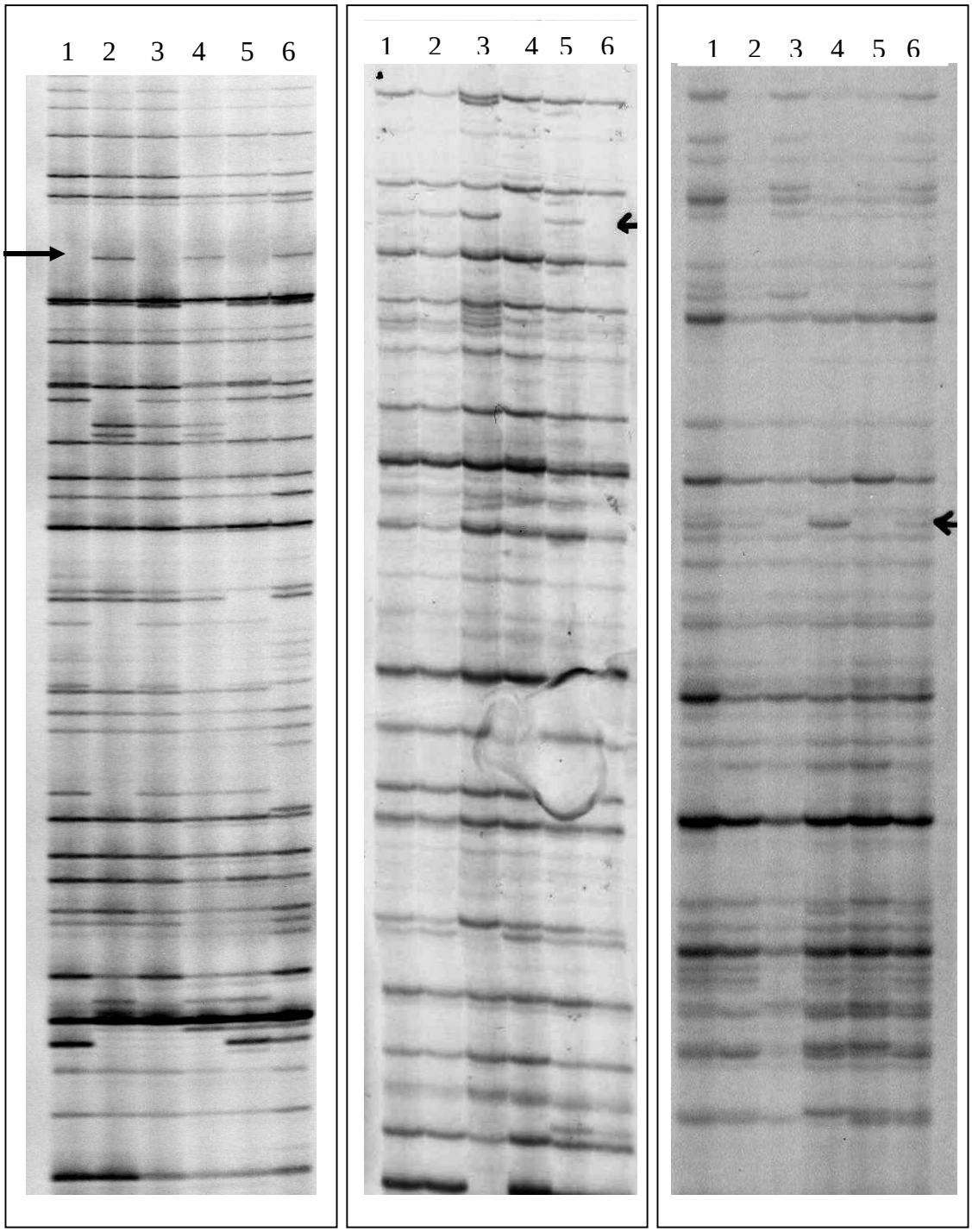


Figure 4.21: AFLP analysis of pooled and parents DNA with primer combinations of *Eco*RI+AAC x *Mse*I+AGT, *Pst*I+AA x *Mse*I+GAC and *Eco*RI+AAC x *Mse*I+AGT

Lane 1-6 consists of pooled and parents DNA (in a sequence of Pr, Ps, R_2, S_1, Fr and Fs) showing potential markers with (a) in a pattern of NYNYNY in primer combination of *Eco*RI+AAC x *Mse*I+AGT (b) NYNY in primer combination of *Pst*I+AA x *Mse*I+GAC and (c) YNYN in primer combination of *Eco*RI+AAC x *Mse*I+ACC

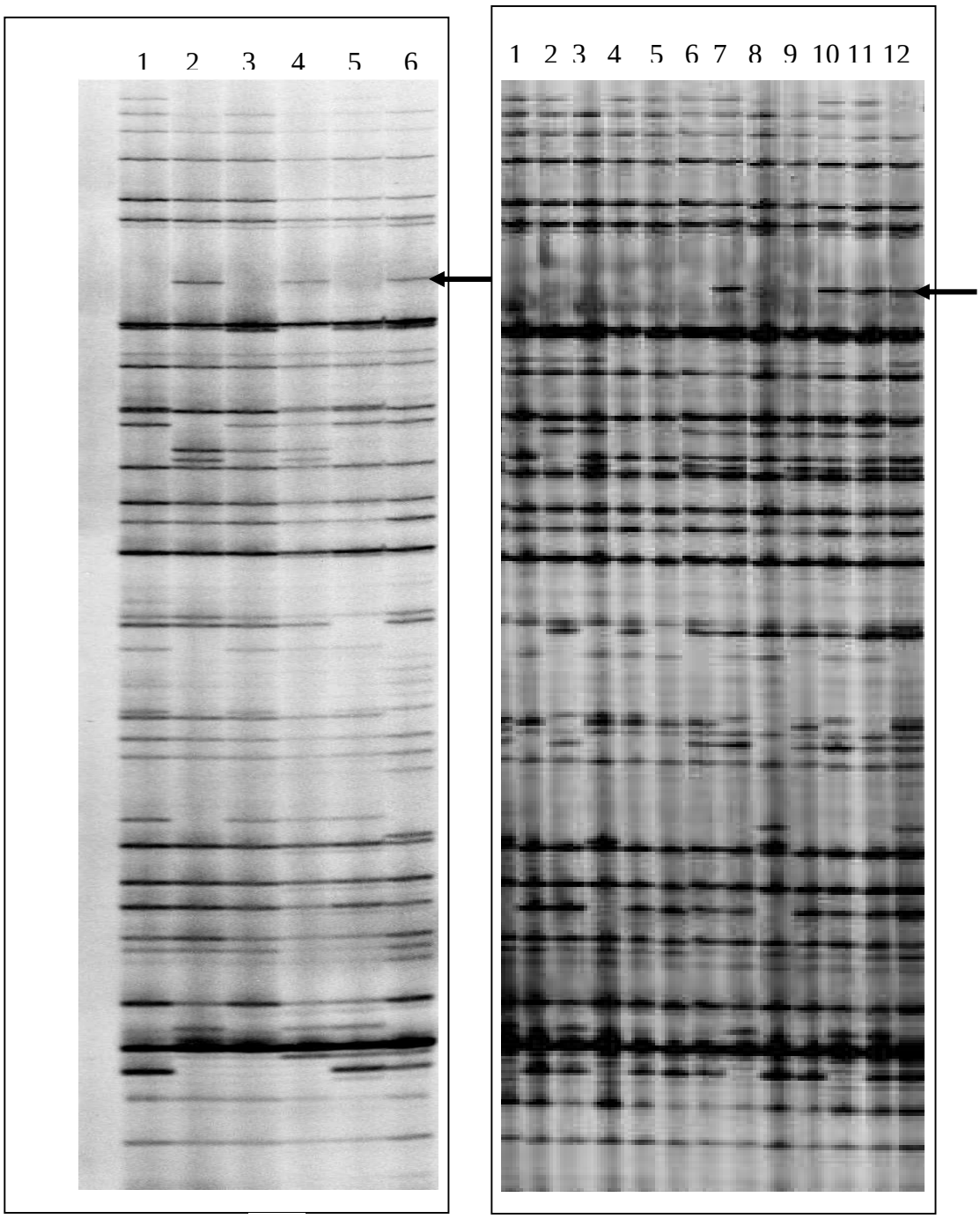


Figure 4.22: Detection and verification of potential `susceptible band' with the primer combination of *Eco*RI+AAC x *Mse*I+AGT

(a) Potential `susceptible' band observed with the presence of band in susceptible parent S_1, susceptible parents bulk Ps and susceptible hybrid bulks Fs but absent in resistant parent, R_2, resistant parent bulk, Pr and resistant hybrid bulk, Fr with a pattern of NYNYNY (lane 1-6) with primer combination of *Eco*RI+AAC x *Mse*I+AGT (b) Verification of potential markers showed that the potentially susceptible band was absent in individuals of Fr bulks (Lane 1-6) but did not consistently appear in all individuals of the Fs bulk (lane 7-12).

Table 4.18: Total number of bands, polymorphic bands and percentage of polymorphism detected by using 53 primer combinations in the F_1 population

Primer combination *Pst*I+2 x *Mse*I+3	Total no. of Bands	Polymorphic marker		Primer combination *Eco*R1+3 x *Mse*I+2/3	Total no. of Bands	Polymorphic marker	
		No. of markers	%			No. of markers	%
***P*+AA x *M*+AAC**	81	14	17.28	***E*+AAC x *M*+CA**	109	24	22.02
***P*+AA x *M*+AAG**	77	10	12.99	***E*+AAC x *M*+AAC**	46	17	12.99
***P*+AA x *M*+AAT**	97	6	6.19	***E*+AAC x *M*+ACC**	65	17	26.17
***P*+AA x *M*+ACT**	*71*	12	*16.90*	***E*+AAC x *M*+ACT**	53	14	26.42
***P*+AA x *M*+AGG**	70	11	15.71	***E*+AAC x *M*+AGT**	*56*	19	*33.93*
***P*+AA x *M*+CCG**	*34*	10	*29.41*	***E*+AAC x *M*+ATG**	66	*20*	30.30
***P*+AA x *M*+CCT**	84	12	*14.29*	***E*+AAC x *M*+CAG**	39	12	30.77
***P*+AA x *M*+CTA**	88	*15*	17.05	***E*+AAC x *M*+GAA**	70	17	24.29
***P*+AA x *M*+CTT**	*51*	6	11.76	***E*+AAC x *M*+GAT**	65	10	15.38
***P*+AA x *M*+GAC**	*54*	9	16.67	***E*+ACA x *M*+AAT**	58	13	22.41
***P*+AA x *M*+GAG**	70	8	11.43	***E*+ACA x *M*+ACG**	59	9	15.25
***P*+AA x *M*+GAC**	65	11	*16.72*	***E*+ACA x *M*+ATT**	60	19	31.67
***P*+AC x *M*+ACC**	*55*	14	25.45	***E*+ACA x *M*+CAG**	38	10	26.32
***P*+AC x *M*+ACT**	60	12	*20.00*	***E*+ACA x *M*+CTG**	43	7	16.28
***P*+AC x *M*+AGA**	64	16	25.00				
***P*+AC x *M*+CAC**	33	*9*	*27.27*				
***P*+AC x *M*+CCA**	68	*10*	14.71				
***P*+AC x *M*+CCG**	46	7	15.22				
***P*+AC x *M*+CGG**	60	6	*10.00*				
***P*+AC x *M*+GCA**	60	10	16.67				
***P*+AG x *M*+AAC**	71	15	21.13				
***P*+AG x *M*+AAT**	101	14	13.86				
***P*+AG x *M*+ACC**	52	14	26.92				
***P*+AG x *M*+AGA**	87	10	11.49				
***P*+AG x *M*+CGC**	50	7	14.00				
***P*+AG x *M*+GCA**	87	17	19.54				
***P*+AT x *M*+AAC**	102	15	14.71				
***P*+AT x *M*+AAT**	86	7	8.14				
***P*+AT x *M*+ACT**	72	9	12.50				
***P*+AT x *M*+AGG**	85	16	18.82				
***P*+CA x *M*+ACT**	79	4	5.06				
***P*+CA x *M*+AGA**	51	6	11.76				
***P*+CA x *M*+CAC**	69	6	8.70				
***P*+CA x *M*+CAG**	92	8	8.70				
***P*+CA x *M*+CCC**	58	7	12.07				
***P*+CA x *M*+CCT**	82	11	13.41				
***P*+CA x *M*+CGT**	45	7	15.56				
***P*+CA x *M*+CTA**	76	9	11.84				
***P*+CA x *M*+GAC**	78	9	11.54				
Total	**2711**	**399**	**-**		**827**	**208**	**-**
Mean	**69.51**	**10.23**	**-**		**57.81**	**13**	**-**

24 in *Eco*R1+AAC x *Mse*I+CA and an average of 11 polymorphic fragments per primer pair. The size of the amplified fragments ranged from 76bp to 540bp while the percentage of polymorphic bands observed per primer combination ranged between 5.06% in *Pst*I+CA x *Mse*I+ACT to 33.93% in *Eco*R1+AAC x *Mse*I+AGT (Table 4.18).

Markers generated from the AFLP analysis were genotyped based on their presence and absence in the parents while segregating in their hybrids. Markers that present in resistant parent but absent in susceptible parent and segregate in their hybrids were grouped as *r*-markers while markers segregate in the hybrids but absent in resistant parent and present in susceptible parent were grouped as *s*-markers. The other sets of markers that present in both parents but segregate in their F_1 hybrids were grouped as *h*-markers (Figure 4.23 & 4.24). A total of 166 AFLP markers consisting of 53 *Eco*-markers and 113 *Pst*-markers had been grouped as *r*-markers while 215 markers consisting of 65 *Eco*-markers and 150 *Pst*-markers were grouped as *s*-markers (Table 4.19 & 4.20). Another 226 markers (90 *Eco*-markers and 136 *Pst*-markers) were grouped as *h*-markers. Many of the polymorphic DNA fragments that were close to each other and difficult to identify were discarded.

4.4.8 Linkage Analysis and the Map Construction

All genotyped AFLP markers and markers generated from RAPD and STMS analysis was then used for linkage analysis in an attempt to construct a linkage map. The 639 markers which consisting of 607 AFLPs, 14 SSRs and 18 RAPDs markers (Table 4.21) were determined for two mapping population (resistance and susceptibility to FOC TR4). A total of 471 markers (286 *Pst*- markers, 155 *Eco*-markers, 17 RAPD markers and 13 STMS markers) were analyzed for susceptible mapping population while 414 markers (249 *Pst*- markers, 143 *Eco*-markers, 9 RAPD markers and 13 STMS markers) were analyzed for resistant mapping population (Table 4.21). The process for

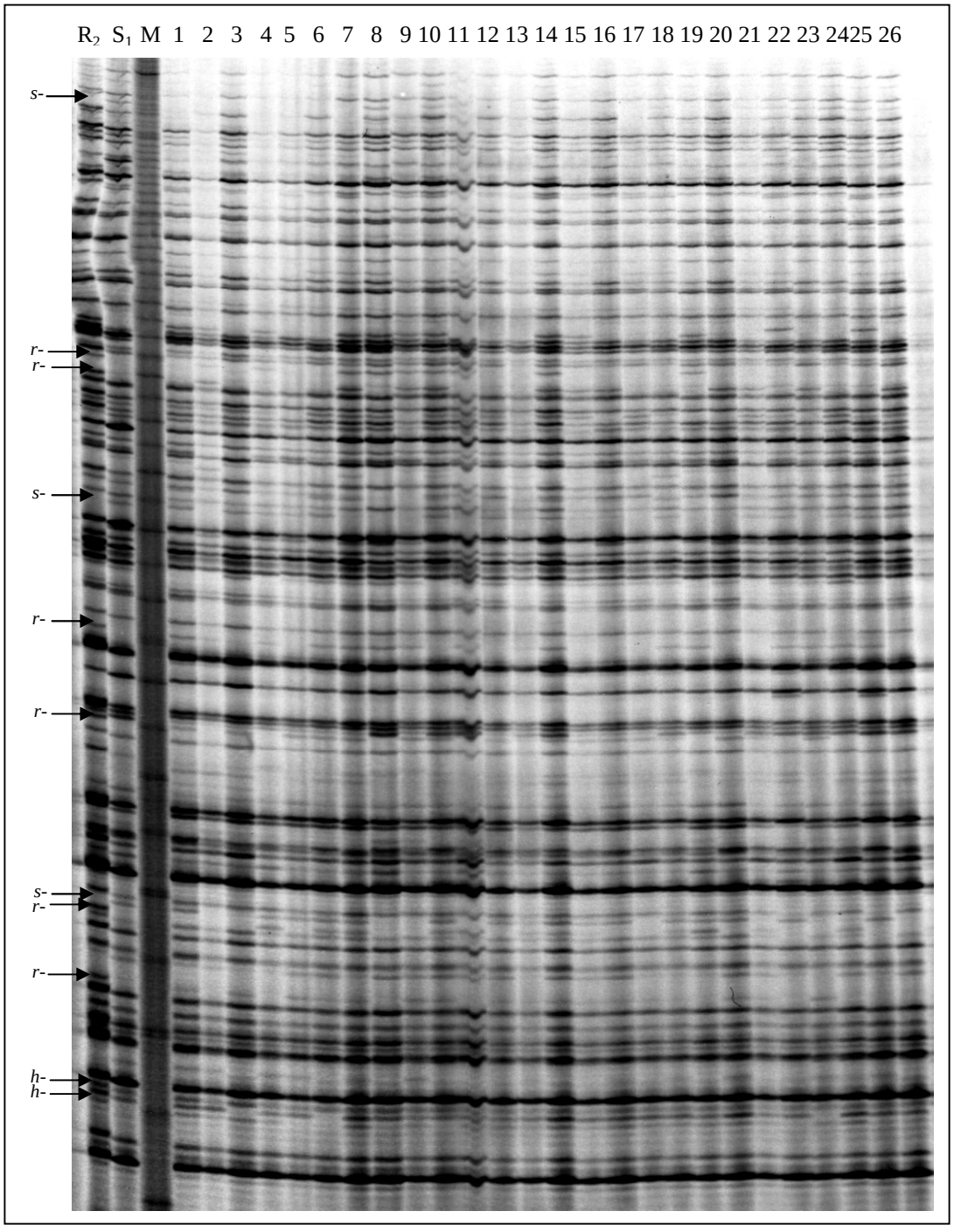


Figure 4.23: AFLP markers generated from *Eco*R1+AAC x *Mse*I+CA primer combination.

R_2, S_1, and M consists of the resistant parent, susceptible parent and molecular marker respectively while lane 1-26 consisted of individual samples of RS populations. Markers were genotyped into *r*-markers, *s*-markers and *h*-markers based on the presence or absence in the parents but segregates in their hybrids.

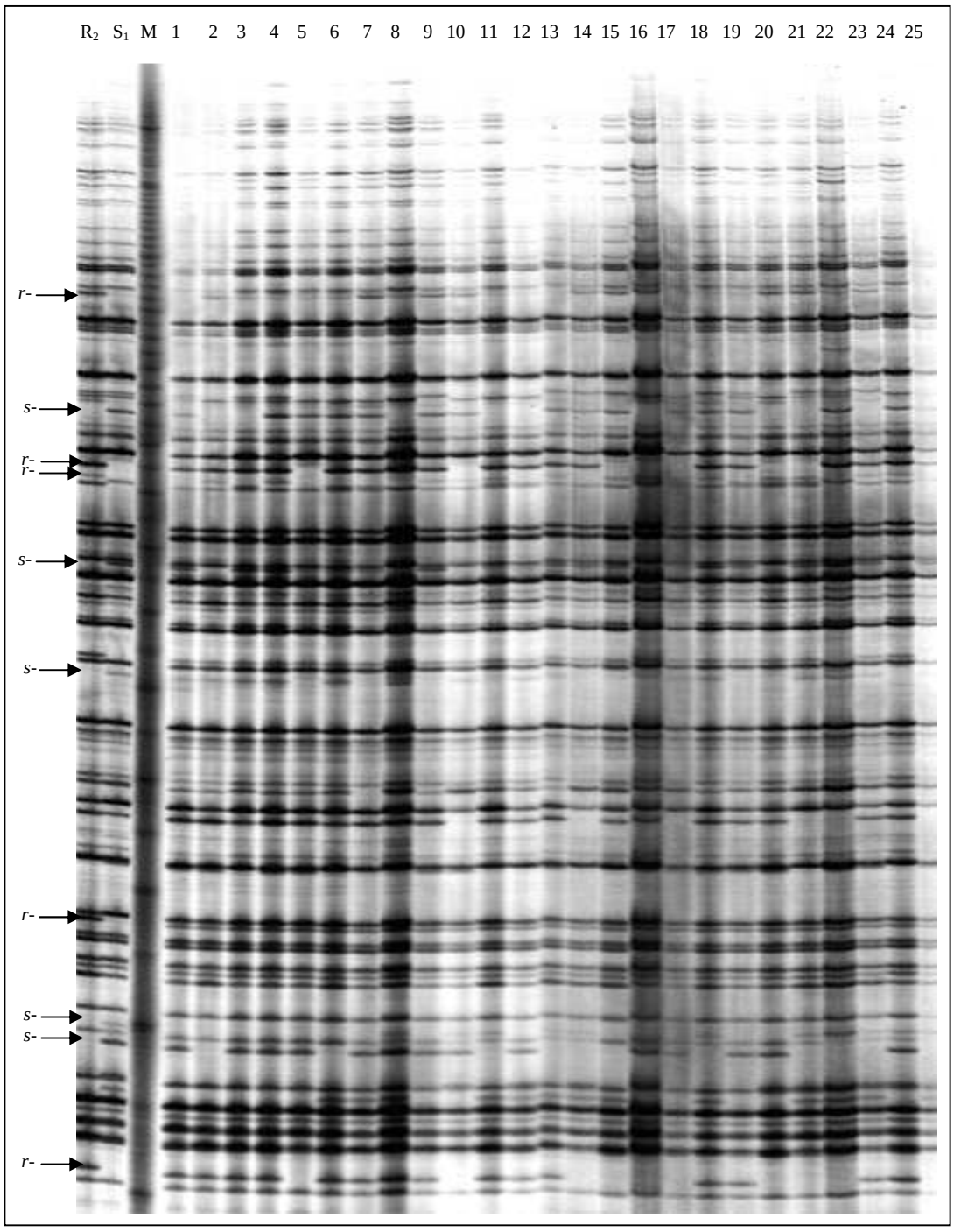


Figure 4.24: AFLP markers generated from *Eco*R1+AAC x *Mse*I+ATG primer combination.

Markers were genotyped into *r*-markers and *s*-markers based on the presence or absence in the parents but segregates in their hybrids. R_2, S_1, and M consists of the resistant parent, susceptible parent and molecular marker respectively while lane 1-25 consisting of individual samples of RS populations.

Table 4.19: Total number of *r*-markers, *s*-markers and *h*-markers scored in hybrid RS population by using *Eco*RI+2 x *Mse*I+2 or 3 primer combinations

No	Primer Combination	*r*-markers	*s*-markers	*h*-markers	Total Markers
1	*E*+AAC x *M*+CA	8	4	12	24
2	*E*+AAC x *M*+AAC	3	6	8	17
3	*E*+AAC x *M*+ACC	7	5	5	17
4	*E*+AAC x *M*+ACT	4	6	4	14
5	*E*+AAC x *M*+AGT	4	5	10	19
6	*E*+AAC x *M*+ATG	5	9	6	20
7	*E*+AAC x *M*+CAG	5	2	5	12
8	*E*+AAC x *M*+GAA	4	3	10	17
9	*E*+AAC x *M*+GAT	1	3	6	10
10	*E*+ACA x *M*+AAT	2	8	3	13
11	*E*+ACA x *M*+ACG	6	1	2	9
12	*E*+ACA x *M*+ATT	1	8	10	19
13	*E*+ACA x *M*+CAG	1	4	5	10
14	*E*+ACA x *M*+CTG	2	1	4	7
	Total	**53**	**65**	**90**	**208**

Table 4.20: Total number of *r*-markers, *s*-markers and *h*-markers scored in hybrid RS population using 39 *Pst*1+2 x *Mse*I+3 primer combinations

No	Primer Combination	*r*-markers	*s*-markers	*h*-markers	Total Markers
1	***P*+AA x *M*+AAC**	5	9	0	14
2	***P*+AA x *M*+AAG**	0	4	6	10
3	***P*+AA x *M*+AAT**	1	1	4	6
4	***P*+AA x *M*+ACT**	2	7	3	12
5	***P*+AA x *M*+AGG**	2	4	5	11
6	***P*+AA x *M*+CCG**	5	2	3	10
7	***P*+AA x *M*+CCT**	3	5	4	12
8	***P*+AA x *M*+CTA**	5	3	7	15
9	***P*+AA x *M*+CTT**	3	0	3	6
10	***P*+AA x *M*+GAC**	3	4	2	9
11	***P*+AA x *M*+GAG**	2	0	6	8
12	***P*+AA x *M*+GCA**	2	4	5	11
13	***P*+AC x *M*+ACC**	4	5	5	14
14	***P*+AC x *M*+ACT**	6	2	4	12
15	***P*+AC x *M*+AGA**	1	10	5	16
16	***P*+AC x *M*+CAC**	2	3	4	9
17	***P*+AC x *M*+CCA**	4	3	3	10
18	***P*+AC x *M*+CCG**	0	5	2	7
19	***P*+AC x *M*+CGG**	4	1	1	6
20	***P*+AC x *M*+GCA**	2	7	1	10
21	***P*+AG x *M*+AAC**	2	7	6	15
22	***P*+AG x *M*+AAT**	5	4	5	14
23	***P*+AG x *M*+ACC**	1	7	6	14
24	***P*+AG x *M*+AGA**	4	2	4	10
25	***P*+AG x *M*+CGC**	1	1	5	7
26	***P*+AG x *M*+GCA**	7	6	4	17
27	***P*+AT x *M*+AAC**	4	7	4	15
28	***P*+AT x *M*+AAT**	2	3	2	7
29	***P*+AT x *M*+ACT**	3	3	3	9
30	***P*+AT x *M*+AGG**	4	7	5	16
31	***P*+CA x *M*+ACT**	3	1	0	4
32	***P*+CA x *M*+AGA**	1	2	3	6
33	***P*+CA x *M*+CAC**	2	3	1	6
34	***P*+CA x *M*+CAG**	3	1	4	8
35	***P*+CA x *M*+CCC**	1	3	3	7
36	***P*+CA x *M*+CCT**	5	5	1	11
37	***P*+CA x *M*+CGT**	4	2	1	7
38	***P*+CA x *M*+CTA**	3	2	4	9
39	***P*+CA x *M*+GAC**	2	5	2	9
	Total	**113**	**150**	**136**	**399**

Table 4.21: Total number of different groups of markers (*Pst*I and *Eco*R1-AFLP, RAPD and STMS) analyzed in the construction of linkage maps

Type of markers	Group of markers			Total no. of markers	No. of markers for JoinMap analysis	
	r-markers	s-markers	h-markers		Resistant mapping population	Susceptible mapping population
***Pst*I+2 x *Mse*I+3**	113	150	136	399	249	186
***Eco*R1+3 x *Mse*I+2 and 3**	53	65	90	208	143	155
RAPD	1	9	8	18	9	17
STMS	1	1	12	14	13	13
Total	**168**	**225**	**246**	**639**	**414**	**471**

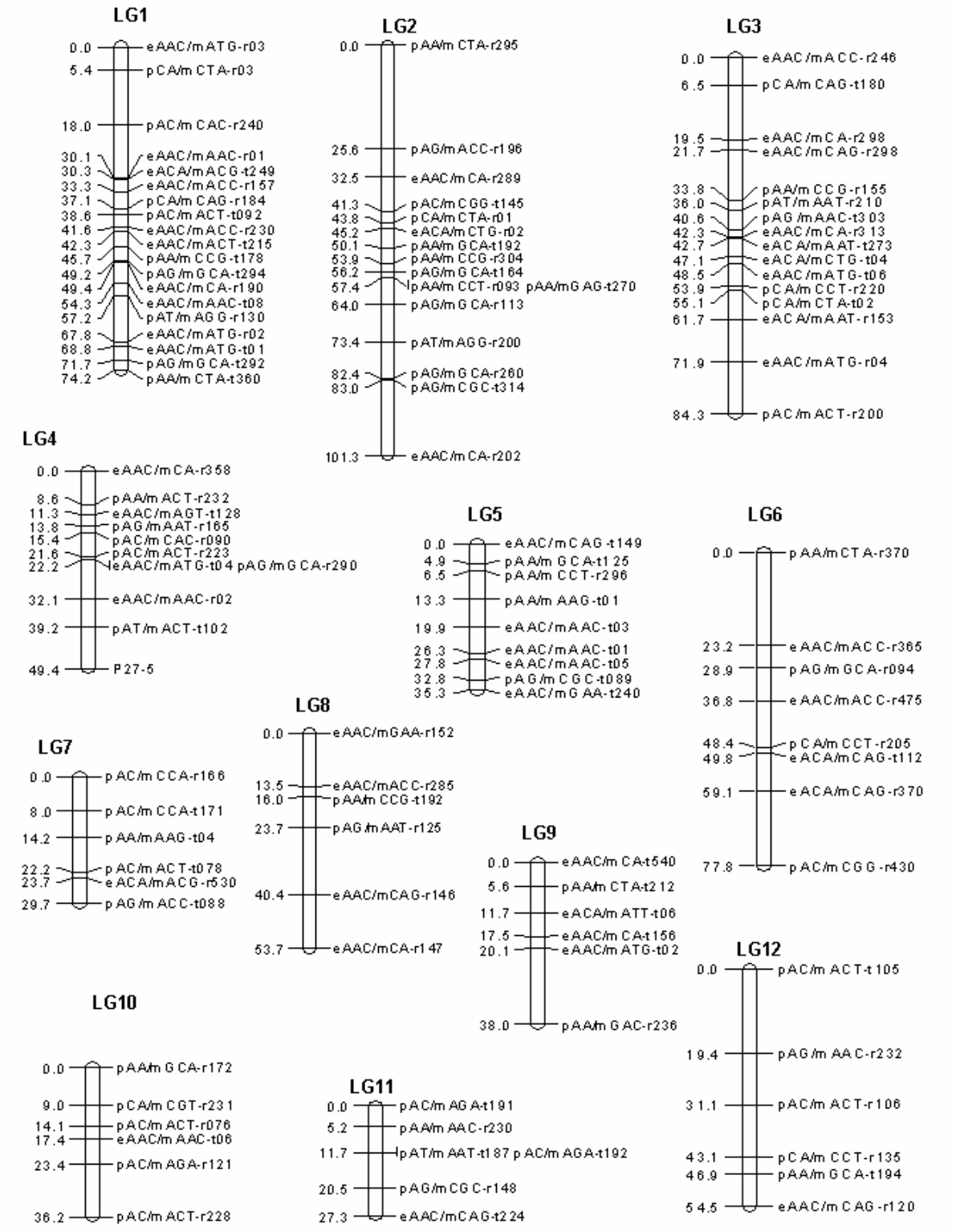
map construction consists of identifying linkage groups using two-point analysis by observing the recombination frequencies between each marker taken two by two. Then, the order of the markers within each linkage group was determined by a three-point analysis followed by a multipoint analysis.

The χ^2 values and locus genotype frequency of markers analyzed for resistant linkage mapping and susceptible linkage mapping were shown in Appendix A and Appendix B respectively. A high degree of segregation distortion was observed in most of the markers (66.7% and 61.4% for resistant and susceptible markers groups respectively). This phenomenon was possibly a consequence of the relatively small number of individuals (only 53) being used and also possibly due to the effect of inbreeding since the percentage of undeveloped seeds were high (almost 50%). Linkage analysis of the data however had resulted in two groups of linkage maps consisting of 32 linkage groups for resistant mapping population (Figure 4.25) and 37 linkage groups for susceptible mapping population (Figure 4.26). Those markers could not be placed on the map during the `first round' and `second round' of JoinMap-mapping procedure were omitted from the map. Indeed, adding new markers might well lead to the segregation some of the current groups.

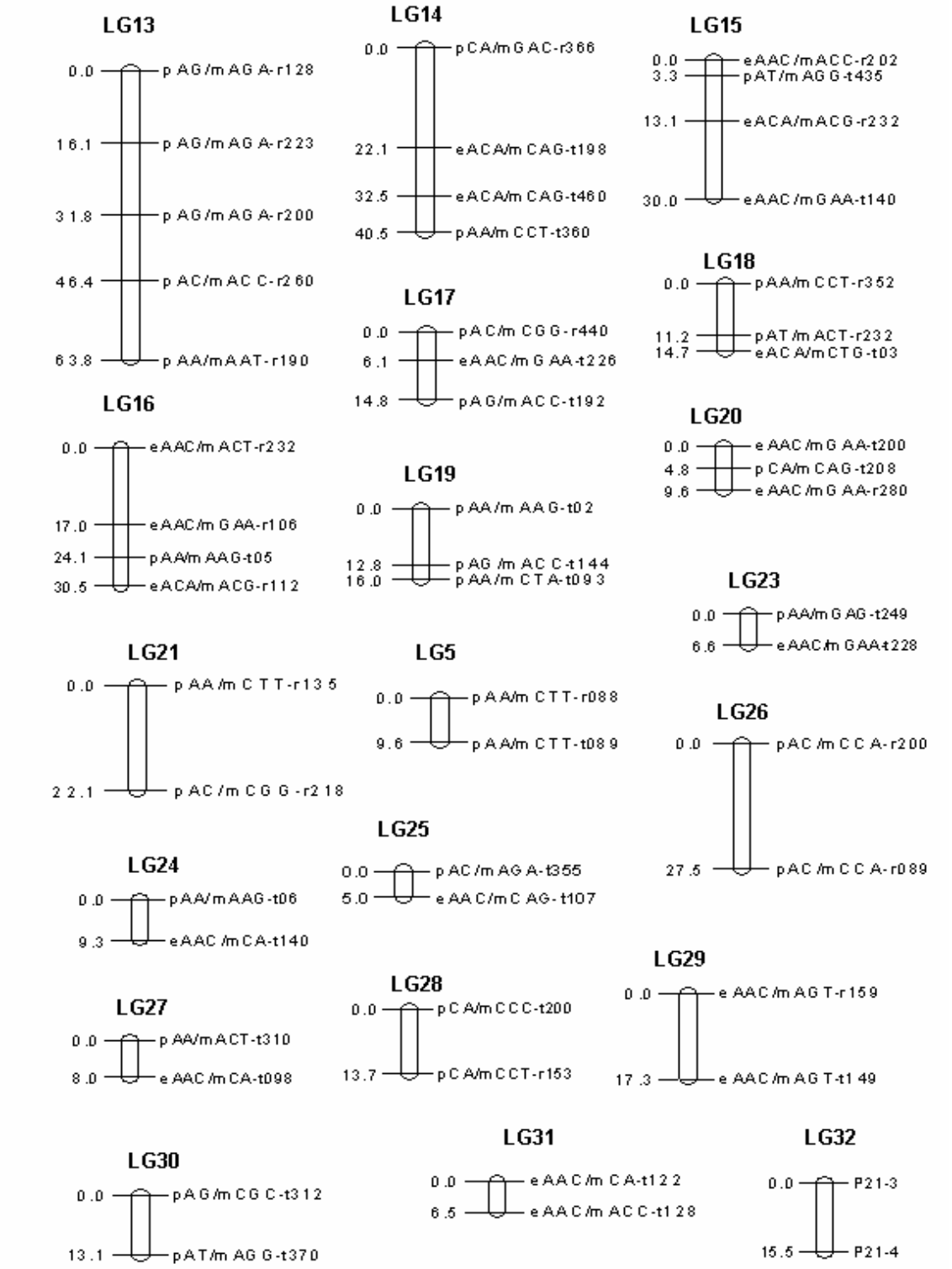
4.4.8.1 Linkage of the Resistant Mapping Population

The resistant mapping population presented a total of 168 loci comprising 67 *Eco*-AFLPs, 98 *Pst*-AFLP and 3 RAPD markers from 414 markers analyzed and were assigned into 32 linkage groups with LOD value 3.2 (Figure 4.25) while the remaining 246 loci (227 AFLPs, 13 SSRs and 6 RAPDs) remained unassigned. Allowing lower LOD value resulted into longer linkage groups while higher LOD value break markers into smaller linkage groups. Out of 168 markers in the resistant linkage map, 88 markers (52.38%) had shown significant distortion from the Mendelian expectation. The percentage of distorted loci in *Eco*-AFLP markers was relatively high (55.22%). In *Pst*-

Figure 4.25: Linkage map obtained for markers with 32 linkage groups using LOD 3.2 developed from resistant mapping population



(Figure 4.25 – cont.)



AFLP markers, the percentage is 51.02% while 33.33% had been observed for RAPD markers (Table 4.22). The total map distance within the 32 linkage groups was 1035.8cM with an average interval of 9.87cM. Linkage groups distance range from 5.0cM in LG25 to 101.3 cM in LG 2 with an average of 5.25 markers per group. The average marker interval was range from 4.12 in LG1 to 27.5cM in LG27 (Table 4.23). Linkage groups could be classified into 3 categories;

(a) large linkage groups with the length of (74.2 to 101.3) cM and consisting of eight to nineteen loci in LG 1, 2, 3 and 6

(b) medium linkage groups with the length of (35.3 to 63.8) cM and consisting of four to ten loci in LG 4, 5, 8, 9, 10, 12, 13 and 14

(c) small linkage groups with the length of (5.0 to 30.5) cM and consisting of two to seven loci in LG 7, 11, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 and 32.

The minimum marker interval of 0.0cM (between *Pst*I+AA x *Mse*I+CCT-r093 and *Pst*I+AA x *Mse*I+GAG-t270 in LG2; *Eco*R1+AAC/*Mse*I+ATG-t04 and *Pst*I+AG x *Mse*I+GCA-r290 in LG4 and *Pst*I+AT x *Mse*I+AAT-t187; *Pst*I+AC/*Mse*I+AGA-t192 in LG11 and the maximum marker interval of 27.5cM (between *Pst*I+AC x *Mse*I+CCA-r200 and *Pst*I+AC x *Mse*I+CCA-r089 in LG 26) had been observed among the linkage groups.

4.4.8.2 Linkage of the Susceptible Mapping Population

The susceptible mapping population presented a total of 194 loci comprising 73 *Eco*-AFLPs, 116 *Pst*-AFLP, 1 SSR and 4 RAPD markers from 471 markers analyzed. Markers were assigned into 37 linkage groups with LOD value 3.2 (Figure 4.26) while the remaining 277 loci (247 AFLPs, 13 SSRs and 17 RAPDs) were remained unassigned. 100 of markers being mapped (51.55%) showed significant distortion from the Mendelian expectation. Among the marker type, *Eco*-AFLP markers had shown the

Table 4.22: Marker distribution and segregation distortion in resistant mapping population

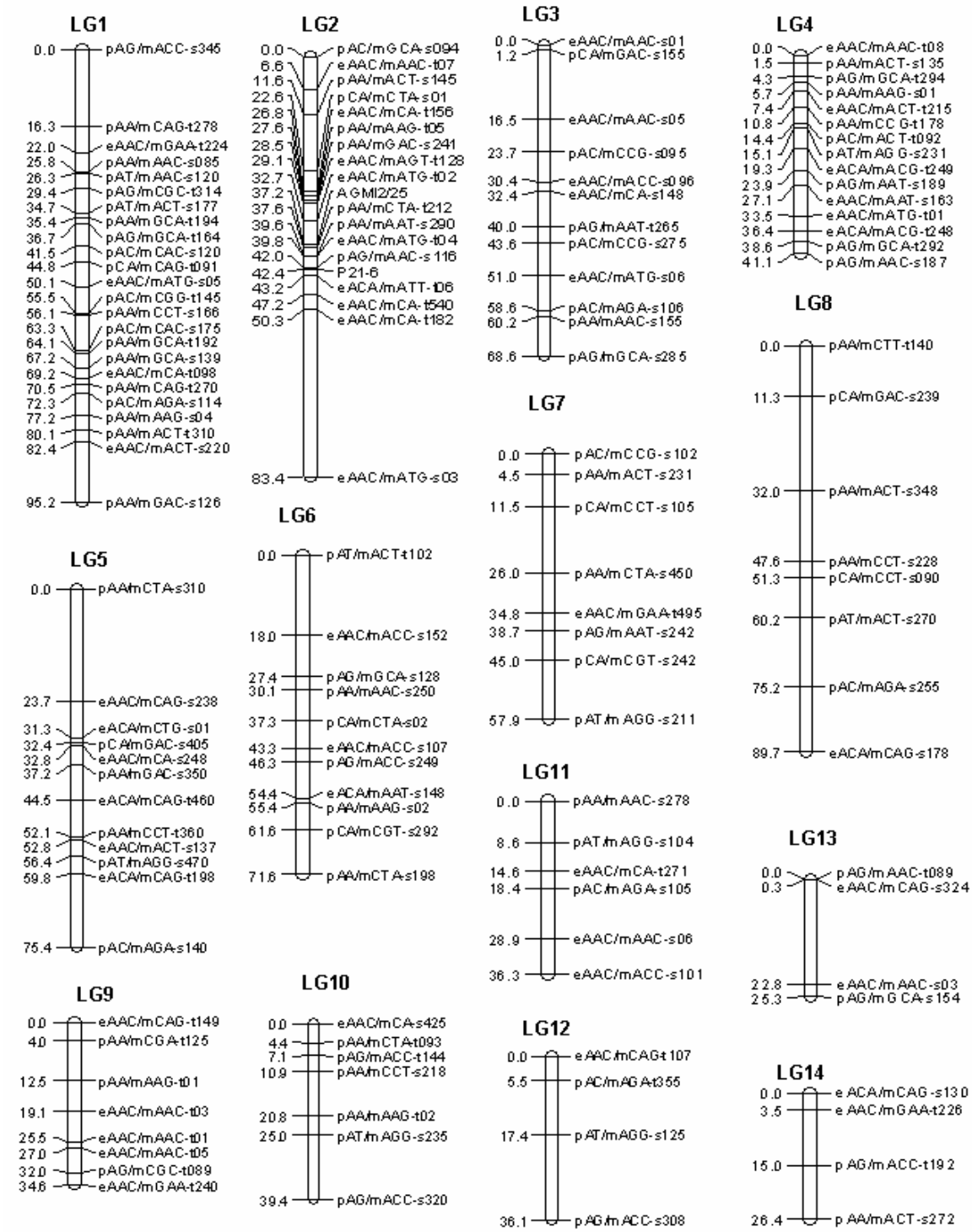
Linkage Group	*Eco*-AFLP Markers	*Pst*-AFLP markers	STMS markers	RAPD markers	Total markers
1	10 *(5)**	9 *(5)*	-	-	19 *(10)*
2	3 *(-)*	13 *(9)*	-	-	16 *(9)*
3	9 *(3)*	7 *(3)*	-	-	16 *(6)*
4	4 *(2)*	6 *(1)*	-	1 *(1)*	11 *(4)*
5	5 *(5)*	4 *(3)*	-	-	9 *(8)*
6	4 *(1)*	4 *(-)*	-	-	8 *(1)*
7	1 *(-)*	5 *(4)*	-	-	6 *(4)*
8	4 *(-)*	2 *(1)*	-	-	6 *(1)*
9	4 *(4)*	2 *(1)*	-	-	6 *(5)*
10	1 *(1)*	5 *(-)*	-	-	6 *(1)*
11	1 *(1)*	5 *(3)*	-	-	6 *(4)*
12	1 *(-)*	5 *(3)*	-	-	6 *(3)*
13	-	5 *(-)*	-	-	5 *(-)*
14	2 *(2)*	2 *(1)*	-	-	4 *(3)*
15	3 *(2)*	1 *(1)*	-	-	4 *(3)*
16	3 *(-)*	1 *(1)*	-	-	4 *(1)*
17	1 *(1)*	2 *(1)*	-	-	3 *(2)*
18	1*(1)*	2 *(-)*	-	-	3 *(1)*
19	-	3 *(3)*	-	-	3 *(3)*
20	2 *(2)*	1 *(1)*	-	-	3 *(3)*
21	-	2 *(-)*	-	-	2 *(-)*
22	-	2 *(1)*	-	-	2 *(1)*
23	1 *(1)*	1 *(1)*	-	-	2 *(2)*
24	1 *(1)*	1 *(1)*	-	-	2 *(2)*
25	1 *(1)*	1 *(1)*	-	-	2 *(2)*
26	-	2 *(1)*	-	-	2 *(1)*
27	1 *(1)*	1 *(1)*	-	-	2 *(2)*
28	-	2 *(1)*	-	-	2 *(1)*
29	2 *(1)*	-	-	-	2 *(1)*
30	-	2 *(2)*	-	-	2 *(2)*
31	2 *(2)*	-	-	-	2 *(2)*
32	-	-	-	2 *(-)*	2 *(-)*
Total	**67 *(37)***	**98 *(50)***	**-**	**3 *(1)***	**168 *(88)***
% distorted	**55.22%**	**51.02%**	**0%**	**33.33%**	**52.38%**

(#)* *number of distorted markers*

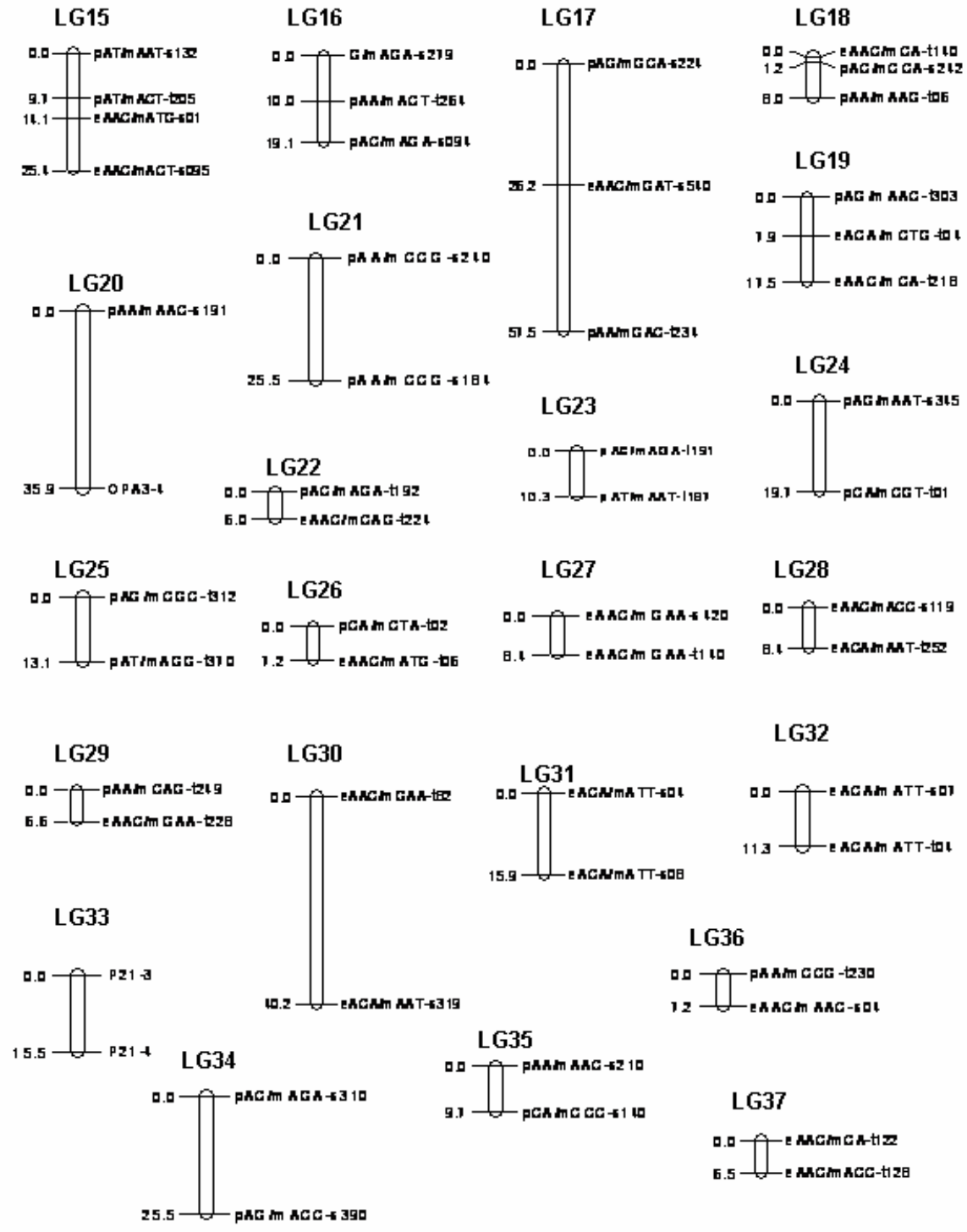
Table 4.23: Main characteristics (size, number of markers and distances) of the resistant mapping population

Linkage group	Length (cM)	Number of markers	Means distances (cM)	Min-Max distances (cM)
1	74.2	19	4.12	0.2-12.6
2	101.3	16	6.75	0.0-25.6
3	84.3	16	5.62	0.4-13.0
4	49.4	11	4.94	0.0-10.2
5	35.3	9	4.41	1.5-6.8
6	77.8	8	11.11	1.4-23.2
7	29.7	6	5.94	1.5-8.0
8	53.7	6	10.74	2.5-16.7
9	38.0	6	7.6	2.6-17.9
10	36.2	6	7.24	3.3-12.8
11	27.3	6	5.46	5.2-8.8
12	54.5	6	10.9	3.8-19.4
13	63.8	5	15.95	14.6-17.4
14	40.5	4	13.5	8.0-22.1
15	30.0	4	10.0	3.3-16.9
16	30.5	4	10.17	6.4-17.0
17	14.8	3	7.4	6.1-8.7
18	14.7	3	7.35	3.5-11.2
19	16.0	3	8.0	3.2-12.8
20	9.6	3	4.8	4.8
21	22.1	2	22.1	22.1
22	9.6	2	9.6	9.6
23	6.6	2	6.6	6.6
24	9.3	2	9.3	9.3
25	5.0	2	5.0	5.0
26	27.5	2	27.5	27.5
27	8.0	2	8.0	8.0
28	13.7	2	13.7	13.7
29	17.3	2	17.3	17.3
30	13.1	2	13.1	13.1
31	6.5	2	6.5	6.5
32	15.5	2	15.1	15.5
Total	**1035.8**	**168**	**315.8**	**-**
Mean	**32.37**	**5.25**	**9.87**	

Figure 4.26: The map obtained for markers with 37 linkage groups using LOD 3.2, develop from susceptible mapping population



(Figure 4.26 – cont.)



higher percentage of distorted loci (64.38%) compared to *Pst*-AFLP markers (45.69%) while no distortion was observed in SSR markers and RAPD markers (Table 4.24). The map covers a total length of 1181.4 cM with an average interval distance of 11.46 cM. Linkage groups range from 6.0cM in LG22 to 95.2 cM in LG1 with an average of 5.24 markers per group. The average marker interval range was from 2.94 in LG4 to 40.2 cM in LG30 (Table 4.25). Linkage groups could be classified into three categories;

(a) large-size linkage groups with the length of (68.6 to 95.2 cM) and consisting of six to twenty four loci in LG 1, 2, 3, 5, 6 and 10;

(b) medium-sized linkage groups with the length of (34.6 to 57.9 cM) and consisting of two to five loci in LG 4, 7, 8, 9, 11, 12, 17, 20 and 30;

(c) small-sized linkage groups with the length of (6.0 to 26.4 cM) and consisting of two to four loci in LG 13, 14, 15, 16, 18, 19, 21, 22, 23, 24, 25, 26, 27, 28, 29, 31, 32, 33, 34, 35, 36 and 37.

The minimum marker interval in this linkage groups was 0.2 cM (between *Pst*I+AA x *Mse*I+AAT-s290 and *Eco*R1+AAC x *Mse*I+ATG-t04 in LG2 and the maximum marker interval was 40.2 cM (between *Eco*R1+AAC x *Mse*I+GAA-t82 and *Eco*R1+ACA x *Mse*I+AAT-s319 in LG 30).

Table 4.24: Marker distribution and segregation distortion in linkage groups of susceptible mapping population

Linkage Group	*Eco*-AFLP markers	*Pst*-AFLP markers	STMS markers	RAPD markers	Total markers
1	4 *(2)*	20 *(9)*	-	-	24 *(11)*
2	9 *(9)*	8 *(4)*	1 *(-)*	1 *(-)*	19 *(13)*
3	5 *(2)*	7 *(1)*	-	-	12 *(3)*
4	6 *(5)*	9 *(5)*	-	-	15 *(10)*
5	6 *(3)*	6 *(1)*	-	-	12 *(4)*
6	3 *(-)*	8 *(1)*	-	-	11 *(1)*
7	1 *(1)*	7 *(2)*	-	-	8 *(3)*
8	1 *(-)*	6 *(3)*	-	-	7 *(3)*
9	5 *(5)*	3 *(3)*	-	-	8 *(8)*
10	1 *(-)*	7 *(1)*	-	-	8 *(1)*
11	3 *(1)*	3 *(-)*	-	-	6 *(1)*
12	1 *(1)*	3 *(1)*	-	-	4 *(2)*
13	2 *(-)*	2 *(1)*	-	-	4 *(1)*
14	2 *(2)*	2 *(1)*	-	-	4 *(3)*
15	2 *(1)*	2 *(2)*	-	-	4 *(3)*
16	-	3 *(1)*	-	-	3 *(1)*
17	1 *(-)*	2 *(2)*	-	-	3 *(2)*
18	1 *(1)*	2 *(1)*	-	-	3 *(2)*
19	2 *(2)*	1 *(1)*	-	-	3 *(3)*
20	-	1 *(1)*	-	1 *(-)*	2 *(1)*
21	-	2 *(1)*	-	-	2 *(1)*
22	1 *(1)*	1 *(1)*	-	-	2 *(2)*
23	-	2 *(2)*	-	-	2 *(2)*
24	-	2 *(1)*	-	-	2 *(1)*
25	-	2 *(2)*	-	-	2 *(2)*
26	1 *(1)*	1 *(1)*	-	-	2 *(2)*
27	2 *(1)*	-	-	-	2 *(1)*
28	2 *(1)*	-	-	-	2 *(1)*
29	1 *(1)*	1 *(1)*	-	-	2 *(2)*
30	2 *(2)*	-	-	-	2 *(2)*
31	2 *(2)*	-	-	-	2 *(2)*
32	2 *(1)*	-	-	-	2 *(1)*
33	-	-	-	2 *(-)*	2 *(-)*
34	-	2 *(-)*	-	-	2 *(-)*
35	-	2 (2)	-	-	2 *(2)*
36	1 *(-)*	1 *(1)*	-	-	2 *(1)*
37	2 *(2)*	-	-	-	2 *(2)*
Total	**73** ***(47)***	**116** ***(53)***	**1** ***(-)***	**4** ***(-)***	**194** ***(100)***
% distorted	***64.38%***	***45.69%***	***0%***	***0%***	***51.55%***

(#)* *number of distorted markers*

Table 4.25: Main characteristics (size, number of markers and distances) of the linkage groups in susceptible mapping population

Linkage group	Length (cM)	Number of markers	Means distances (cM)	Min-Max distances (cM)
1	95.2	24	4.14	0.5-16.3
2	83.4	19	4.63	0.2-33.1
3	68.6	12	6.24	1.2-15.3
4	41.1	15	2.94	0.7-6.4
5	75.4	12	6.86	0.4-23.7
6	71.6	11	7.16	1.0-18.0
7	57.9	8	8.27	3.9-14.5
8	39.4	7	6.57	2.7-14.4
9	34.6	8	4.94	1.5-8.5
10	89.7	8	12.81	3.7-20.7
11	36.3	6	7.26	3.8-10.5
12	36.1	4	12.03	5.5-18.7
13	25.3	4	8.43	0.3-22.5
14	26.4	4	8.8	3.5-12.0
15	25.4	4	8.47	4.4-11.3
16	19.1	3	9.55	9.1-10.0
17	57.5	3	19.17	26.2-31.3
18	8.0	3	4.00	1.2-6.8
19	17.5	3	8.75	7.9-9.6
20	35.9	2	35.9	35.9
21	25.5	2	25.5	25.5
22	6.0	2	6.0	6.0
23	10.3	2	10.3	10.3
24	19.7	2	19.7	19.7
25	13.1	2	13.1	13.1
26	7.2	2	7.2	7.2
27	8.4	2	8.4	8.4
28	8.4	2	8.4	8.4
29	6.6	2	6.6	6.6
30	40.2	2	40.2	40.2
31	15.9	2	15.9	15.9
32	11.3	2	11.3	11.3
33	15.5	2	15.5	15.5
34	25.5	2	25.5	25.5
35	9.7	2	9.7	9.7
36	7.2	2	7.2	7.2
37	6.5	2	6.5	6.5
Total	**1181.4**	**192**	**423.92**	**-**
Mean	**31.93**	**5.19**	**11.46**	**-**

5.0 DISCUSSION AND CONCLUSION

5.1 Strategy for Selection of Segregation Population

Musa breeding programs have been developed in various countries based on different populations and for different targets. A series of crosses for Sigatoka resistance, bunch position, chromosome rearrangement and parthenocarphy have been carried out by CIRAD (Centre de Cooperation Internationale en Recherché Agronomique pour le Developpement), CARBAP (Centre Africain de Recherches sur Bananiers et Plantains) in Cameroon, IITA (International Institute of Tropical Agriculture) in Nigeria (Pillay & Tripathi, 2007), NARO (National Agricultural Research Organization) in Uganda and in IIHR (Indian Institute of Horticulture Research) in India. The overall strategy in banana breeding is to incorporate the desired traits from wild and cultivated banana into the existing cultivars rather than selection of genetic materials that are completely different from the existing cultivars. All of the studies have concentrated on traits or problems which are of particular importance in the regions concerned. It is imperative that breeding programs be carried out to address specific problems and needs of banana growers in each particular country or region.

In this study, I had attempted to develop an F_1 segregating population of a diploid wild banana *Musa acuminata* ssp. *malaccensis* (AA) for the major pathogen of banana in Malaysia which is *Fusarium oxysporum* ssp *malaccensis* Tropical Race 4. This approach was based on a breeding strategy developed by FHIA and now adopted by other programs which is focused on the production of improved diploids possessing useful resistance characteristics from wild sources in an improved genetic background (Stover & Buddenhagen, 1986; Escalant & Jain, 2004). For that purpose, a major contribution of the FHIA program has been the development of the protocol for creating synthetic diploid hybrids using pollen parents, SH lines (Rowe, 1998a; Ploetz, 2005) with male and female fertile and low rate heterozygosity which ensures the heritability

of their interesting characters. Their genetic variability offers a large genetic base to the breeders and sources of genetic resistance to major banana diseases (Novak, 1992). The diploids are improved by crosses of selected parents for desired traits that present fertile male and/or fertile female gametes, therefore obtaining improved diploid hybrids (Ferreira *et al*., 2004). The long process has been successful in producing many improved diploids after many crosses between different natural diploids and diploid hybrids (Montcel *et al.*, 1996).

The major and most destructive disease in Malaysia Indonesia, China Taiwan, the Philippines, South Africa and parts of Australia is caused by a unique population consisting of VCG 01213/16 from the Southeast Asian region and also known as Tropical race 4 (TR4) which was believed to have coevolved with its diverse hosts in Asia (Molina, 2006; Ploetz & Pegg, 2000). In addition to focusing on the need to develop new populations with traits specific to this pathogen, it was also proposed that a good strategy would be to derive the trait from local wild banana resources which had co-evolved with the pathogen. In this study the population used was the indigenous wild banana *Musa acuminata* ssp *malaccensis* (AA) which has previously been shown to have very high resistance to FOC TR4 (Javed *et al*., 2004). Crop wild relatives have been recognized in breeding programs of major crops since the 1940s and 1950s and the use of wild genes crop improvement gained in prominence by the 1970s and 1980s with their usage being investigated in a wide range of crops (Hajjar & Hodgkin, 2007). Tanksley and McCouch (1997) suggested that the continued sampling of wild germplasm would result in new gene discoveries and use.

The development of inbred lines segregating for resistance and susceptibility (in terms of their response towards the Fusarium wilt) from these resource would not only be very useful to get better understanding of the disease behavior and their genetic basis

but may allow for further applications such as for map based cloning of potential resistance gene candidates or for developing markers for marker assisted selection.

Initially, mature fruit bunch of four random open cross populations of wild banana *Musa acuminata* ssp. *malaccensis* had been selected from three different locations of central and southern Peninsular Malaysia were selected for the study. The populations were previously identified to be naturally segregating to FOC (A. Javed pers. Comm.). Matured seeds were extracted and seed progenies were raised through zygotic embryo culture. In this study, *in vitro* cultured seed progenies of the wild banana *Musa acuminata* ssp. *malaccensis* were also shown to be segregating for apical dominance.

It has been previously reported that seed germination in *Musa* was found to be difficult to achieve under natural conditions (Asif *et al.,* 2001). One difficulty faced early in this study was in getting adequate numbers of germinated seeds and replicates for fusarium screening. Decrease in seed viability due to long term storage (more than a month) had greatly reduced the number of progenies to 30% of the developed seeds harvested (See section 4.3). Germinated seeds were also required to go through *in-vitro* stages before planting in order to generate replicates and uniform plantlets for disease screening. Most of individuals (more than 50%) showed a propensity for very high apical dominance which required a longer for development of the clonal population.

Vuylsteke and Swennen (1993) reported that low seed germination was due to malformed embryos, absence of endosperm, seed coat being softer than the normal and missing the embryo despite the presence of fully developed endosperm and chalazal mass. Javed *et al*. (2001) however had reported that *in vi*tro grown zygotic embryos resulted in more than 90% germination within one week. Germination and growth can be affected by the media composition and culture conditions and in this study the low to moderated percentage of embryo germination observed was achieved without any seed

treatment as described by Asif *et al*. (2001). Escalant and Teisson (1987) reported that seed germination of diploid banana is highly dependent on the maturity of the fruits during harvesting and the conditions prior germination. It could be said therefore that the low seed germination under natural conditions could be affected by many different factors. It was also observed in this study that embryo germination was not affected by light conditions but light however appeared to affect the embryo growth. Cultures maintained in the dark produced more roots with longer shoots and roots compared to grown under light. Light also appeared to affect the root growth which further causing a delayed appearance of shoots. Light could be inhibitory to auxin production and may also offset the balance of growth hormones necessary for root initiation. An optimized technique of *in vitro Musa* embryo germination such as that utilized for this study offers advantages for the study of wild banana populations that can be exploited in *Musa* breeding programs. Silva *et al*. (1999) and Asif *et al*. (2001) both reported the successful use of embryo culture in banana breeding where hybrid seeds were obtained as a result of pollination of diploids and triploids *Musa* clones. The embryos were maintained in the dark for two weeks before being placed under light. Multiplication of individual progeny enables studies on susceptible resistance seed progenies which had been sacrificed during the FOC TR4 screening by providing sufficient numbers of replicates that also facilitate crosses among progenies for segregation studies.

In actual practice, controlled crosses are not easy to perform because of the time differences of female and male reproductive organs maturity in banana (Fawcett, 1921; Purseglove, 1988). However, this drawback was overcome by planting several shoots of the same individuals (clones) to achieve synchronization as was carried out in this study. It was observed that at least two shoots per replicate were needed in the field in order to get synchronization. In this study we were able to generate three type of crosses which was between selected resistant male and female susceptible and two

crosses of selected resistant male and female (See Section 4.4). The differential degree of responses to *Fusarium oxysporum* f. sp. *cubense* TR4 in the hybrid F_1 populations observed in this study provided a potentially useful genetic resource for development of disease resistance markers which could be exploited in marker assisted selection.

5.1.1 Fusarium Screening

Individual pathogens vary in their potential range of host species, adaptation to biological and non-biological factors such as plant vigour, condition and type of soil, temperature, humidity and time exposure. Screening for resistance requires procedures capable of screening large populations efficiently and cheaply. Screening of banana cultivars in Fusarium infested soil or `Hot Spot' has been found to be useful in selecting tolerant plants (Hwang & Ko, 1987; Ho, 1999). However, the disease expression takes a long time to observe (4 to 5 months) and there were also problems related to quarantine practices to avoid disease spread, disease escape due to uneven distribution of pathogen, soil variables and environmental influences. Pegg *et al.* (1996) reported that inoculation at seedling stage could produce severe symptom that is not expressed in the field. In this study the double tray method reported by Mohamed *et al*. (1999) was used for screening of the regenerated embryos and subsequent clones and seedlings. The method was reported to give reliable results and was easy to handle compared to double cup method (Mak *et al*., 2004) which was established earlier. Seedlings survived in double tray screening planted in the `Hot Spot' still showed their resistance after a year in the field. The technique can be adapted for mass screening besides being a rapid method for early screening of Fusarium wilt. It is also amendable to modifications to allow investigations on the effects of different inoculum concentrations or environmental variation on infection and disease expression.

Javed *et al*. (2004) had reported that wild banana seed progenies screened for FOC TR4 were found to be segregating for disease compared to suckers. This was not

always observed in the natural habitat as presumably, natural selection in the field had eliminated susceptible progenies in the wild populations. Vakili (1965) indicated that the main source of variation in different plots of seedlings was based on genetic rather than the screening method. The factors which could affect the seedling response to FOC TR4 could be related to the heterogeneity of the seed and pathogen variability. Variation in population size and location of sample collected could affect the breeding behaviour of wild banana *Musa acuminata* ssp. *malaccensis* populations. Seed progenies derived from different populations and locations could have different genetic make up (Vakili, 1965) and the seed progenies derived from the same fruit bunch could have been produced as a result of both inbreeding and outcrossing.

Although the genetic mechanism of resistance to FOC TR4 is not clear, Rowe and Rosales (1993) suggested that the resistance seemed to be under polygenic control. However more recent studies by Javed (2004) on five wild banana *Musa acuminata* ssp *malaccensis* populations has shown that the chi-square analysis on the FOC screening result of three populations from five wild banana populations tested had data that fit to a monogenic ratio. This suggests a single recessive gene was associated with resistance to FOC TR4 resistance (Javed *et al*., 2004). Some discrepancies can be observed due to a mixture of sibs and cross pollination occurring within the same fruit bunch or/and among different accessions, or the size of population used.

Another factor which has to be taken into account is the influence of the environment and other factors on the uniformity of the population under study. In addition to population size this further affects the accuracy of testing. Inadequate replicates caused by losses during micropropagation and acclimatization is one factor in this study that may limit the accuracy of the *in vitro* pathogenicity testing as the size of plantlets varied (between 15 to 30 cm). Adequate replicates are required to minimize the error of environment influence to the disease expression thus may effect the accuracy of

the phenotype data. However, analyzing hundreds or thousands of segregating progeny is costly and time consuming and was not possible for the scope of this study. Taking all the limiting factors into account it was not possible to adequately analyze segregation patterns of the population developed in this study based on phenotypic characteristics.

5.2 Developing Markers for resistance to FOC TR4

Relying on morphological characters to select and cross plants carrying desired traits for cultivar improvement is practically slow and produces highly unpredictable progenies. The expression of morphological characters are also affected by environment and sometimes altered by epistatic and pleiotropic interaction which results in unreliable data (Pillay & Tripathi, 2007). Molecular markers based on DNA polymorphisms in the nucleotide sequences of genome regions detected by restriction enzymes or two priming sites offer plant breeders the potential of making genetic progress more rapidly and precisely. Differences in DNA known as DNA polymorphisms within genes have the potential to affect the gene function and hence the phenotype of the individual (Escalant & Panis, 2002).

Crop wild relatives including the progenitors of crops as well as other species more or less closely related to them provide plant breeders with a broad pool of potentially useful genetic resources and is well exploited in breeding programs of major crops like maize, rice, potato, wheat, tomatoes and others (Hajjar & Hodgkin, 2007). In this study, preliminary screening on a wild banana *Musa acuminata* ssp. *malaccensis* population with RAPD markers had revealed a large amount of polymorphism even though only four primers were used and provided good early evidence for polymorphism and its usefulness for the segregation studies.

Two criteria had been taken into account in deciding which marker system to use for this study.

(i) Which markers fit the genetic assumptions which underlie the method by which data be analyzed.

(ii) The practical ease of use of the marker system.

RAPD is a very fast way to obtain early information about genetic variation. Despite its reported disadvantages which are the dominant nature of the marker system and reproducibility problems, which may limit their application in marker assisted selection (MAS), it has been widely applied on *Musa* (Howell *et al.,* 1994; Pillay *et al*., 2006) and other crops (Gupta & Roy, 2002). High polymorphism observed from four RAPD primers in this study had provided good early evidence for polymorphism and segregation studies. The 18 RAPD markers identified were included in further analysis for construction of the linkage map. Previously reported studies have also utilized RAPD markers in the development of linkage maps of diploid bananas (Escalant & Panis, 2002; Faure *et al*., 1993).

Despite the fact that several microsatellite markers had been published in banana (Kaemmer *et al.*, 1997; Lagoda *et al.,* 1998; Grapin *et al.,* 1998; Crouch *et al*., 1999a; Creste *et al*., 2003) the lack of amplification of products in some genotypes has been common in *Musa* and it may reflect the divergences in the sequences flanking the microsatellite loci leading to production of null alleles. There were also problems to assign the exact length of some alleles which may results from the denaturation conditions and gel concentration during electrophoresis (Creste *et al.,* 2003). However, STMS markers have proved to be useful because of their highly reproducibility. However apart from the high cost of production of these markers, optimization of PCR condition is still often needed to avoid null alleles as experienced in this study. Several attempts were carried out including altering the annealing temperatures, applying touch down program and adjusting the $MgCl_2$ concentration. To ensure that the occurrence of

null allele was not a failure of reaction, the assays were repeated several times. As a result in this study 10 markers were generated from ten selected primer combinations and were utilized for linkage analysis. Ideally more microsatellite markers are still needed for QTL analysis and mapping in order to facilitate the effectiveness of these markers for MAS. However it was decided that a more efficient system would be used in subsequent analysis in the interest of time and efficiency.

AFLP methods was chosen for the final analysis as they allow for generation of a high number of molecular markers relatively quickly (several markers can be detected in a single PCR assay) with only modest experimental effort (Cai *et al.,* 2004; Hori *et al.,* 2003). Intergration of AFLP markers to RFLP markers had shown an effectively saturated linkage map in alfalfa (Barcaccia *et al.,* 1999), barley (Becker *et al.,* 1995) and rye (Saal & Wricke, 2002). Unfortunately, the information content of these banding patterns is restricted, as they must initially be treated as dominant markers. Because genetic mapping relies on the estimation of recombination frequencies between pairs of markers and implies to be able to distinguish parental from recombinant gametes, missing genotypes (unable to distinguish AA from Aa) would hamper the good success of establishing a genetic map. Despite of some disadvantages, RAPD and AFLP markers are still preferred for bulk segregant analysis as they allow a highly efficient generation of markers (Kema *et al.,* 2002; Seefelder *et al.,* 2000). Between the two, studies on genetic linkage maps have shown that AFLP markers are more reliable than RAPD markers (Kema *et al.,* 2002). In this study, a total of 607 of AFLP markers were generated from thirty *Eco*R1+3 x *Mse*I+3, one *Eco*R1+3 x *Mse*I+2 and forty three *Pst*I+2 x *Mse*I+3 primer combinations and had provided a more reasonable number of markers for linkage mapping. Additionally the markers generated via RAPD and STMS analysis were also incorporated in the study.

5.3 Linkage Mapping

A high density map is needed to locate the desired trait such as resistance to diseases and pests. Genetic mapping in *Musa* is not very far advanced (Pillay & Tripathi, 2007). The construction of a linkage map requires a segregating population (Collard *et al*., 2005) derived from a cross between two diverse parents, differing for the character of interest (Gupta, 2002). The first low density map was developed based on an F_2 progeny of a F_1 hybrid plant derived from a cross between SF265 (AA) x Banksii (AA) which segregating for parthenocarpy (Faure *et al.*, 1993; Heslop-Harrison & Schwarzacher, 2007; Pillay & Tripathi, 2007). Although some series of crosses for other traits like Sigatoka resistance, bunch position, chromosome rearrangement and parthenocarphy had been carried out by CIRAD, NARO, IITA and IIHR, no high density linkage map is yet available.

The IITA is developing several populations based on the A and B genomes from crosses between *Musa acuminata* (Calcutta 4) x *Musa balbisiana* (Pillay & Tripathi, 2007). The Indian Institute of Horticulture Research (IIHR) developed segregating populations from crosses between *Musa acuminata* x *Musa balbisiana,* ABB cultivated type with AA and AAA cultivars and wild BB types (Beeheekela x Bhimathia) for mapping purpose and contrasting cultivars/wild accession for fusarium wilt (Sub Tropical Race) and nematode resistance. NARO is developing a segregating population for parthenocarpy by crossing Calcutta 4 and Pisang Lilin. However, until now, the mapping populations are limited in number despite several attempts to develop suitable segregating populations at various research institutes (Pillay & Tripathi, 2007). No other cross for FOC TR4 such as for this study has been reported.

Since, none of the potential markers could distinguish clearly for any marker linked to resistance or susceptibility, data from markers generated from RAPD, STMS and AFLP analysis were genotyped to generate a localized linkage map (See section

4.4.6). In linkage analysis, markers that co-segregate (always present or absent together) must be linked, i.e. they must be located in vicinity to each others in the genome. However, in some cases due to recombination events, the linkage between the markers may be lost. The frequency with which the linkage between co-segregating markers is broken is an indication of the genetic distance between the markers (Schmidt *et al.,* 1995).

Genetic linkage maps consist of ordering molecular markers across the genome and require a high number of markers for a good coverage of the genome. AFLP markers are usually preferred for increasing marker density (Vos *et al.,* 1995; Collard *et al.,* 2005). *Pst*-AFLP markers have been shown to be more efficient in detecting polymorphisms than *Eco*-AFLP markers as the *Eco*-AFLP mainly clustered in the centromic regions while *Pst*-AFLP were randomly distributed across the chromosomes regions (Vuylsteke *et al*., 1999; Young, 1999). Combination of both *Pst*-AFLP and *Eco*-AFLP markers would provide complementing coverage of both target regions (Yuan *et al*., 2004). The overall rate of polymorphism reported in this study ranged from 5.06% - 29.41% with an average of 11 markers in *Pst*I+CA x *Mse*I+ACT and 12.99% - 33.93% with an average of 13 markers in *Eco*R1+AAC x *Mse*I+CA. It is generally accepted that establishing the correct number of linkage groups in outbreeding species by using only dominant markers such as AFLPs is difficult especially for species having a large number of chromosomes. Therefore, codominant markers such as RFLPs and SSRs will still be needed in order to refine the maps (Cai *et al*., 2004). Addition of RAPD and STMS markers into the map as used in this study could therefore increase the marker density.

As a DNA-based molecular marker, AFLP loci theoretically should segregate in a Mendelian manner in F_1 hybrid due to the direct consequence of gametophytic or sporophytic selection without environmental influence. However, segregation deviation

of the molecular markers from Mendelian ratio often happens. In general, the results of the marker analysis showed a high degree of segregation distortion (about 50% on both maps). Skewed segregation ratios have been reported frequently in many plants (Lambrides, *et al.*, 2004) including oil palm (Billotte *et al.*, 2005) and coffee (Ky *et al.*, 2000). Segregation distortion could be due to self-incompatibility alleles (Bert *et al.*, 1999) or gametic, zygotic or/and post-zygotic selection (Ky *et al.*, 2000; Virk *et al.*, 1998). Allelic disequilibrium might also influence by the small number of sample population (only 53 individuals) used in this study. In small populations, allele frequencies can be altered by random genetic drift, which refer to random changes in allele frequencies due to sampling error. In other words, allele frequencies may drift from generation to generations as matter of chance. Division of population into sub-populations or groups as observed in case of wild bananas reduces the genetic variability and increases homozygosity. Since mating between similar individuals (inbreeding) takes place in subpopulations, genetic variability within the group decreases with some genes fixed while some are eliminated thus increasing homozygosity (Wright, 1951). Lyttle (1991) considered this phenomenon as the evolutionary force of an organism, while Yu and Pauls (1992) suggested that it may be attributed to either by chloroplast or mitochondrial DNA contamination or by some degree of preferential pairing, gametophytic selection or linked deleterious mutations (Li *et al.*, 2004).

The accuracy of measuring the genetic distance and determining marker order is directly related to the number of individuals studied in the mapping population. Larger population size gives more accurate mapping study. Ideally, mapping populations should consist of a minimum of 50 individuals for constructing linkage maps (Collard *et al*., 2005). Genotyping error and missing data can affect the order and distance between markers within linkage map. A large proportion of missing data for a marker may

indicate that the marker was difficult to score and such marker should be excluded from the data. This is critical for dominant marker especially when faint bands are score as missing thus influences the segregation ratio (become greater or smaller) depending on phenotype associated with the corresponding band. In practice, it can hardly be avoided that for some markers or individuals not all observations can be made, for instance due to problems with gel quality or other technical difficulties in the laboratory.

As a consequence of using a relatively small sample size in this study (only 53 individuals) with a high percentage of missing value and some genotyping error that may occur during scoring, these may lead to the formation of short map length in the linkage groups (LG 21 – LG 32 in resistant mapping population and LG 20 – LG 37 in susceptible mapping population) in order to get more widely separated markers especially in the presence of distorted segregation (unequal transmission ratio of alternative alleles from parents to offspring). Therefore, higher number of individuals (at least 100) with more replicates (at least 3) would be needed to minimize the error. Ideally, number of linkage groups is equal to the number of chromosomes, but in practice it may be smaller or larger (Jansen *et al*., 2001). In this study, both sets of linkage groups (37 for susceptible mapping population and 32 for resistant mapping population) did not appear to correspond to the 22 pairs of chromosomes of the diploid bananas *Musa acuminata* ssp. *malaccensis* (x=11) and the high percentage of unassigned loci (40% and 35% respectively) showed that the genome was not fully covered. It should also be noted that distance on a linkage map is not directly related to the physical distance of DNA between genetic markers but depends on the genome size of the plant species (Collard *et al*., 2005).

5.4 Conclusions and Suggestions for future work

The accuracy of any mapping procedures not only depends on the ability of the statistical method to determine the location and estimate the genetic effect of the QTL

but is also influenced by the experimental design (type of segregating population), its size, heritability of the trait, the number of QTLs and contribution of each QTL to the total genotype variance, their interactions and distribution throughout the genome, the number and distance between consecutive markers, percentage of co-dominant marker, the reliability of the order of markers in the genetic linkage map and many others (Asins, 2002; Collard *et al*., 2005). Disease incidence or severity may be rated on an ordinal scale rather than a truly quantitative evaluation that has the normal distribution required for most statistical approaches. Genetic differences between strains or isolates, the timing and method of inoculation may also result in different QTL profiles (Asins, 2002). Parentage analysis should also ideally be performed to analyze the same family for genetic and QTL mapping (Lallias, 2007).

Development of a segregating population is required for the construction of a linkage map. Larger populations are required for higher resolution mapping (Collard *et al.*, 2005). A single segregating population such as that developed in this study provides only partial information. In this project, only a set of F_1 (from crosses between selected male resistant and selected female susceptible) had been analyzed. Another set of F_1 from the reciprocal cross or selfing of each individual parent may provide more informative value for further study. Concentrating more efforts on a single family in future studies may allow more numbers of progenies to be genotyped and thus can minimize any potential errors. Screening banana against FOC at the seedling stage as mention in this study allow selection in a larger population and requires less time.

Markers that are not adequately tested before use in MAS programs may not be reliable for predicting phenotype. Therefore, high resolution mapping, validation of markers and marker conversion are required for development of markers for use in MAS. More tightly-linked markers can be identified by using larger population sizes and greater number of markers (Snowdon & Friedt, 2004; Collard *et al.*, 2005).

Validation of markers is needed to test the effectiveness of markers to predict phenotype and should reveal polymorphism in different populations from a wide range of different parental genotypes. Testing for the presence of the markers on a range of cultivars and other important genotypes are needed (Collard, *et al*., 2005). In order to be useful in breeding programs they should reveal polymorphism in different populations derived from a wide range of different parental genotypes. Marker conversion by the development of sequence characterized amplified regions (SCARs) may also be applied when there are problems of reproducibility (e.g. RAPD) and when the marker technique is complicated, time consuming or expensive (e.g. RFLP or AFLP). Finally, it is clear that larger population size, more accurate phenotypic data, multiple replications and environments, various genetic backgrounds, appropriate quantitative genetic analysis and independent verification are necessary in order to develop reliable markers for MAS. Additional and confirmatory experiments should be performed in other families and populations from several locations.

Despite the various limitations described, the population and processes developed and the basic linkage map generated in this study represents a starting point for a more comprehensive programme for segregation analysis for this important trait in banana. This area of research presents a viable and important approach which may lead to the selection or development of FOC TR4 resistant banana clones. This outcome is much needed for the survival of the Malaysian banana industry in the future which is under increasing threat from this and other pathogens.

Appendix A: Locus genotype frequency and χ^2 value of markers analyzed by JoinMap 3.0 in the construction of resistant mapping population

Locus Genotype Freq rdata [r+h] of AFLP+RAPD+STMS

Nr	Locus	Seg.type	ac	ad	bc	bd	ee	ef	eg	fg	hh	hk	kk	h-	k-	ll	lm	nn	np	--	χ 2	Df	Signif.	Classes
1	pAA/mAAC-r370	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	15	36	0	0	2	8.7	1	****	[ll:lm]
2	pAA/mAAC-r230	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	21	32	0	0	0	2.3	1	-	[ll:lm]
3	pAA/mAAC-r185	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	31	22	0	0	0	1.5	1	-	[ll:lm]
4	pAA/mAAC-r178	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	14	39	0	0	0	11.8	1	*****	[ll:lm]
5	pAA/mAAC-r141	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	24	29	0	0	0	0.5	1	-	[ll:lm]
6	pAA/mAAG-t01	<hkxhk>	0	0	0	0	0	0	0	0	17	24	0	0	0	0	0	0	0	12	15.3	2	******	[hh:hk:kk]
7	pAA/mAAG-t02	<hkxhk>	0	0	0	0	0	0	0	0	18	23	0	0	0	0	0	0	0	12	16.4	2	******	[hh:hk:kk]
8	pAA/mAAG-t03	<hkxhk>	0	0	0	0	0	0	0	0	13	28	0	0	0	0	0	0	0	12	13.7	2	****	[hh:hk:kk]
9	pAA/mAAG-t04	<hkxhk>	0	0	0	0	0	0	0	0	16	25	0	0	0	0	0	0	0	12	14.5	2	*****	[hh:hk:kk]
10	pAA/mAAG-t05	<hkxhk>	0	0	0	0	0	0	0	0	12	29	0	0	0	0	0	0	0	12	14.1	2	*****	[hh:hk:kk]
11	pAA/mAAG-t06	<hkxhk>	0	0	0	0	0	0	0	0	7	34	0	0	0	0	0	0	0	12	20.2	2	*******	[hh:hk:kk]
12	pAA/mAAT-r190	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	25	23	0	0	5	0.1	1	-	[ll:lm]
13	pAA/mAAT-t227	<hkxhk>	0	0	0	0	0	0	0	0	10	35	0	0	0	0	0	0	0	8	18.3	2	******	[hh:hk:kk]
14	pAA/mAAT-t206	<hkxhk>	0	0	0	0	0	0	0	0	18	27	0	0	0	0	0	0	0	8	16.2	2	******	[hh:hk:kk]
15	pAA/mAAT-t157	<hkxhk>	0	0	0	0	0	0	0	0	11	39	0	0	0	0	0	0	0	3	20.5	2	*******	[hh:hk:kk]
16	pAA/mAAT-t128	<hkxhk>	0	0	0	0	0	0	0	0	18	32	0	0	0	0	0	0	0	3	16.9	2	******	[hh:hk:kk]
17	pAA/mACT-r355	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	24	28	0	0	1	0.3	1	-	[ll:lm]
18	pAA/mACT-r232	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	22	30	0	0	1	1.2	1	-	[ll:lm]
19	pAA/mACT-t310	<hkxhk>	0	0	0	0	0	0	0	0	15	37	0	0	0	0	0	0	0	1	18	2	******	[hh:hk:kk]
20	pAA/mACT-t264	<hkxhk>	0	0	0	0	0	0	0	0	15	37	0	0	0	0	0	0	0	1	18	2	******	[hh:hk:kk]
21	pAA/mACT-t169	<hkxhk>	0	0	0	0	0	0	0	0	13	40	0	0	0	0	0	0	0	0	20.1	2	*******	[hh:hk:kk]
22	pAA/mAGG-r146	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	21	30	0	0	2	1.6	1	-	[ll:lm]
23	pAA/mAGG-r091	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	21	31	0	0	1	1.9	1	-	[ll:lm]
24	pAA/mAGG-t240	<hkxhk>	0	0	0	0	0	0	0	0	27	21	0	0	0	0	0	0	0	5	31.1	2	*******	[hh:hk:kk]
25	pAA/mAGG-t174	<hkxhk>	0	0	0	0	0	0	0	0	32	19	0	0	0	0	0	0	0	2	43.5	2	*******	[hh:hk:kk]
26	pAA/mAGG-t170	<hkxhk>	0	0	0	0	0	0	0	0	26	25	0	0	0	0	0	0	0	2	26.5	2	*******	[hh:hk:kk]
27	pAA/mAGG-t143	<hkxhk>	0	0	0	0	0	0	0	0	16	35	0	0	0	0	0	0	0	2	17.1	2	******	[hh:hk:kk]
28	pAA/mAGG-t101	<hkxhk>	0	0	0	0	0	0	0	0	11	40	0	0	0	0	0	0	0	2	21.2	2	*******	[hh:hk:kk]
29	pAA/mCCG-r345	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	26	8	0	0	19	9.5	1	****	[ll:lm]
30	pAA/mCCG-r304	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	12	24	0	0	17	4	1	**	[ll:lm]
31	pAA/mCCG-r239	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	25	26	0	0	2	0	1	-	[ll:lm]
32	pAA/mCCG-r155	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	28	25	0	0	0	0.2	1	-	[ll:lm]
33	pAA/mCCG-r100	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	18	35	0	0	0	5.5	1	**	[ll:lm]
34	pAA/mCCG-t230	<hkxhk>	0	0	0	0	0	0	0	0	14	37	0	0	0	0	0	0	0	2	18.1	2	******	[hh:hk:kk]
35	pAA/mCCG-t192	<hkxhk>	0	0	0	0	0	0	0	0	18	33	0	0	0	0	0	0	0	2	17.1	2	******	[hh:hk:kk]
36	pAA/mCCG-t178	<hkxhk>	0	0	0	0	0	0	0	0	21	31	0	0	0	0	0	0	0	1	18.9	2	*******	[hh:hk:kk]
37	pAA/mCCT-r352	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	28	20	0	0	5	1.3	1	-	[ll:lm]
38	pAA/mCCT-r296	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	20	29	0	0	4	1.6	1	-	[ll:lm]

39	pAA/mCCT-r093	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	18	35	0	0	0	5.5	1	**	[ll:lm]
40	pAA/mCCT-t360	<hkxhk>	0	0	0	0	0	0	0	0	23	25	0	0	0	0	0	0	0	5	22.1	2	*******	[hh:hk:kk]
41	pAA/mCCT-t249	<hkxhk>	0	0	0	0	0	0	0	0	12	41	0	0	0	0	0	0	0	0	21.3	2	*******	[hh:hk:kk]
42	pAA/mCCT-t211	<hkxhk>	0	0	0	0	0	0	0	0	14	39	0	0	0	0	0	0	0	0	19.2	2	*******	[hh:hk:kk]
43	pAA/mCCT-t209	<hkxhk>	0	0	0	0	0	0	0	0	20	33	0	0	0	0	0	0	0	0	18.3	2	******	[hh:hk:kk]
44	pAA/mCTA-r370	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	21	31	0	0	1	1.9	1	-	[ll:lm]
45	pAA/mCTA-r295	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	15	38	0	0	0	10	1	****	[ll:lm]
46	pAA/mCTA-r226	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	9	44	0	0	0	23.1	1	*******	[ll:lm]
47	pAA/mCTA-r216	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	15	38	0	0	0	10	1	****	[ll:lm]
48	pAA/mCTA-r112	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	23	30	0	0	0	0.9	1	-	[ll:lm]
49	pAA/mCTA-t405	<hkxhk>	0	0	0	0	0	0	0	0	14	38	0	0	0	0	0	0	0	1	18.6	2	*******	[hh:hk:kk]
50	pAA/mCTA-t360	<hkxhk>	0	0	0	0	0	0	0	0	18	34	0	0	0	0	0	0	0	1	17.4	2	******	[hh:hk:kk]
51	pAA/mCTA-t212	<hkxhk>	0	0	0	0	0	0	0	0	15	38	0	0	0	0	0	0	0	0	18.5	2	*******	[hh:hk:kk]
52	pAA/mCTA-t207	<hkxhk>	0	0	0	0	0	0	0	0	17	36	0	0	0	0	0	0	0	0	17.7	2	******	[hh:hk:kk]
53	pAA/mCTA-t111	<hkxhk>	0	0	0	0	0	0	0	0	16	37	0	0	0	0	0	0	0	0	18	2	******	[hh:hk:kk]
54	pAA/mCTA-t104	<hkxhk>	0	0	0	0	0	0	0	0	10	43	0	0	0	0	0	0	0	0	24.3	2	*******	[hh:hk:kk]
55	pAA/mCTA-t093	<hkxhk>	0	0	0	0	0	0	0	0	7	46	0	0	0	0	0	0	0	0	30.6	2	*******	[hh:hk:kk]
56	pAA/mCTT-r278	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	24	28	0	0	1	0.3	1	-	[ll:lm]
57	pAA/mCTT-r135	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	24	29	0	0	0	0.5	1	-	[ll:lm]
58	pAA/mCTT-r088	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	22	31	0	0	0	1.5	1	-	[ll:lm]
59	pAA/mCTT-t140	<hkxhk>	0	0	0	0	0	0	0	0	29	24	0	0	0	0	0	0	0	0	32.2	2	*******	[hh:hk:kk]
60	pAA/mCTT-t114	<hkxhk>	0	0	0	0	0	0	0	0	32	21	0	0	0	0	0	0	0	0	40.9	2	*******	[hh:hk:kk]
61	pAA/mCTT-t089	<hkxhk>	0	0	0	0	0	0	0	0	21	32	0	0	0	0	0	0	0	0	18.9	2	*******	[hh:hk:kk]
62	pAA/mGAC-r236	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	16	34	0	0	3	6.5	1	**	[ll:lm]
63	pAA/mGAC-r149	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	33	17	0	0	3	5.1	1	**	[ll:lm]
64	pAA/mGAC-r139	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	38	12	0	0	3	13.5	1	******	[ll:lm]
65	pAA/mGAC-t234	<hkxhk>	0	0	0	0	0	0	0	0	22	28	0	0	0	0	0	0	0	3	20.1	2	*******	[hh:hk:kk]
66	pAA/mGAC-t210	<hkxhk>	0	0	0	0	0	0	0	0	19	31	0	0	0	0	0	0	0	3	17.3	2	******	[hh:hk:kk]
67	pAA/mGAG-r228	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	24	15	0	0	14	2.1	1	-	[ll:lm]
68	pAA/mGAG-r136	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	16	25	0	0	12	2	1	-	[ll:lm]
69	pAA/mGAG-t306	<hkxhk>	0	0	0	0	0	0	0	0	20	20	0	0	0	0	0	0	0	13	20	2	*******	[hh:hk:kk]
70	pAA/mGAG-t278	<hkxhk>	0	0	0	0	0	0	0	0	16	24	0	0	0	0	0	0	0	13	14.4	2	*****	[hh:hk:kk]
71	pAA/mGAG-t270	<hkxhk>	0	0	0	0	0	0	0	0	16	23	0	0	0	0	0	0	0	14	14.4	2	*****	[hh:hk:kk]
72	pAA/mGAG-t249	<hkxhk>	0	0	0	0	0	0	0	0	12	27	0	0	0	0	0	0	0	14	13.2	2	****	[hh:hk:kk]
73	pAA/mGAG-t155	<hkxhk>	0	0	0	0	0	0	0	0	14	27	0	0	0	0	0	0	0	12	13.7	2	****	[hh:hk:kk]
74	pAA/mGAG-t154	<hkxhk>	0	0	0	0	0	0	0	0	15	26	0	0	0	0	0	0	0	12	13.9	2	*****	[hh:hk:kk]
75	pAA/mGCA-r225	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	14	38	0	0	1	11.1	1	*****	[ll:lm]
76	pAA/mGCA-r172	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	23	30	0	0	0	0.9	1	-	[ll:lm]
77	pAA/mGCA-t372	<hkxhk>	0	0	0	0	0	0	0	0	23	29	0	0	0	0	0	0	0	1	21	2	*******	[hh:hk:kk]
78	pAA/mGCA-t340	<hkxhk>	0	0	0	0	0	0	0	0	15	37	0	0	0	0	0	0	0	1	18	2	******	[hh:hk:kk]
79	pAA/mGCA-t194	<hkxhk>	0	0	0	0	0	0	0	0	17	36	0	0	0	0	0	0	0	0	17.7	2	******	[hh:hk:kk]
80	pAA/mGCA-t192	<hkxhk>	0	0	0	0	0	0	0	0	17	36	0	0	0	0	0	0	0	0	17.7	2	******	[hh:hk:kk]
81	pAA/mGCA-t125	<hkxhk>	0	0	0	0	0	0	0	0	8	45	0	0	0	0	0	0	0	0	28.3	2	*******	[hh:hk:kk]
82	pAC/mACC-r435	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	27	19	0	0	7	1.4	1	-	[ll:lm]

83	pAC/mACC-r260	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	27	24	0	0	2	0.2	1	-	[ll:lm]
84	pAC/mACC-r179	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	34	17	0	0	2	5.7	1	**	[ll:lm]
85	pAC/mACC-r134	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	7	44	0	0	2	26.8	1	*******	[ll:lm]
86	pAC/mACC-t415	<hkxhk>	0	0	0	0	0	0	0	0	18	29	0	0	0	0	0	0	0	6	16.4	2	******	[hh:hk:kk]
87	pAC/mACC-t370	<hkxhk>	0	0	0	0	0	0	0	0	5	46	0	0	0	0	0	0	0	2	33.9	2	*******	[hh:hk:kk]
88	pAC/mACC-t268	<hkxhk>	0	0	0	0	0	0	0	0	19	33	0	0	0	0	0	0	0	1	17.6	2	******	[hh:hk:kk]
89	pAC/mACC-t156	<hkxhk>	0	0	0	0	0	0	0	0	19	32	0	0	0	0	0	0	0	2	17.5	2	******	[hh:hk:kk]
90	pAC/mACC-t120	<hkxhk>	0	0	0	0	0	0	0	0	18	33	0	0	0	0	0	0	0	2	17.1	2	******	[hh:hk:kk]
91	pAC/mACT-r228	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	24	24	0	0	5	0	1	-	[ll:lm]
92	pAC/mACT-r230	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	21	27	0	0	5	0.8	1	-	[ll:lm]
93	pAC/mACT-r223	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	19	29	0	0	5	2.1	1	-	[ll:lm]
94	pAC/mACT-r200	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	17	31	0	0	5	4.1	1	**	[ll:lm]
95	pAC/mACT-r106	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	27	21	0	0	5	0.8	1	-	[ll:lm]
96	pAC/mACT-r076	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	19	29	0	0	5	2.1	1	-	[ll:lm]
97	pAC/mACT-t272	<hkxhk>	0	0	0	0	0	0	0	0	13	35	0	0	0	0	0	0	0	5	17.1	2	******	[hh:hk:kk]
98	pAC/mACT-t105	<hkxhk>	0	0	0	0	0	0	0	0	12	36	0	0	0	0	0	0	0	5	18	2	******	[hh:hk:kk]
99	pAC/mACT-t092	<hkxhk>	0	0	0	0	0	0	0	0	15	33	0	0	0	0	0	0	0	5	16.1	2	******	[hh:hk:kk]
100	pAC/mACT-t078	<hkxhk>	0	0	0	0	0	0	0	0	16	32	0	0	0	0	0	0	0	5	16	2	******	[hh:hk:kk]
101	pAC/mAGA-r121	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	19	34	0	0	0	4.3	1	**	[ll:lm]
102	pAC/mAGA-t355	<hkxhk>	0	0	0	0	0	0	0	0	14	38	0	0	0	0	0	0	0	1	18.6	2	*******	[hh:hk:kk]
103	pAC/mAGA-t218	<hkxhk>	0	0	0	0	0	0	0	0	12	41	0	0	0	0	0	0	0	0	21.3	2	*******	[hh:hk:kk]
104	pAC/mAGA-t192	<hkxhk>	0	0	0	0	0	0	0	0	9	44	0	0	0	0	0	0	0	0	26.2	2	*******	[hh:hk:kk]
105	pAC/mAGA-t191	<hkxhk>	0	0	0	0	0	0	0	0	18	35	0	0	0	0	0	0	0	0	17.7	2	******	[hh:hk:kk]
106	pAC/mAGA-t148	<hkxhk>	0	0	0	0	0	0	0	0	24	29	0	0	0	0	0	0	0	0	22.2	2	*******	[hh:hk:kk]
107	pAC/mCAC-r240	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	27	20	0	0	6	1	1	-	[ll:lm]
108	pAC/mCAC-r090	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	24	23	0	0	6	0	1	-	[ll:lm]
109	pAC/mCAC-t384	<hkxhk>	0	0	0	0	0	0	0	0	21	22	0	0	0	0	0	0	0	10	20.5	2	*******	[hh:hk:kk]
110	pAC/mCAC-t283	<hkxhk>	0	0	0	0	0	0	0	0	18	25	0	0	0	0	0	0	0	10	16.2	2	******	[hh:hk:kk]
111	pAC/mCAC-t232	<hkxhk>	0	0	0	0	0	0	0	0	13	34	0	0	0	0	0	0	0	6	16.6	2	******	[hh:hk:kk]
112	pAC/mCAC-t183	<hkxhk>	0	0	0	0	0	0	0	0	26	21	0	0	0	0	0	0	0	6	29.3	2	*******	[hh:hk:kk]
113	pAC/mCCA-r200	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	35	18	0	0	0	5.5	1	**	[ll:lm]
114	pAC/mCCA-r189	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	23	30	0	0	0	0.9	1	-	[ll:lm]
115	pAC/mCCA-r166	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	24	29	0	0	0	0.5	1	-	[ll:lm]
116	pAC/mCCA-r089	<hkxhk>	0	0	0	0	0	0	0	0	16	37	0	0	0	0	0	0	0	0	18	2	******	[hh:hk:kk]
117	pAC/mCCA-t218	<hkxhk>	0	0	0	0	0	0	0	0	22	30	0	0	0	0	0	0	0	1	19.9	2	*******	[hh:hk:kk]
118	pAC/mCCA-t171	<hkxhk>	0	0	0	0	0	0	0	0	18	35	0	0	0	0	0	0	0	0	17.7	2	******	[hh:hk:kk]
119	pAC/mCCA-t088	<hkxhk>	0	0	0	0	0	0	0	0	12	41	0	0	0	0	0	0	0	0	21.3	2	*******	[hh:hk:kk]
120	pAC/mCCG-t290	<hkxhk>	0	0	0	0	0	0	0	0	14	39	0	0	0	0	0	0	0	0	19.2	2	*******	[hh:hk:kk]
121	pAC/mCCG-t085	<hkxhk>	0	0	0	0	0	0	0	0	16	37	0	0	0	0	0	0	0	0	18	2	******	[hh:hk:kk]
122	pAC/mCGG-r440	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	16	21	0	0	16	0.7	1	-	[ll:lm]
123	pAC/mCGG-r430	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	24	26	0	0	3	0.1	1	-	[ll:lm]
124	pAC/mCGG-r355	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	41	12	0	0	0	15.9	1	*******	[ll:lm]
125	pAC/mCGG-r218	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	26	27	0	0	0	0	1	-	[ll:lm]
126	pAC/mCGG-t145	<hkxhk>	0	0	0	0	0	0	0	0	23	30	0	0	0	0	0	0	0	0	20.9	2	*******	[hh:hk:kk]

127	pAC/mGCA-r250	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	22	27	0	0	4	0.5	1	-	[ll:lm]
128	pAC/mGCA-r083	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	15	38	0	0	0	10	1	****	[ll:lm]
129	pAC/mGCA-t088	<hkxhk>	0	0	0	0	0	0	0	0	17	36	0	0	0	0	0	0	0	0	17.7	2	******	[hh:hk:kk]
130	pAG/mAAC-r232	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	17	32	0	0	4	4.6	1	**	[ll:lm]
131	pAG/mAAC-r108	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	25	26	0	0	2	0	1	-	[ll:lm]
132	pAG/mAAC-t303	<hkxhk>	0	0	0	0	0	0	0	0	14	35	0	0	0	0	0	0	0	4	17	2	******	[hh:hk:kk]
133	pAG/mAAC-t250	<hkxhk>	0	0	0	0	0	0	0	0	13	36	0	0	0	0	0	0	0	4	17.7	2	******	[hh:hk:kk]
134	pAG/mAAC-t238	<hkxhk>	0	0	0	0	0	0	0	0	31	18	0	0	0	0	0	0	0	4	42.7	2	*******	[hh:hk:kk]
135	pAG/mAAC-t138	<hkxhk>	0	0	0	0	0	0	0	0	19	33	0	0	0	0	0	0	0	1	17.6	2	******	[hh:hk:kk]
136	pAG/mAAC-t127	<hkxhk>	0	0	0	0	0	0	0	0	4	47	0	0	0	0	0	0	0	2	36.9	2	*******	[hh:hk:kk]
137	pAG/mAAC-t089	<hkxhk>	0	0	0	0	0	0	0	0	11	41	0	0	0	0	0	0	0	1	22	2	*******	[hh:hk:kk]
138	pAG/mAAT-r410	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	23	23	0	0	7	0	1	-	[ll:lm]
139	pAG/mAAT-r288	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	23	27	0	0	3	0.3	1	-	[ll:lm]
140	pAG/mAAT-r165	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	25	27	0	0	1	0.1	1	-	[ll:lm]
141	pAG/mAAT-r125	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	23	28	0	0	2	0.5	1	-	[ll:lm]
142	pAG/mAAT-r123	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	22	28	0	0	3	0.7	1	-	[ll:lm]
143	pAG/mAAT-t358	<hkxhk>	0	0	0	0	0	0	0	0	16	31	0	0	0	0	0	0	0	6	15.7	2	******	[hh:hk:kk]
144	pAG/mAAT-t265	<hkxhk>	0	0	0	0	0	0	0	0	16	33	0	0	0	0	0	0	0	4	16.4	2	******	[hh:hk:kk]
145	pAG/mAAT-t254	<hkxhk>	0	0	0	0	0	0	0	0	10	39	0	0	0	0	0	0	0	4	21.2	2	*******	[hh:hk:kk]
146	pAG/mAAT-t226	<hkxhk>	0	0	0	0	0	0	0	0	22	27	0	0	0	0	0	0	0	4	20.3	2	*******	[hh:hk:kk]
147	pAG/mAAT-t153	<hkxhk>	0	0	0	0	0	0	0	0	16	35	0	0	0	0	0	0	0	2	17.1	2	******	[hh:hk:kk]
148	pAG/mACC-r196	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	24	29	0	0	0	0.5	1	-	[ll:lm]
149	pAG/mACC-t192	<hkxhk>	0	0	0	0	0	0	0	0	20	33	0	0	0	0	0	0	0	0	18.3	2	******	[hh:hk:kk]
150	pAG/mACC-t174	<hkxhk>	0	0	0	0	0	0	0	0	21	32	0	0	0	0	0	0	0	0	18.9	2	*******	[hh:hk:kk]
151	pAG/mACC-t144	<hkxhk>	0	0	0	0	0	0	0	0	10	43	0	0	0	0	0	0	0	0	24.3	2	*******	[hh:hk:kk]
152	pAG/mACC-t101	<hkxhk>	0	0	0	0	0	0	0	0	20	33	0	0	0	0	0	0	0	0	18.3	2	******	[hh:hk:kk]
153	pAG/mACC-t091	<hkxhk>	0	0	0	0	0	0	0	0	18	35	0	0	0	0	0	0	0	0	17.7	2	******	[hh:hk:kk]
154	pAG/mACC-t088	<hkxhk>	0	0	0	0	0	0	0	0	12	41	0	0	0	0	0	0	0	0	21.3	2	*******	[hh:hk:kk]
155	pAG/mAGA-r445	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	20	21	0	0	12	0	1	-	[ll:lm]
156	pAG/mAGA-r223	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	24	27	0	0	2	0.2	1	-	[ll:lm]
157	pAG/mAGA-r200	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	20	31	0	0	2	2.4	1	-	[ll:lm]
158	pAG/mAGA-r128	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	23	29	0	0	1	0.7	1	-	[ll:lm]
159	pAG/mAGA-t358	<hkxhk>	0	0	0	0	0	0	0	0	16	25	0	0	0	0	0	0	0	12	14.5	2	*****	[hh:hk:kk]
160	pAG/mAGA-t147	<hkxhk>	0	0	0	0	0	0	0	0	19	33	0	0	0	0	0	0	0	1	17.6	2	******	[hh:hk:kk]
161	pAG/mAGA-t087	<hkxhk>	0	0	0	0	0	0	0	0	9	43	0	0	0	0	0	0	0	1	25.4	2	*******	[hh:hk:kk]
162	pAG/mAGA-t086	<hkxhk>	0	0	0	0	0	0	0	0	13	39	0	0	0	0	0	0	0	1	19.5	2	*******	[hh:hk:kk]
163	pAG/mCGC-r148	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	16	29	0	0	8	3.8	1	*	[ll:lm]
164	pAG/mCGC-t314	<hkxhk>	0	0	0	0	0	0	0	0	17	21	0	0	0	0	0	0	0	15	15.6	2	******	[hh:hk:kk]
165	pAG/mCGC-t312	<hkxhk>	0	0	0	0	0	0	0	0	21	17	0	0	0	0	0	0	0	15	23.6	2	*******	[hh:hk:kk]
166	pAG/mCGC-t115	<hkxhk>	0	0	0	0	0	0	0	0	9	36	0	0	0	0	0	0	0	8	19.8	2	*******	[hh:hk:kk]
167	pAG/mCGC-t089	<hkxhk>	0	0	0	0	0	0	0	0	8	37	0	0	0	0	0	0	0	8	21.5	2	*******	[hh:hk:kk]
168	pAG/mCGC-t088	<hkxhk>	0	0	0	0	0	0	0	0	16	29	0	0	0	0	0	0	0	8	15.1	2	*****	[hh:hk:kk]
169	pAG/mGCA-r362	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	14	37	0	0	2	10.4	1	****	[ll:lm]
170	pAG/mGCA-r290	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	32	19	0	0	2	3.3	1	*	[ll:lm]

171	pAG/mGCA-r260	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	15	37	0	0	1	9.3	1	****	[ll:lm]
172	pAG/mGCA-r255	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	26	26	0	0	1	0	1	-	[ll:lm]
173	pAG/mGCA-r252	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	14	38	0	0	1	11.1	1	*****	[ll:lm]
174	pAG/mGCA-r113	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	22	31	0	0	0	1.5	1	-	[ll:lm]
175	pAG/mGCA-r094	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	26	27	0	0	0	0	1	-	[ll:lm]
176	pAG/mGCA-t294	<hkxhk>	0	0	0	0	0	0	0	0	22	29	0	0	0	0	0	0	0	2	19.9	2	*******	[hh:hk:kk]
177	pAG/mGCA-t292	<hkxhk>	0	0	0	0	0	0	0	0	11	40	0	0	0	0	0	0	0	2	21.2	2	*******	[hh:hk:kk]
178	pAG/mGCA-t164	<hkxhk>	0	0	0	0	0	0	0	0	12	41	0	0	0	0	0	0	0	0	21.3	2	*******	[hh:hk:kk]
179	pAG/mGCA-t091	<hkxhk>	0	0	0	0	0	0	0	0	12	41	0	0	0	0	0	0	0	0	21.3	2	*******	[hh:hk:kk]
180	pAT/mAAC-r271	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	28	19	0	0	6	1.7	1	-	[ll:lm]
181	pAT/mAAC-r147	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	27	24	0	0	2	0.2	1	-	[ll:lm]
182	pAT/mAAC-r138	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	19	32	0	0	2	3.3	1	*	[ll:lm]
183	pAT/mAAC-r103	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	24	28	0	0	1	0.3	1	-	[ll:lm]
184	pAT/mAAC-t128	<hkxhk>	0	0	0	0	0	0	0	0	16	35	0	0	0	0	0	0	0	2	17.1	2	******	[hh:hk:kk]
185	pAT/mAAC-t099	<hkxhk>	0	0	0	0	0	0	0	0	19	33	0	0	0	0	0	0	0	1	17.6	2	******	[hh:hk:kk]
186	pAT/mAAC-t083	<hkxhk>	0	0	0	0	0	0	0	0	19	33	0	0	0	0	0	0	0	1	17.6	2	******	[hh:hk:kk]
187	pAT/mAAC-t075	<hkxhk>	0	0	0	0	0	0	0	0	6	47	0	0	0	0	0	0	0	0	33.1	2	*******	[hh:hk:kk]
188	pAT/mAAT-r210	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	26	27	0	0	0	0	1	-	[ll:lm]
189	pAT/mAAT-r098	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	19	34	0	0	0	4.3	1	**	[ll:lm]
190	pAT/mAAT-t187	<hkxhk>	0	0	0	0	0	0	0	0	20	33	0	0	0	0	0	0	0	0	18.3	2	******	[hh:hk:kk]
191	pAT/mAAT-t131	<hkxhk>	0	0	0	0	0	0	0	0	7	46	0	0	0	0	0	0	0	0	30.6	2	*******	[hh:hk:kk]
192	pAT/mACT-r232	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	31	18	0	0	4	3.5	1	*	[ll:lm]
193	pAT/mACT-r144	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	27	24	0	0	2	0.2	1	-	[ll:lm]
194	pAT/mACT-r096	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	14	37	0	0	2	10.4	1	****	[ll:lm]
195	pAT/mACT-t208	<hkxhk>	0	0	0	0	0	0	0	0	18	31	0	0	0	0	0	0	0	4	16.7	2	******	[hh:hk:kk]
196	pAT/mACT-t205	<hkxhk>	0	0	0	0	0	0	0	0	35	14	0	0	0	0	0	0	0	4	59	2	*******	[hh:hk:kk]
197	pAT/mACT-t102	<hkxhk>	0	0	0	0	0	0	0	0	29	22	0	0	0	0	0	0	0	2	33.9	2	*******	[hh:hk:kk]
198	pAT/mAGG-r435	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	28	25	0	0	0	0.2	1	-	[ll:lm]
199	pAT/mAGG-r200	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	16	37	0	0	0	8.3	1	****	[ll:lm]
200	pAT/mAGG-r130	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	30	23	0	0	0	0.9	1	-	[ll:lm]
201	pAT/mAGG-r097	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	25	28	0	0	0	0.2	1	-	[ll:lm]
202	pAT/mAGG-435	<hkxhk>	0	0	0	0	0	0	0	0	21	32	0	0	0	0	0	0	0	0	18.9	2	*******	[hh:hk:kk]
203	pAT/mAGG-t370	<hkxhk>	0	0	0	0	0	0	0	0	22	31	0	0	0	0	0	0	0	0	19.8	2	*******	[hh:hk:kk]
204	pAT/mAGG-t195	<hkxhk>	0	0	0	0	0	0	0	0	10	43	0	0	0	0	0	0	0	0	24.3	2	*******	[hh:hk:kk]
205	pAT/mAGG-t168	<hkxhk>	0	0	0	0	0	0	0	0	20	33	0	0	0	0	0	0	0	0	18.3	2	******	[hh:hk:kk]
206	pAT/mAGG-t145	<hkxhk>	0	0	0	0	0	0	0	0	22	31	0	0	0	0	0	0	0	0	19.8	2	*******	[hh:hk:kk]
207	pCA/mACT-r400	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	16	22	0	0	15	0.9	1	-	[ll:lm]
208	pCA/mACT-r238	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	20	19	0	0	14	0	1	-	[ll:lm]
209	pCA/mACT-r226	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	24	17	0	0	12	1.2	1	-	[ll:lm]
210	pCA/mAGA-r01	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	32	20	0	0	1	2.8	1	*	[ll:lm]
211	pCA/mAGA-t01	<hkxhk>	0	0	0	0	0	0	0	0	9	43	0	0	0	0	0	0	0	1	25.4	2	*******	[hh:hk:kk]
212	pCA/mAGA-t02	<hkxhk>	0	0	0	0	0	0	0	0	12	40	0	0	0	0	0	0	0	1	20.6	2	*******	[hh:hk:kk]
213	pCA/mAGA-t03	<hkxhk>	0	0	0	0	0	0	0	0	13	39	0	0	0	0	0	0	0	1	19.5	2	*******	[hh:hk:kk]
214	pCA/mCAC-r124	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	26	21	0	0	6	0.5	1	-	[ll:lm]

215	pCA/mCAC-r111	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	25	22	0	0	6	0.2	1	-	[ll:lm]
216	pCA/mCAC-t148	<hkxhk>	0	0	0	0	0	0	0	0	14	33	0	0	0	0	0	0	0	6	16	2	******	[hh:hk:kk]
217	pCA/mCAG-r214	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	12	27	0	0	14	5.8	1	**	[ll:lm]
218	pCA/mCAG-r188	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	12	27	0	0	14	5.8	1	**	[ll:lm]
219	pCA/mCAG-r184	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	23	16	0	0	14	1.3	1	-	[ll:lm]
220	pCA/mCAG-t208	<hkxhk>	0	0	0	0	0	0	0	0	3	36	0	0	0	0	0	0	0	14	28.4	2	*******	[hh:hk:kk]
221	pCA/mCAG-t180	<hkxhk>	0	0	0	0	0	0	0	0	15	24	0	0	0	0	0	0	0	14	13.6	2	****	[hh:hk:kk]
222	pCA/mCAG-t126	<hkxhk>	0	0	0	0	0	0	0	0	9	31	0	0	0	0	0	0	0	13	16.1	2	******	[hh:hk:kk]
223	pCA/mCAG-t091	<hkxhk>	0	0	0	0	0	0	0	0	7	33	0	0	0	0	0	0	0	13	19.4	2	*******	[hh:hk:kk]
224	pCA/mCCC-r125	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	12	40	0	0	1	15.1	1	******	[ll:lm]
225	pCA/mCCC-t300	<hkxhk>	0	0	0	0	0	0	0	0	8	40	0	0	0	0	0	0	0	5	24	2	*******	[hh:hk:kk]
226	pCA/mCCC-t200	<hkxhk>	0	0	0	0	0	0	0	0	15	36	0	0	0	0	0	0	0	2	17.5	2	******	[hh:hk:kk]
227	pCA/mCCC-t141	<hkxhk>	0	0	0	0	0	0	0	0	15	36	0	0	0	0	0	0	0	2	17.5	2	******	[hh:hk:kk]
228	pCA/mCCT-r220	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	20	32	0	0	1	2.8	1	*	[ll:lm]
229	pCA/mCCT-r212	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	29	23	0	0	1	0.7	1	-	[ll:lm]
230	pCA/mCCT-r205	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	24	28	0	0	1	0.3	1	-	[ll:lm]
231	pCA/mCCT-r153	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	30	22	0	0	1	1.2	1	-	[ll:lm]
232	pCA/mCCT-r135	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	14	38	0	0	1	11.1	1	*****	[ll:lm]
233	pCA/mCCT-t141	<hkxhk>	0	0	0	0	0	0	0	0	5	47	0	0	0	0	0	0	0	1	34.9	2	*******	[hh:hk:kk]
234	pCA/mCGT-r530	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	23	18	0	0	12	0.6	1	-	[ll:lm]
235	pCA/mCGT-r445	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	24	17	0	0	12	1.2	1	-	[ll:lm]
236	pCA/mCGT-r248	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	5	36	0	0	12	23.4	1	*******	[ll:lm]
237	pCA/mCGT-r231	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	20	21	0	0	12	0	1	-	[ll:lm]
238	pCA/mCGT-t338	<hkxhk>	0	0	0	0	0	0	0	0	18	23	0	0	0	0	0	0	0	12	16.4	2	******	[hh:hk:kk]
239	pCA/mCTA-r01	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	16	36	0	0	1	7.7	1	***	[ll:lm]
240	pCA/mCTA-r02	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	31	21	0	0	1	1.9	1	-	[ll:lm]
241	pCA/mCTA-r03	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	24	28	0	0	1	0.3	1	-	[ll:lm]
242	pCA/mCTA-t01	<hkxhk>	0	0	0	0	0	0	0	0	12	40	0	0	0	0	0	0	0	1	20.6	2	*******	[hh:hk:kk]
243	pCA/mCTA-t02	<hkxhk>	0	0	0	0	0	0	0	0	13	39	0	0	0	0	0	0	0	1	19.5	2	*******	[hh:hk:kk]
244	pCA/mCTA-t03	<hkxhk>	0	0	0	0	0	0	0	0	12	40	0	0	0	0	0	0	0	1	20.6	2	*******	[hh:hk:kk]
245	pCA/mCTA-t04	<hkxhk>	0	0	0	0	0	0	0	0	9	43	0	0	0	0	0	0	0	1	25.4	2	*******	[hh:hk:kk]
246	pCA/mGAC-r366	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	21	29	0	0	3	1.3	1	-	[ll:lm]
247	pCA/mGAC-r148	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	27	23	0	0	3	0.3	1	-	[ll:lm]
248	pCA/mGAC-t340	<hkxhk>	0	0	0	0	0	0	0	0	15	35	0	0	0	0	0	0	0	3	17	2	******	[hh:hk:kk]
249	pCA/mGAC-t232	<hkxhk>	0	0	0	0	0	0	0	0	11	39	0	0	0	0	0	0	0	3	20.5	2	*******	[hh:hk:kk]
250	eAAC/mCA-r358	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	22	19	0	0	12	0.2	1	-	[ll:lm]
251	eAAC/mCA-r313	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	19	22	0	0	12	0.2	1	-	[ll:lm]
252	eAAC/mCA-r298	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	27	14	0	0	12	4.1	1	**	[ll:lm]
253	eAAC/mCA-r289	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	25	16	0	0	12	2	1	-	[ll:lm]
254	eAAC/mCA-r202	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	20	21	0	0	12	0	1	-	[ll:lm]
255	eAAC/mCA-r190	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	19	22	0	0	12	0.2	1	-	[ll:lm]
256	eAAC/mCA-r181	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	17	24	0	0	12	1.2	1	-	[ll:lm]
257	eAAC/mCA-r147	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	21	20	0	0	12	0	1	-	[ll:lm]
258	eAAC/mCA-t540	<hkxhk>	0	0	0	0	0	0	0	0	16	25	0	0	0	0	0	0	0	12	14.5	2	*****	[hh:hk:kk]

259	eAAC/mCA-t271	<hkxhk>	0	0	0	0	0	0	0	0	15	26	0	0	0	0	0	0	0	12	13.9	2	*****	[hh:hk:kk]
260	eAAC/mCA-t218	<hkxhk>	0	0	0	0	0	0	0	0	17	24	0	0	0	0	0	0	0	12	15.3	2	******	[hh:hk:kk]
261	eAAC/mCA-t197	<hkxhk>	0	0	0	0	0	0	0	0	9	32	0	0	0	0	0	0	0	12	16.9	2	******	[hh:hk:kk]
262	eAAC/mCA-t182	<hkxhk>	0	0	0	0	0	0	0	0	16	25	0	0	0	0	0	0	0	12	14.5	2	*****	[hh:hk:kk]
263	eAAC/mCA-t174	<hkxhk>	0	0	0	0	0	0	0	0	9	32	0	0	0	0	0	0	0	12	16.9	2	******	[hh:hk:kk]
264	eAAC/mCA-t158	<hkxhk>	0	0	0	0	0	0	0	0	8	33	0	0	0	0	0	0	0	12	18.4	2	******	[hh:hk:kk]
265	eAAC/mCA-t156	<hkxhk>	0	0	0	0	0	0	0	0	4	37	0	0	0	0	0	0	0	12	27.3	2	*******	[hh:hk:kk]
266	eAAC/mCA-t140	<hkxhk>	0	0	0	0	0	0	0	0	14	27	0	0	0	0	0	0	0	12	13.7	2	****	[hh:hk:kk]
267	eAAC/mCA-t122	<hkxhk>	0	0	0	0	0	0	0	0	7	34	0	0	0	0	0	0	0	12	20.2	2	*******	[hh:hk:kk]
268	eAAC/mCA-t121	<hkxhk>	0	0	0	0	0	0	0	0	6	35	0	0	0	0	0	0	0	12	22.3	2	*******	[hh:hk:kk]
269	eAAC/mCA-t098	<hkxhk>	0	0	0	0	0	0	0	0	14	27	0	0	0	0	0	0	0	12	13.7	2	****	[hh:hk:kk]
270	eAAC/mAAC-r01	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	38	13	0	0	2	12.3	1	******	[ll:lm]
271	eAAC/mAAC-r02	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	22	29	0	0	2	1	1	-	[ll:lm]
272	eAAC/mAAC-r03	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	7	45	0	0	1	27.8	1	*******	[ll:lm]
273	eAAC/mAAC-t01	<hkxhk>	0	0	0	0	0	0	0	0	10	37	0	0	0	0	0	0	0	6	19.8	2	*******	[hh:hk:kk]
274	eAAC/mAAC-t02	<hkxhk>	0	0	0	0	0	0	0	0	13	39	0	0	0	0	0	0	0	1	19.5	2	*******	[hh:hk:kk]
275	eAAC/mAAC-t03	<hkxhk>	0	0	0	0	0	0	0	0	17	35	0	0	0	0	0	0	0	1	17.4	2	******	[hh:hk:kk]
276	eAAC/mAAC-t04	<hkxhk>	0	0	0	0	0	0	0	0	9	43	0	0	0	0	0	0	0	1	25.4	2	*******	[hh:hk:kk]
277	eAAC/mAAC-t05	<hkxhk>	0	0	0	0	0	0	0	0	11	41	0	0	0	0	0	0	0	1	22	2	*******	[hh:hk:kk]
278	eAAC/mAAC-t06	<hkxhk>	0	0	0	0	0	0	0	0	17	35	0	0	0	0	0	0	0	1	17.4	2	******	[hh:hk:kk]
279	eAAC/mAAC-t07	<hkxhk>	0	0	0	0	0	0	0	0	10	42	0	0	0	0	0	0	0	1	23.5	2	*******	[hh:hk:kk]
280	eAAC/mAAC-t08	<hkxhk>	0	0	0	0	0	0	0	0	23	29	0	0	0	0	0	0	0	1	21	2	*******	[hh:hk:kk]
281	eAAC/mACC-r475	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	24	17	0	0	12	1.2	1	-	[ll:lm]
282	eAAC/mACC-r365	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	22	19	0	0	12	0.2	1	-	[ll:lm]
283	eAAC/mACC-r285	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	24	17	0	0	12	1.2	1	-	[ll:lm]
284	eAAC/mACC-r246	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	21	20	0	0	12	0	1	-	[ll:lm]
285	eAAC/mACC-r230	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	26	15	0	0	12	3	1	*	[ll:lm]
286	eAAC/mACC-r202	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	7	34	0	0	12	17.8	1	*******	[ll:lm]
287	eAAC/mACC-r157	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	26	15	0	0	12	3	1	*	[ll:lm]
288	eAAC/mACC-t450	<hkxhk>	0	0	0	0	0	0	0	0	14	27	0	0	0	0	0	0	0	12	13.7	2	****	[hh:hk:kk]
289	eAAC/mACC-t310	<hkxhk>	0	0	0	0	0	0	0	0	10	31	0	0	0	0	0	0	0	12	15.6	2	******	[hh:hk:kk]
290	eAAC/mACC-t265	<hkxhk>	0	0	0	0	0	0	0	0	16	25	0	0	0	0	0	0	0	12	14.5	2	*****	[hh:hk:kk]
291	eAAC/mACC-t203	<hkxhk>	0	0	0	0	0	0	0	0	8	33	0	0	0	0	0	0	0	12	18.4	2	******	[hh:hk:kk]
292	eAAC/mACC-t128	<hkxhk>	0	0	0	0	0	0	0	0	12	29	0	0	0	0	0	0	0	12	14.1	2	*****	[hh:hk:kk]
293	eAAC/mACT-r580	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	37	12	0	0	4	12.8	1	******	[ll:lm]
294	eAAC/mACT-r438	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	33	16	0	0	4	5.9	1	**	[ll:lm]
295	eAAC/mACT-r232	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	31	22	0	0	0	1.5	1	-	[ll:lm]
296	eAAC/mACT-r152	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	35	18	0	0	0	5.5	1	**	[ll:lm]
297	eAAC/mACT-t384	<hkxhk>	0	0	0	0	0	0	0	0	21	28	0	0	0	0	0	0	0	4	19	2	*******	[hh:hk:kk]
298	eAAC/mACT-t240	<hkxhk>	0	0	0	0	0	0	0	0	24	29	0	0	0	0	0	0	0	0	22.2	2	*******	[hh:hk:kk]
299	eAAC/mACT-t215	<hkxhk>	0	0	0	0	0	0	0	0	23	30	0	0	0	0	0	0	0	0	20.9	2	*******	[hh:hk:kk]
300	eAAC/mACT-t103	<hkxhk>	0	0	0	0	0	0	0	0	15	38	0	0	0	0	0	0	0	0	18.5	2	*******	[hh:hk:kk]
301	eAAC/mAGT-r315	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	29	22	0	0	2	1	1	-	[ll:lm]
302	eAAC/mAGT-r200	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	25	26	0	0	2	0	1	-	[ll:lm]

303	eAAC/mAGT-r178	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	18	33	0	0	2	4.4	1	**	[ll:lm]
304	eAAC/mAGT-r159	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	33	18	0	0	2	4.4	1	**	[ll:lm]
305	eAAC/mAGT-t455	<hkxhk>	0	0	0	0	0	0	0	0	23	27	0	0	0	0	0	0	0	3	21.5	2	*******	[hh:hk:kk]
306	eAAC/mAGT-t280	<hkxhk>	0	0	0	0	0	0	0	0	13	38	0	0	0	0	0	0	0	2	18.9	2	*******	[hh:hk:kk]
307	eAAC/mAGT-t254	<hkxhk>	0	0	0	0	0	0	0	0	15	36	0	0	0	0	0	0	0	2	17.5	2	******	[hh:hk:kk]
308	eAAC/mAGT-t245	<hkxhk>	0	0	0	0	0	0	0	0	14	38	0	0	0	0	0	0	0	1	18.6	2	*******	[hh:hk:kk]
309	eAAC/mAGT-t201	<hkxhk>	0	0	0	0	0	0	0	0	21	31	0	0	0	0	0	0	0	1	18.9	2	*******	[hh:hk:kk]
310	eAAC/mAGT-t176	<hkxhk>	0	0	0	0	0	0	0	0	15	36	0	0	0	0	0	0	0	2	17.5	2	******	[hh:hk:kk]
311	eAAC/mAGT-t149	<hkxhk>	0	0	0	0	0	0	0	0	12	39	0	0	0	0	0	0	0	2	19.9	2	*******	[hh:hk:kk]
312	eAAC/mAGT-t133	<hkxhk>	0	0	0	0	0	0	0	0	9	42	0	0	0	0	0	0	0	2	24.5	2	*******	[hh:hk:kk]
313	eAAC/mAGT-t128	<hkxhk>	0	0	0	0	0	0	0	0	9	44	0	0	0	0	0	0	0	0	26.2	2	*******	[hh:hk:kk]
314	eAAC/mAGT-t125	<hkxhk>	0	0	0	0	0	0	0	0	10	42	0	0	0	0	0	0	0	1	23.5	2	*******	[hh:hk:kk]
315	eAAC/mATG-r01	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	18	35	0	0	0	5.5	1	**	[ll:lm]
316	eAAC/mATG-r02	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	27	26	0	0	0	0	1	-	[ll:lm]
317	eAAC/mATG-r03	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	28	24	0	0	1	0.3	1	-	[ll:lm]
318	eAAC/mATG-r04	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	21	32	0	0	0	2.3	1	-	[ll:lm]
319	eAAC/mATG-r05	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	33	20	0	0	0	3.2	1	*	[ll:lm]
320	eAAC/mATG-t01	<hkxhk>	0	0	0	0	0	0	0	0	12	41	0	0	0	0	0	0	0	0	21.3	2	*******	[hh:hk:kk]
321	eAAC/mATG-t02	<hkxhk>	0	0	0	0	0	0	0	0	11	42	0	0	0	0	0	0	0	0	22.7	2	*******	[hh:hk:kk]
322	eAAC/mATG-t03	<hkxhk>	0	0	0	0	0	0	0	0	8	45	0	0	0	0	0	0	0	0	28.3	2	*******	[hh:hk:kk]
323	eAAC/mATG-t04	<hkxhk>	0	0	0	0	0	0	0	0	15	37	0	0	0	0	0	0	0	1	18	2	******	[hh:hk:kk]
324	eAAC/mATG-t05	<hkxhk>	0	0	0	0	0	0	0	0	6	47	0	0	0	0	0	0	0	0	33.1	2	*******	[hh:hk:kk]
325	eAAC/mATG-t06	<hkxhk>	0	0	0	0	0	0	0	0	10	43	0	0	0	0	0	0	0	0	24.3	2	*******	[hh:hk:kk]
326	eAAC/mCAG-r316	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	20	33	0	0	0	3.2	1	*	[ll:lm]
327	eAAC/mCAG-r298	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	33	20	0	0	0	3.2	1	*	[ll:lm]
328	eAAC/mCAG-r171	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	19	34	0	0	0	4.3	1	**	[ll:lm]
329	eAAC/mCAG-r146	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	26	27	0	0	0	0	1	-	[ll:lm]
330	eAAC/mCAG-r120	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	22	31	0	0	0	1.5	1	-	[ll:lm]
331	eAAC/mCAG-t224	<hkxhk>	0	0	0	0	0	0	0	0	11	42	0	0	0	0	0	0	0	0	22.7	2	*******	[hh:hk:kk]
332	eAAC/mCAG-t196	<hkxhk>	0	0	0	0	0	0	0	0	8	45	0	0	0	0	0	0	0	0	28.3	2	*******	[hh:hk:kk]
333	eAAC/mCAG-t149	<hkxhk>	0	0	0	0	0	0	0	0	8	45	0	0	0	0	0	0	0	0	28.3	2	*******	[hh:hk:kk]
334	eAAC/mCAG-t123	<hkxhk>	0	0	0	0	0	0	0	0	15	38	0	0	0	0	0	0	0	0	18.5	2	*******	[hh:hk:kk]
335	eAAC/mCAG-t107	<hkxhk>	0	0	0	0	0	0	0	0	15	38	0	0	0	0	0	0	0	0	18.5	2	*******	[hh:hk:kk]
336	eAAC/mGAA-r280	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	9	34	0	0	10	14.5	1	******	[ll:lm]
337	eAAC/mGAA-r152	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	18	21	0	0	14	0.2	1	-	[ll:lm]
338	eAAC/mGAA-r106	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	26	18	0	0	9	1.4	1	-	[ll:lm]
339	eAAC/mGAA-r078	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	5	42	0	0	6	29.1	1	*******	[ll:lm]
340	eAAC/mGAA-t495	<hkxhk>	0	0	0	0	0	0	0	0	13	30	0	0	0	0	0	0	0	10	14.6	2	*****	[hh:hk:kk]
341	eAAC/mGAA-t240	<hkxhk>	0	0	0	0	0	0	0	0	9	34	0	0	0	0	0	0	0	10	18.3	2	******	[hh:hk:kk]
342	eAAC/mGAA-t238	<hkxhk>	0	0	0	0	0	0	0	0	13	30	0	0	0	0	0	0	0	10	14.6	2	*****	[hh:hk:kk]
343	eAAC/mGAA-t228	<hkxhk>	0	0	0	0	0	0	0	0	17	26	0	0	0	0	0	0	0	10	15.3	2	******	[hh:hk:kk]
344	eAAC/mGAA-t226	<hkxhk>	0	0	0	0	0	0	0	0	21	22	0	0	0	0	0	0	0	10	20.5	2	*******	[hh:hk:kk]
345	eAAC/mGAA-t224	<hkxhk>	0	0	0	0	0	0	0	0	10	33	0	0	0	0	0	0	0	10	16.9	2	******	[hh:hk:kk]
346	eAAC/mGAA-t200	<hkxhk>	0	0	0	0	0	0	0	0	5	38	0	0	0	0	0	0	0	10	26.5	2	*******	[hh:hk:kk]

347	eAAC/mGAA-t140	<hkxhk>	0	0	0	0	0	0	0	0	12	27	0	0	0	0	0	0	0	14	13.2	2	****	[hh:hk:kk]
348	eAAC/mGAA-t113	<hkxhk>	0	0	0	0	0	0	0	0	17	26	0	0	0	0	0	0	0	10	15.3	2	******	[hh:hk:kk]
349	eAAC/mGAA-t082	<hkxhk>	0	0	0	0	0	0	0	0	6	38	0	0	0	0	0	0	0	9	24.9	2	*******	[hh:hk:kk]
350	eAAC/mGAT-r520	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	22	25	0	0	6	0.2	1	-	[ll:lm]
351	eAAC/mGAT-t468	<hkxhk>	0	0	0	0	0	0	0	0	15	29	0	0	0	0	0	0	0	9	14.7	2	*****	[hh:hk:kk]
352	eAAC/mGAT-t320	<hkxhk>	0	0	0	0	0	0	0	0	10	39	0	0	0	0	0	0	0	4	21.2	2	*******	[hh:hk:kk]
353	eAAC/mGAT-t183	<hkxhk>	0	0	0	0	0	0	0	0	7	46	0	0	0	0	0	0	0	0	30.6	2	*******	[hh:hk:kk]
354	eAAC/mGAT-t163	<hkxhk>	0	0	0	0	0	0	0	0	28	25	0	0	0	0	0	0	0	0	29.8	2	*******	[hh:hk:kk]
355	eAAC/mGAT-t159	<hkxhk>	0	0	0	0	0	0	0	0	26	27	0	0	0	0	0	0	0	0	25.5	2	*******	[hh:hk:kk]
356	eAAC/mGAT-t132	<hkxhk>	0	0	0	0	0	0	0	0	4	49	0	0	0	0	0	0	0	0	38.8	2	*******	[hh:hk:kk]
357	eACA/mAAT-r279	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	14	19	0	0	20	0.8	1	-	[ll:lm]
358	eACA/mAAT-r153	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	17	16	0	0	20	0	1	-	[ll:lm]
359	eACA/mAAT-t273	<hkxhk>	0	0	0	0	0	0	0	0	20	13	0	0	0	0	0	0	0	20	25.7	2	*******	[hh:hk:kk]
360	eACA/mAAT-t252	<hkxhk>	0	0	0	0	0	0	0	0	15	18	0	0	0	0	0	0	0	20	13.9	2	*****	[hh:hk:kk]
361	eACA/mAAT-t203	<hkxhk>	0	0	0	0	0	0	0	0	19	14	0	0	0	0	0	0	0	20	22.6	2	*******	[hh:hk:kk]
362	eACA/mACG-r530	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	26	26	0	0	1	0	1	-	[ll:lm]
363	eACA/mACG-r350	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	17	36	0	0	0	6.8	1	***	[ll:lm]
364	eACA/mACG-r303	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	32	21	0	0	0	2.3	1	-	[ll:lm]
365	eACA/mACG-r232	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	17	35	0	0	1	6.2	1	**	[ll:lm]
366	eACA/mACG-r138	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	20	33	0	0	0	3.2	1	*	[ll:lm]
367	eACA/mACG-r112	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	25	28	0	0	0	0.2	1	-	[ll:lm]
368	eACA/mACG-t249	<hkxhk>	0	0	0	0	0	0	0	0	26	27	0	0	0	0	0	0	0	0	25.5	2	*******	[hh:hk:kk]
369	eACA/mACG-t248	<hkxhk>	0	0	0	0	0	0	0	0	10	42	0	0	0	0	0	0	0	1	23.5	2	*******	[hh:hk:kk]
370	eACA/mATT-r01	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	4	48	0	0	1	37.2	1	*******	[ll:lm]
371	eACA/mATT-t01	<hkxhk>	0	0	0	0	0	0	0	0	28	23	0	0	0	0	0	0	0	2	31.2	2	*******	[hh:hk:kk]
372	eACA/mATT-t02	<hkxhk>	0	0	0	0	0	0	0	0	27	25	0	0	0	0	0	0	0	1	28.1	2	*******	[hh:hk:kk]
373	eACA/mATT-t03	<hkxhk>	0	0	0	0	0	0	0	0	29	23	0	0	0	0	0	0	0	1	33	2	*******	[hh:hk:kk]
374	eACA/mATT-t04	<hkxhk>	0	0	0	0	0	0	0	0	18	34	0	0	0	0	0	0	0	1	17.4	2	******	[hh:hk:kk]
375	eACA/mATT-t05	<hkxhk>	0	0	0	0	0	0	0	0	15	37	0	0	0	0	0	0	0	1	18	2	******	[hh:hk:kk]
376	eACA/mATT-t06	<hkxhk>	0	0	0	0	0	0	0	0	15	38	0	0	0	0	0	0	0	0	18.5	2	*******	[hh:hk:kk]
377	eACA/mATT-t07	<hkxhk>	0	0	0	0	0	0	0	0	11	42	0	0	0	0	0	0	0	0	22.7	2	*******	[hh:hk:kk]
378	eACA/mATT-t08	<hkxhk>	0	0	0	0	0	0	0	0	11	42	0	0	0	0	0	0	0	0	22.7	2	*******	[hh:hk:kk]
379	eACA/mATT-t09	<hkxhk>	0	0	0	0	0	0	0	0	12	41	0	0	0	0	0	0	0	0	21.3	2	*******	[hh:hk:kk]
380	eACA/mATT-t10	<hkxhk>	0	0	0	0	0	0	0	0	8	45	0	0	0	0	0	0	0	0	28.3	2	*******	[hh:hk:kk]
381	eACA/mCAG-r370	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	25	23	0	0	5	0.1	1	-	[ll:lm]
382	eACA/mCAG-t460	<hkxhk>	0	0	0	0	0	0	0	0	20	25	0	0	0	0	0	0	0	8	18.3	2	******	[hh:hk:kk]
383	eACA/mCAG-t264	<hkxhk>	0	0	0	0	0	0	0	0	14	35	0	0	0	0	0	0	0	4	17	2	******	[hh:hk:kk]
384	eACA/mCAG-t198	<hkxhk>	0	0	0	0	0	0	0	0	18	32	0	0	0	0	0	0	0	3	16.9	2	******	[hh:hk:kk]
385	eACA/mCAG-t137	<hkxhk>	0	0	0	0	0	0	0	0	8	42	0	0	0	0	0	0	0	3	25.7	2	*******	[hh:hk:kk]
386	eACA/mCAG-t112	<hkxhk>	0	0	0	0	0	0	0	0	17	33	0	0	0	0	0	0	0	3	16.7	2	******	[hh:hk:kk]
387	eACA/mCTG-r01	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	12	40	0	0	1	15.1	1	******	[ll:lm]
388	eACA/mCTG-r02	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	27	25	0	0	1	0.1	1	-	[ll:lm]
389	eACA/mCTG-t01	<hkxhk>	0	0	0	0	0	0	0	0	20	32	0	0	0	0	0	0	0	1	18.1	2	******	[hh:hk:kk]
390	eACA/mCTG-t02	<hkxhk>	0	0	0	0	0	0	0	0	13	39	0	0	0	0	0	0	0	1	19.5	2	*******	[hh:hk:kk]

391	eACA/mCTG-t03	<hkxhk>	0	0	0	0	0	0	0	0	26	26	0	0	0	0	0	0	0	1	26	2	*******	[hh:hk:kk]
392	eACA/mCTG-t04	<hkxhk>	0	0	0	0	0	0	0	0	13	39	0	0	0	0	0	0	0	1	19.5	2	*******	[hh:hk:kk]
393	P27-1	<hkxhk>	0	0	0	0	0	0	0	0	21	16	0	0	0	0	0	0	0	16	24.5	2	*******	[hh:hk:kk]
394	P27-2	<hkxhk>	0	0	0	0	0	0	0	0	4	33	0	0	0	0	0	0	0	16	23.6	2	*******	[hh:hk:kk]
395	P27-5	<hkxhk>	0	0	0	0	0	0	0	0	26	11	0	0	0	0	0	0	0	16	42.6	2	*******	[hh:hk:kk]
396	P27-4	<hkxhk>	0	0	0	0	0	0	0	0	8	29	0	0	0	0	0	0	0	16	15.4	2	******	[hh:hk:kk]
397	P27-6	<hkxhk>	0	0	0	0	0	0	0	0	22	15	0	0	0	0	0	0	0	16	27.5	2	*******	[hh:hk:kk]
398	OPA3-1	<hkxhk>	0	0	0	0	0	0	0	0	25	14	0	0	0	0	0	0	0	14	35.1	2	*******	[hh:hk:kk]
399	OPA3-2	<hkxhk>	0	0	0	0	0	0	0	0	23	16	0	0	0	0	0	0	0	14	28.4	2	*******	[hh:hk:kk]
400	OPA3-3	<hkxhk>	0	0	0	0	0	0	0	0	9	30	0	0	0	0	0	0	0	14	15.5	2	******	[hh:hk:kk]
401	OPA3-6	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	18	21	0	0	14	0.2	1	-	[ll:lm]
402	STMS8fp/rpa	<hkxhk>	0	0	0	0	0	0	0	0	12	29	0	0	0	0	0	0	0	12	14.1	2	*****	[hh:hk:kk]
403	STMS8fp/rpb	<hkxhk>	0	0	0	0	0	0	0	0	8	34	0	0	0	0	0	0	0	11	19.1	2	*******	[hh:hk:kk]
404	AGMI9-93a	<hkxhk>	0	0	0	0	0	0	0	0	2	48	0	0	0	0	0	0	0	3	42.5	2	*******	[hh:hk:kk]
405	AGMI10-103a	<hkxhk>	0	0	0	0	0	0	0	0	4	41	0	0	0	0	0	0	0	8	31.1	2	*******	[hh:hk:kk]
406	AGMI10-103b	<hkxhk>	0	0	0	0	0	0	0	0	9	36	0	0	0	0	0	0	0	8	19.8	2	*******	[hh:hk:kk]
407	AGMI35/6a	<hkxhk>	0	0	0	0	0	0	0	0	5	37	0	0	0	0	0	0	0	11	25.6	2	*******	[hh:hk:kk]
408	AGMI35/6b	<hkxhk>	0	0	0	0	0	0	0	0	2	40	0	0	0	0	0	0	0	11	34.6	2	*******	[hh:hk:kk]
409	AGMI95/6a	<hkxhk>	0	0	0	0	0	0	0	0	11	36	0	0	0	0	0	0	0	6	18.4	2	*******	[hh:hk:kk]
410	AGMI95/6b	<hkxhk>	0	0	0	0	0	0	0	0	3	44	0	0	0	0	0	0	0	6	36.1	2	*******	[hh:hk:kk]
411	AGMI101/2a	<hkxhk>	0	0	0	0	0	0	0	0	9	40	0	0	0	0	0	0	0	4	22.9	2	*******	[hh:hk:kk]
412	AGMI101/2b	<hkxhk>	0	0	0	0	0	0	0	0	9	40	0	0	0	0	0	0	0	4	22.9	2	*******	[hh:hk:kk]
413	AGMI105/8a	<lmxll>	0	0	0	0	0	0	0	0	0	0	0	0	0	10	32	0	0	11	11.5	1	*****	[ll:lm]
414	AGMI105/8b	<hkxhk>	0	0	0	0	0	0	0	0	8	34	0	0	0	0	0	0	0	11	19.1	2	*******	[hh:hk:kk]

Appendix B: Locus genotype frequency and χ^2 value of markers analyzed by JoinMap 3.0 in the construction of susceptible mapping population

Locus Genotype Freq sdata [s+h]- of AFLP+RAPD+STMS

Nr	Locus	Seg.type	ac	ad	bc	bd	ee	ef	eg	fg	hh	hk	kk	h-	k-	ll	lm	nn	np	--	χ 2	Df	Signif.	Classes
1	pAA/mAAC-s278	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	30	23	0	0.9	1	-	[nn:np]
2	pAA/mAAC-s250	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	27	26	0	0	1	-	[nn:np]
3	pAA/mAAC-s224	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	22	31	0	1.5	1	-	[nn:np]
4	pAA/mAAC-s210	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	44	0	23.1	1	*******	[nn:np]
5	pAA/mAAC-s191	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	38	0	10	1	****	[nn:np]
6	pAA/mAAC-s167	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	42	0	18.1	1	*******	[nn:np]
7	pAA/mAAC-s155	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	21	32	0	2.3	1	-	[nn:np]
8	pAA/mAAC-s096	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	28	25	0	0.2	1	-	[nn:np]
9	pAA/mAAC-s085	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	27	26	0	0	1	-	[nn:np]
10	pAA/mAAG-s01	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	26	15	12	3	1	*	[nn:np]
11	pAA/mAAG-s02	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	25	12	2	1	-	[nn:np]
12	pAA/mAAG-s03	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	26	12	3	1	*	[nn:np]
13	pAA/mAAG-s04	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	22	19	12	0.2	1	-	[nn:np]
14	pAA/mAAG-t01	<hkxhk>	0	0	0	0	0	0	0	0	17	24	0	0	0	0	0	0	0	12	15.3	2	******	[hh:hk:kk]
15	pAA/mAAG-t02	<hkxhk>	0	0	0	0	0	0	0	0	18	23	0	0	0	0	0	0	0	12	16.4	2	******	[hh:hk:kk]
16	pAA/mAAG-t03	<hkxhk>	0	0	0	0	0	0	0	0	13	28	0	0	0	0	0	0	0	12	13.7	2	****	[hh:hk:kk]
17	pAA/mAAG-t04	<hkxhk>	0	0	0	0	0	0	0	0	16	25	0	0	0	0	0	0	0	12	14.5	2	*****	[hh:hk:kk]
18	pAA/mAAG-t05	<hkxhk>	0	0	0	0	0	0	0	0	12	29	0	0	0	0	0	0	0	12	14.1	2	*****	[hh:hk:kk]
19	pAA/mAAG-t06	<hkxhk>	0	0	0	0	0	0	0	0	7	34	0	0	0	0	0	0	0	12	20.2	2	*******	[hh:hk:kk]
20	pAA/mAAT-s290	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18	19	16	0	1	-	[nn:np]
21	pAA/mAAT-t227	<hkxhk>	0	0	0	0	0	0	0	0	10	35	0	0	0	0	0	0	0	8	18.3	2	******	[hh:hk:kk]
22	pAA/mAAT-t206	<hkxhk>	0	0	0	0	0	0	0	0	18	27	0	0	0	0	0	0	0	8	16.2	2	******	[hh:hk:kk]
23	pAA/mAAT-t157	<hkxhk>	0	0	0	0	0	0	0	0	11	39	0	0	0	0	0	0	0	3	20.5	2	*******	[hh:hk:kk]
24	pAA/mAAT-t128	<hkxhk>	0	0	0	0	0	0	0	0	18	32	0	0	0	0	0	0	0	3	16.9	2	******	[hh:hk:kk]
25	pAA/mACT-s348	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	24	27	2	0.2	1	-	[nn:np]
26	pAA/mACT-s272	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25	27	1	0.1	1	-	[nn:np]
27	pAA/mACT-s266	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	44	1	24.9	1	*******	[nn:np]
28	pAA/mACT-s231	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	38	1	11.1	1	*****	[nn:np]
29	pAA/mACT-s163	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	37	0	8.3	1	****	[nn:np]
30	pAA/mACT-s145	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	34	19	0	4.3	1	**	[nn:np]
31	pAA/mACT-s135	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	17	36	0	6.8	1	***	[nn:np]
32	pAA/mACT-t310	<hkxhk>	0	0	0	0	0	0	0	0	15	37	0	0	0	0	0	0	0	1	18	2	******	[hh:hk:kk]
33	pAA/mACT-t264	<hkxhk>	0	0	0	0	0	0	0	0	15	37	0	0	0	0	0	0	0	1	18	2	******	[hh:hk:kk]
34	pAA/mACT-t159	<hkxhk>	0	0	0	0	0	0	0	0	13	40	0	0	0	0	0	0	0	0	20.1	2	*******	[hh:hk:kk]
35	pAA/mAGG-s295	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	32	6	6.2	1	**	[nn:np]
36	pAA/mAGG-s252	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	31	15	7	5.6	1	**	[nn:np]

37	pAA/mAGG-s210	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13	36	4	10.8	1	****	[nn:np]
38	pAA/mAGG-s136	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	32	19	2	3.3	1	*	[nn:np]
39	pAA/mAGG-t240	<hkxhk>	0	0	0	0	0	0	0	0	27	21	0	0	0	0	0	0	0	5	31.1	2	*******	[hh:hk:kk]
40	pAA/mAGG-t174	<hkxhk>	0	0	0	0	0	0	0	0	32	19	0	0	0	0	0	0	0	2	43.5	2	*******	[hh:hk:kk]
41	pAA/mAGG-t170	<hkxhk>	0	0	0	0	0	0	0	0	26	25	0	0	0	0	0	0	0	2	26.5	2	*******	[hh:hk:kk]
42	pAA/mAGG-t143	<hkxhk>	0	0	0	0	0	0	0	0	16	35	0	0	0	0	0	0	0	2	17.1	2	******	[hh:hk:kk]
43	pAA/mAGG-t101	<hkxhk>	0	0	0	0	0	0	0	0	11	40	0	0	0	0	0	0	0	2	21.2	2	*******	[hh:hk:kk]
44	pAA/mCCG-s240	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	36	2	8.7	1	****	[nn:np]
45	pAA/mCCG-s184	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	26	26	1	0	1	-	[nn:np]
46	pAA/mCCG-t230	<hkxhk>	0	0	0	0	0	0	0	0	14	37	0	0	0	0	0	0	0	2	18.1	2	******	[hh:hk:kk]
47	pAA/mCCG-t192	<hkxhk>	0	0	0	0	0	0	0	0	18	33	0	0	0	0	0	0	0	2	17.1	2	******	[hh:hk:kk]
48	pAA/mCCG-t178	<hkxhk>	0	0	0	0	0	0	0	0	21	31	0	0	0	0	0	0	0	1	18.9	2	*******	[hh:hk:kk]
49	pAA/mCCT-s294	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	39	10	4	17.2	1	*******	[nn:np]
50	pAA/mCCT-s228	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	30	23	0	0.9	1	-	[nn:np]
51	pAA/mCCT-s218	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	28	25	0	0.2	1	-	[nn:np]
52	pAA/mCCT-s166	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	28	25	0	0.2	1	-	[nn:np]
53	pAA/mCCT-s099	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	30	23	0	0.9	1	-	[nn:np]
54	pAA/mCCT-t360	<hkxhk>	0	0	0	0	0	0	0	0	23	25	0	0	0	0	0	0	0	5	22.1	2	*******	[hh:hk:kk]
55	pAA/mCCT-t249	<hkxhk>	0	0	0	0	0	0	0	0	12	41	0	0	0	0	0	0	0	0	21.3	2	*******	[hh:hk:kk]
56	pAA/mCCT-t211	<hkxhk>	0	0	0	0	0	0	0	0	14	39	0	0	0	0	0	0	0	0	19.2	2	*******	[hh:hk:kk]
57	pAA/mCCT-t209	<hkxhk>	0	0	0	0	0	0	0	0	20	33	0	0	0	0	0	0	0	0	18.3	2	******	[hh:hk:kk]
58	pAA/mCTA-s450	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	23	29	1	0.7	1	-	[nn:np]
59	pAA/mCTA-s310	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	29	24	0	0.5	1	-	[nn:np]
60	pAA/mCTA-s198	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	22	31	0	1.5	1	-	[nn:np]
61	pAA/mCTA-t405	<hkxhk>	0	0	0	0	0	0	0	0	14	38	0	0	0	0	0	0	0	1	18.6	2	*******	[hh:hk:kk]
62	pAA/mCTA-t360	<hkxhk>	0	0	0	0	0	0	0	0	18	34	0	0	0	0	0	0	0	1	17.4	2	******	[hh:hk:kk]
63	pAA/mCTA-t212	<hkxhk>	0	0	0	0	0	0	0	0	15	38	0	0	0	0	0	0	0	0	18.5	2	*******	[hh:hk:kk]
64	pAA/mCTA-t207	<hkxhk>	0	0	0	0	0	0	0	0	17	36	0	0	0	0	0	0	0	0	17.7	2	******	[hh:hk:kk]
65	pAA/mCTA-t111	<hkxhk>	0	0	0	0	0	0	0	0	16	37	0	0	0	0	0	0	0	0	18	2	******	[hh:hk:kk]
66	pAA/mCTA-t104	<hkxhk>	0	0	0	0	0	0	0	0	10	43	0	0	0	0	0	0	0	0	24.3	2	*******	[hh:hk:kk]
67	pAA/mCTA-t093	<hkxhk>	0	0	0	0	0	0	0	0	7	46	0	0	0	0	0	0	0	0	30.6	2	*******	[hh:hk:kk]
68	pAA/mCTT-t140	<hkxhk>	0	0	0	0	0	0	0	0	29	24	0	0	0	0	0	0	0	0	32.2	2	*******	[hh:hk:kk]
69	pAA/mCTT-t114	<hkxhk>	0	0	0	0	0	0	0	0	32	21	0	0	0	0	0	0	0	0	40.9	2	*******	[hh:hk:kk]
70	pAA/mCTT-t089	<hkxhk>	0	0	0	0	0	0	0	0	21	32	0	0	0	0	0	0	0	0	18.9	2	*******	[hh:hk:kk]
71	pAA/mGAC-s492	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	31	17	5	4.1	1	**	[nn:np]
72	pAA/mGAC-s350	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	29	19	5	2.1	1	-	[nn:np]
73	pAA/mGAC-s241	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	23	27	3	0.3	1	-	[nn:np]
74	pAA/mGAC-s126	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	22	28	3	0.7	1	-	[nn:np]
75	pAA/mGAC-t234	<hkxhk>	0	0	0	0	0	0	0	0	22	28	0	0	0	0	0	0	0	3	20.1	2	*******	[hh:hk:kk]
76	pAA/mGAC-t210	<hkxhk>	0	0	0	0	0	0	0	0	19	31	0	0	0	0	0	0	0	3	17.3	2	******	[hh:hk:kk]
77	pAA/mCAG-t306	<hkxhk>	0	0	0	0	0	0	0	0	20	20	0	0	0	0	0	0	0	13	20	2	*******	[hh:hk:kk]
78	pAA/mCAG-t278	<hkxhk>	0	0	0	0	0	0	0	0	16	24	0	0	0	0	0	0	0	13	14.4	2	*****	[hh:hk:kk]

79	pAA/mCAG-t270	<hkxhk>	0	0	0	0	0	0	0	0	16	23	0	0	0	0	0	0	0	14	14.4	2	*****	[hh:hk:kk]
80	pAA/mCAG-t249	<hkxhk>	0	0	0	0	0	0	0	0	12	27	0	0	0	0	0	0	0	14	13.2	2	****	[hh:hk:kk]
81	pAA/mCAG-t155	<hkxhk>	0	0	0	0	0	0	0	0	14	27	0	0	0	0	0	0	0	12	13.7	2	****	[hh:hk:kk]
82	pAA/mCAG-t154	<hkxhk>	0	0	0	0	0	0	0	0	15	26	0	0	0	0	0	0	0	12	13.9	2	*****	[hh:hk:kk]
83	pAA/mGCA-s380	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	28	24	1	0.3	1	-	[nn:np]
84	pAA/mGCA-s350	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	31	21	1	1.9	1	-	[nn:np]
85	pAA/mGCA-s270	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	29	23	1	0.7	1	-	[nn:np]
86	pAA/mGCA-s139	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	24	29	0	0.5	1	-	[nn:np]
87	pAA/mGCA-t372	<hkxhk>	0	0	0	0	0	0	0	0	23	29	0	0	0	0	0	0	0	1	21	2	*******	[hh:hk:kk]
88	pAA/mGCA-t340	<hkxhk>	0	0	0	0	0	0	0	0	15	37	0	0	0	0	0	0	0	1	18	2	******	[hh:hk:kk]
89	pAA/mGCA-t194	<hkxhk>	0	0	0	0	0	0	0	0	17	36	0	0	0	0	0	0	0	0	17.7	2	******	[hh:hk:kk]
90	pAA/mGCA-t192	<hkxhk>	0	0	0	0	0	0	0	0	17	36	0	0	0	0	0	0	0	0	17.7	2	******	[hh:hk:kk]
91	pAA/mCGA-t125	<hkxhk>	0	0	0	0	0	0	0	0	8	45	0	0	0	0	0	0	0	0	28.3	2	*******	[hh:hk:kk]
92	pAC/mACC-s417	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18	22	13	0.4	1	-	[nn:np]
93	pAC/mACC-s269	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	30	16	7	4.3	1	**	[nn:np]
94	pAC/mACC-s185	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	26	25	2	0	1	-	[nn:np]
95	pAC/mACC-s184	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	21	31	1	1.9	1	-	[nn:np]
96	pAC/mACC-s178	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	33	18	2	4.4	1	**	[nn:np]
97	pAC/mACC-t415	<hkxhk>	0	0	0	0	0	0	0	0	18	29	0	0	0	0	0	0	0	6	16.4	2	******	[hh:hk:kk]
98	pAC/mACC-t370	<hkxhk>	0	0	0	0	0	0	0	0	5	46	0	0	0	0	0	0	0	2	33.9	2	*******	[hh:hk:kk]
99	pAC/mACC-t268	<hkxhk>	0	0	0	0	0	0	0	0	19	33	0	0	0	0	0	0	0	1	17.6	2	******	[hh:hk:kk]
100	pAC/mACC-t156	<hkxhk>	0	0	0	0	0	0	0	0	19	32	0	0	0	0	0	0	0	2	17.5	2	******	[hh:hk:kk]
101	pAC/mACC-t120	<hkxhk>	0	0	0	0	0	0	0	0	18	33	0	0	0	0	0	0	0	2	17.1	2	******	[hh:hk:kk]
102	pAC/mACT-s151	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	21	27	5	0.8	1	-	[nn:np]
103	pAC/mACT-s117	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	28	5	1.3	1	-	[nn:np]
104	pAC/mACT-t272	<hkxhk>	0	0	0	0	0	0	0	0	13	35	0	0	0	0	0	0	0	5	17.1	2	******	[hh:hk:kk]
105	pAC/mACT-t105	<hkxhk>	0	0	0	0	0	0	0	0	12	36	0	0	0	0	0	0	0	5	18	2	******	[hh:hk:kk]
106	pAC/mACT-t092	<hkxhk>	0	0	0	0	0	0	0	0	15	33	0	0	0	0	0	0	0	5	16.1	2	******	[hh:hk:kk]
107	pAC/mACT-t078	<hkxhk>	0	0	0	0	0	0	0	0	16	32	0	0	0	0	0	0	0	5	16	2	******	[hh:hk:kk]
108	pAC/mAGA-s310	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	22	30	1	1.2	1	-	[nn:np]
109	pAC/mAGA-s305	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	28	24	1	0.3	1	-	[nn:np]
110	pAC/mAGA-s255	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	34	18	1	4.9	1	**	[nn:np]
111	pAC/mAGA-s169	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	28	25	0	0.2	1	-	[nn:np]
112	pAC/mAGA-s146	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18	35	0	5.5	1	**	[nn:np]
113	pAC/mAGA-s140	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	27	26	0	0	1	-	[nn:np]
114	pAC/mAGA-s114	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	24	29	0	0.5	1	-	[nn:np]
115	pAC/mAGA-s106	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18	35	0	5.5	1	**	[nn:np]
116	pAC/mAGA-s105	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	27	26	0	0	1	-	[nn:np]
117	pAC/mAGA-s094	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	24	29	0	0.5	1	-	[nn:np]
118	pAC/mAGA-t355	<hkxhk>	0	0	0	0	0	0	0	0	14	38	0	0	0	0	0	0	0	1	18.6	2	*******	[hh:hk:kk]
119	pAC/mAGA-t218	<hkxhk>	0	0	0	0	0	0	0	0	12	41	0	0	0	0	0	0	0	0	21.3	2	*******	[hh:hk:kk]
120	pAC/mAGA-t192	<hkxhk>	0	0	0	0	0	0	0	0	9	44	0	0	0	0	0	0	0	0	26.2	2	*******	[hh:hk:kk]

121	pAC/mAGA-t191	<hkxhk>	0	0	0	0	0	0	0	0	18	35	0	0	0	0	0	0	0	0	17.7	2	******	[hh:hk:kk]
122	pAC/mAGA-t148	<hkxhk>	0	0	0	0	0	0	0	0	24	29	0	0	0	0	0	0	0	0	22.2	2	*******	[hh:hk:kk]
123	pAC/mCAC-s206	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25	22	6	0.2	1	-	[nn:np]
124	pAC/mCAC-s175	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18	29	6	2.6	1	-	[nn:np]
125	pAC/mCAC-s120	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18	29	6	2.6	1	-	[nn:np]
126	pAC/mCAC-t384	<hkxhk>	0	0	0	0	0	0	0	0	21	22	0	0	0	0	0	0	0	10	20.5	2	*******	[hh:hk:kk]
127	pAC/mCAC-t283	<hkxhk>	0	0	0	0	0	0	0	0	18	25	0	0	0	0	0	0	0	10	16.2	2	******	[hh:hk:kk]
128	pAC/mCAC-t233	<hkxhk>	0	0	0	0	0	0	0	0	13	34	0	0	0	0	0	0	0	6	16.6	2	******	[hh:hk:kk]
129	pAC/mCAC-t183	<hkxhk>	0	0	0	0	0	0	0	0	26	21	0	0	0	0	0	0	0	6	29.3	2	*******	[hh:hk:kk]
130	pAC/mCCA-s290	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25	27	1	0.1	1	-	[nn:np]
131	pAC/mCCA-s242	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	32	20	1	2.8	1	*	[nn:np]
132	pAC/mCCA-s084	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	46	0	28.7	1	*******	[nn:np]
133	pAC/mCCA-t218	<hkxhk>	0	0	0	0	0	0	0	0	22	30	0	0	0	0	0	0	0	1	19.9	2	*******	[hh:hk:kk]
134	pAC/mCCA-t171	<hkxhk>	0	0	0	0	0	0	0	0	18	35	0	0	0	0	0	0	0	0	17.7	2	******	[hh:hk:kk]
135	pAC/mCCA-t088	<hkxhk>	0	0	0	0	0	0	0	0	12	41	0	0	0	0	0	0	0	0	21.3	2	*******	[hh:hk:kk]
136	pAC/mCCG-s275	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18	35	0	5.5	1	**	[nn:np]
137	pAC/mCCG-s210	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	41	0	15.9	1	*******	[nn:np]
138	pAC/mCCG-s120	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	38	0	10	1	****	[nn:np]
139	pAC/mCCG-s102	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	23	30	0	0.9	1	-	[nn:np]
140	pAC/mCCG-s095	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25	28	0	0.2	1	-	[nn:np]
141	pAC/mCCG-t290	<hkxhk>	0	0	0	0	0	0	0	0	14	39	0	0	0	0	0	0	0	0	19.2	2	*******	[hh:hk:kk]
142	pAC/mCCG-t085	<hkxhk>	0	0	0	0	0	0	0	0	16	37	0	0	0	0	0	0	0	0	18	2	******	[hh:hk:kk]
143	pAC/mCGG-s121	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	37	0	8.3	1	****	[nn:np]
144	pAC/mCGG-t145	<hkxhk>	0	0	0	0	0	0	0	0	23	30	0	0	0	0	0	0	0	0	20.9	2	*******	[hh:hk:kk]
145	pAC/mGCA-s334	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	27	23	3	0.3	1	-	[nn:np]
146	pAC/mGCA-s330	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	27	21	5	0.8	1	-	[nn:np]
147	pAC/mGCA-s223	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	30	21	2	1.6	1	-	[nn:np]
148	pAC/mGCA-s214	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	30	23	0	0.9	1	-	[nn:np]
149	pAC/mGCA-s150	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	34	19	0	4.3	1	**	[nn:np]
150	pAC/mGCA-s094	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	37	0	8.3	1	****	[nn:np]
151	pAC/mGCA-s084	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	37	0	8.3	1	****	[nn:np]
152	pAC/mGCA-t088	<hkxhk>	0	0	0	0	0	0	0	0	17	36	0	0	0	0	0	0	0	0	17.7	2	******	[hh:hk:kk]
153	pAG/mAAC-s237	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	38	4	14.9	1	******	[nn:np]
154	pAG/mAAC-s210	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	40	3	18	1	*******	[nn:np]
155	pAG/mAAC-s187	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25	26	2	0	1	-	[nn:np]
156	pAG/mAAC-s158	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	31	2	2.4	1	-	[nn:np]
157	pAG/mAAC-s135	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	23	28	2	0.5	1	-	[nn:np]
158	pAG/mAAC-s116	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	37	2	10.4	1	****	[nn:np]
159	pAG/mAAC-s106	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	39	12	2	14.3	1	******	[nn:np]
160	pAG/mAAC-t303	<hkxhk>	0	0	0	0	0	0	0	0	14	35	0	0	0	0	0	0	0	4	17	2	******	[hh:hk:kk]
161	pAG/mAAC-t250	<hkxhk>	0	0	0	0	0	0	0	0	13	36	0	0	0	0	0	0	0	4	17.7	2	******	[hh:hk:kk]
162	pAG/mAAC-t238	<hkxhk>	0	0	0	0	0	0	0	0	31	18	0	0	0	0	0	0	0	4	42.7	2	*******	[hh:hk:kk]

163	pAG/mAAC-t138	<hkxhk>	0	0	0	0	0	0	0	0	19	33	0	0	0	0	0	0	0	1	17.6	2	******	[hh:hk:kk]
164	pAG/mAAC-t127	<hkxhk>	0	0	0	0	0	0	0	0	4	47	0	0	0	0	0	0	0	2	36.9	2	*******	[hh:hk:kk]
165	pAG/mAAC-t089	<hkxhk>	0	0	0	0	0	0	0	0	11	41	0	0	0	0	0	0	0	1	22	2	*******	[hh:hk:kk]
166	pAG/mAAT-s345	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25	24	4	0	1	-	[nn:np]
167	pAG/mAAT-s242	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	27	22	4	0.5	1	-	[nn:np]
168	pAG/mAAT-s215	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	35	3	8	1	****	[nn:np]
169	pAG/mAAT-s189	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	31	19	3	2.9	1	*	[nn:np]
170	pAG/mAAT-t358	<hkxhk>	0	0	0	0	0	0	0	0	16	31	0	0	0	0	0	0	0	6	15.7	2	******	[hh:hk:kk]
171	pAG/mAAT-t265	<hkxhk>	0	0	0	0	0	0	0	0	16	33	0	0	0	0	0	0	0	4	16.4	2	******	[hh:hk:kk]
172	pAG/mAAT-t254	<hkxhk>	0	0	0	0	0	0	0	0	10	39	0	0	0	0	0	0	0	4	21.2	2	*******	[hh:hk:kk]
173	pAG/mAAT-t226	<hkxhk>	0	0	0	0	0	0	0	0	22	27	0	0	0	0	0	0	0	4	20.3	2	*******	[hh:hk:kk]
174	pAG/mAAT-t153	<hkxhk>	0	0	0	0	0	0	0	0	16	35	0	0	0	0	0	0	0	2	17.1	2	******	[hh:hk:kk]
175	pAG/mACC-s390	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	33	19	1	3.8	1	*	[nn:np]
176	pAG/mACC-s345	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	31	21	1	1.9	1	-	[nn:np]
177	pAG/mACC-s320	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	28	24	1	0.3	1	-	[nn:np]
178	pAG/mACC-s308	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	29	24	0	0.5	1	-	[nn:np]
179	pAG/mACC-s249	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	31	22	0	1.5	1	-	[nn:np]
180	pAG/mACC-s163	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	23	30	0	0.9	1	-	[nn:np]
181	pAG/mACC-s136	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	41	0	15.9	1	*******	[nn:np]
182	pAG/mACC-t192	<hkxhk>	0	0	0	0	0	0	0	0	20	33	0	0	0	0	0	0	0	0	18.3	2	******	[hh:hk:kk]
183	pAG/mACC-t174	<hkxhk>	0	0	0	0	0	0	0	0	21	32	0	0	0	0	0	0	0	0	18.9	2	*******	[hh:hk:kk]
184	pAG/mACC-t144	<hkxhk>	0	0	0	0	0	0	0	0	10	43	0	0	0	0	0	0	0	0	24.3	2	*******	[hh:hk:kk]
185	pAG/mACC-t101	<hkxhk>	0	0	0	0	0	0	0	0	20	33	0	0	0	0	0	0	0	0	18.3	2	******	[hh:hk:kk]
186	pAG/mACC-t091	<hkxhk>	0	0	0	0	0	0	0	0	18	35	0	0	0	0	0	0	0	0	17.7	2	******	[hh:hk:kk]
187	pAG/mACC-t088	<hkxhk>	0	0	0	0	0	0	0	0	12	41	0	0	0	0	0	0	0	0	21.3	2	*******	[hh:hk:kk]
188	pAG/mAGA-s279	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	22	23	8	0	1	-	[nn:np]
189	pAG/mAGA-s208	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	29	4	1.6	1	-	[nn:np]
190	pAG/mAGA-t358	<hkxhk>	0	0	0	0	0	0	0	0	16	25	0	0	0	0	0	0	0	12	14.5	2	*****	[hh:hk:kk]
191	pAG/mAGA-t147	<hkxhk>	0	0	0	0	0	0	0	0	19	33	0	0	0	0	0	0	0	1	17.6	2	******	[hh:hk:kk]
192	pAG/mAGA-t087	<hkxhk>	0	0	0	0	0	0	0	0	9	43	0	0	0	0	0	0	0	1	25.4	2	*******	[hh:hk:kk]
193	pAG/mAGA-t086	<hkxhk>	0	0	0	0	0	0	0	0	13	39	0	0	0	0	0	0	0	1	19.5	2	*******	[hh:hk:kk]
194	pAG/mCGC-s370	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18	18	17	0	1	-	[nn:np]
195	pAG/mCGC-t314	<hkxhk>	0	0	0	0	0	0	0	0	17	21	0	0	0	0	0	0	0	15	15.6	2	******	[hh:hk:kk]
196	pAG/mCGC-t312	<hkxhk>	0	0	0	0	0	0	0	0	21	17	0	0	0	0	0	0	0	15	23.6	2	*******	[hh:hk:kk]
197	pAG/mCGC-t115	<hkxhk>	0	0	0	0	0	0	0	0	9	36	0	0	0	0	0	0	0	8	19.8	2	*******	[hh:hk:kk]
198	pAG/mCGC-t089	<hkxhk>	0	0	0	0	0	0	0	0	8	37	0	0	0	0	0	0	0	8	21.5	2	*******	[hh:hk:kk]
199	pAG/mCGC-t088	<hkxhk>	0	0	0	0	0	0	0	0	16	29	0	0	0	0	0	0	0	8	15.1	2	*****	[hh:hk:kk]
200	pAG/mGCA-s285	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	31	2	2.4	1	-	[nn:np]
201	pAG/mGCA-s253	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	24	28	1	0.3	1	-	[nn:np]
202	pAG/mGCA-s224	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	37	1	9.3	1	****	[nn:np]
203	pAG/mGCA-s154	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	33	0	3.2	1	*	[nn:np]
204	pAG/mGCA-s130	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	21	32	0	2.3	1	-	[nn:np]

205	pAG/mGCA-s128	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	26	27	0	0	1	-	[nn:np]
206	pAG/mGCA-t294	<hkxhk>	0	0	0	0	0	0	0	0	22	29	0	0	0	0	0	0	0	2	19.9	2	*******	[hh:hk:kk]
207	pAG/mGCA-t292	<hkxhk>	0	0	0	0	0	0	0	0	11	40	0	0	0	0	0	0	0	2	21.2	2	*******	[hh:hk:kk]
208	pAG/mGCA-t164	<hkxhk>	0	0	0	0	0	0	0	0	12	41	0	0	0	0	0	0	0	0	21.3	2	*******	[hh:hk:kk]
209	pAG/mGCA-t091	<hkxhk>	0	0	0	0	0	0	0	0	12	41	0	0	0	0	0	0	0	0	21.3	2	*******	[hh:hk:kk]
210	pAT/mAAC-s260	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	27	20	6	1	1	-	[nn:np]
211	pAT/mAAC-s238	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	30	17	6	3.6	1	*	[nn:np]
212	pAT/mAAC-s175	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	35	16	2	7.1	1	***	[nn:np]
213	pAT/mAAC-s173	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	24	27	2	0.2	1	-	[nn:np]
214	pAT/mAAC-s132	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	17	34	2	5.7	1	**	[nn:np]
215	pAT/mAAC-s120	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	28	24	1	0.3	1	-	[nn:np]
216	pAT/mAAC-s118	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	37	1	9.3	1	****	[nn:np]
217	pAT/mAAC-t128	<hkxhk>	0	0	0	0	0	0	0	0	16	35	0	0	0	0	0	0	0	2	17.1	2	******	[hh:hk:kk]
218	pAT/mAAC-t099	<hkxhk>	0	0	0	0	0	0	0	0	19	33	0	0	0	0	0	0	0	1	17.6	2	******	[hh:hk:kk]
219	pAT/mAAC-t083	<hkxhk>	0	0	0	0	0	0	0	0	19	33	0	0	0	0	0	0	0	1	17.6	2	******	[hh:hk:kk]
220	pAT/mAAC-t075	<hkxhk>	0	0	0	0	0	0	0	0	6	47	0	0	0	0	0	0	0	0	33.1	2	*******	[hh:hk:kk]
221	pAT/mAAT-s141	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	28	25	0	0.2	1	-	[nn:np]
222	pAT/mAAT-s132	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	38	15	0	10	1	****	[nn:np]
223	pAT/mAAT-s104	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	24	29	0	0.5	1	-	[nn:np]
224	pAT/mAAT-t187	<hkxhk>	0	0	0	0	0	0	0	0	20	33	0	0	0	0	0	0	0	0	18.3	2	******	[hh:hk:kk]
225	pAT/mAAT-t131	<hkxhk>	0	0	0	0	0	0	0	0	7	46	0	0	0	0	0	0	0	0	30.6	2	*******	[hh:hk:kk]
226	pAT/mACT-s420	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	26	22	5	0.3	1	-	[nn:np]
227	pAT/mACT-s270	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	24	25	4	0	1	-	[nn:np]
228	pAT/mACT-s177	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18	31	4	3.5	1	*	[nn:np]
229	pAT/mACT-t208	<hkxhk>	0	0	0	0	0	0	0	0	18	31	0	0	0	0	0	0	0	4	16.7	2	******	[hh:hk:kk]
230	pAT/mACT-t205	<hkxhk>	0	0	0	0	0	0	0	0	35	14	0	0	0	0	0	0	0	4	59	2	*******	[hh:hk:kk]
231	pAT/mACT-t102	<hkxhk>	0	0	0	0	0	0	0	0	29	22	0	0	0	0	0	0	0	2	33.9	2	*******	[hh:hk:kk]
232	pAT/mAGG-s470	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	31	22	0	1.5	1	-	[nn:np]
233	pAT/mAGG-s235	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	33	20	0	3.2	1	*	[nn:np]
234	pAT/mAGG-s231	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	24	29	0	0.5	1	-	[nn:np]
235	pAT/mAGG-s211	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18	35	0	5.5	1	**	[nn:np]
236	pAT/mAGG-s172	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	22	31	0	1.5	1	-	[nn:np]
237	pAT/mAGG-s106	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	33	0	3.2	1	*	[nn:np]
238	pAT/mAGG-s104	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	22	31	0	1.5	1	-	[nn:np]
239	pAT/mAGG-t01	<hkxhk>	0	0	0	0	0	0	0	0	21	32	0	0	0	0	0	0	0	0	18.9	2	*******	[hh:hk:kk]
240	pAT/mAGG-t02	<hkxhk>	0	0	0	0	0	0	0	0	22	31	0	0	0	0	0	0	0	0	19.8	2	*******	[hh:hk:kk]
241	pAT/mAGG-t03	<hkxhk>	0	0	0	0	0	0	0	0	10	43	0	0	0	0	0	0	0	0	24.3	2	*******	[hh:hk:kk]
242	pAT/mAGG-t04	<hkxhk>	0	0	0	0	0	0	0	0	20	33	0	0	0	0	0	0	0	0	18.3	2	******	[hh:hk:kk]
243	pAT/mAGG-t05	<hkxhk>	0	0	0	0	0	0	0	0	22	31	0	0	0	0	0	0	0	0	19.8	2	*******	[hh:hk:kk]
244	pCA/mACT-s145	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	36	8	16.2	1	*******	[nn:np]
245	pCA/mAGA-s01	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	36	1	7.7	1	***	[nn:np]
246	pCA/mAGA-s02	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	45	1	27.8	1	*******	[nn:np]

247	pCA/mAGA-t01	<hkxhk>	0	0	0	0	0	0	0	0	9	43	0	0	0	0	0	0	0	1	25.4	2	*******	[hh:hk:kk]
248	pCA/mAGA-t02	<hkxhk>	0	0	0	0	0	0	0	0	12	40	0	0	0	0	0	0	0	1	20.6	2	*******	[hh:hk:kk]
249	pCA/mAGA-t03	<hkxhk>	0	0	0	0	0	0	0	0	13	39	0	0	0	0	0	0	0	1	19.5	2	*******	[hh:hk:kk]
250	pCA/mCAC-s228	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	33	10	12.3	1	******	[nn:np]
251	pCA/mCAC-s145	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	37	6	15.5	1	*******	[nn:np]
252	pCA/mCAC-s110	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	36	6	13.3	1	******	[nn:np]
253	pCA/mCAC-t148	<hkxhk>	0	0	0	0	0	0	0	0	14	33	0	0	0	0	0	0	0	6	16	2	******	[hh:hk:kk]
254	pCA/mCAG-s098	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	24	16	13	1.6	1	-	[nn:np]
255	pCA/mCAG-t208	<hkxhk>	0	0	0	0	0	0	0	0	3	36	0	0	0	0	0	0	0	14	28.4	2	*******	[hh:hk:kk]
256	pCA/mCAG-t180	<hkxhk>	0	0	0	0	0	0	0	0	15	24	0	0	0	0	0	0	0	14	13.6	2	****	[hh:hk:kk]
257	pCA/mCAG-t126	<hkxhk>	0	0	0	0	0	0	0	0	9	31	0	0	0	0	0	0	0	13	16.1	2	******	[hh:hk:kk]
258	pCA/mCAG-t091	<hkxhk>	0	0	0	0	0	0	0	0	7	33	0	0	0	0	0	0	0	13	19.4	2	*******	[hh:hk:kk]
259	pCA/mCCC-s310	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	38	5	16.3	1	*******	[nn:np]
260	pCA/mCCC-s227	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	23	28	2	0.5	1	-	[nn:np]
261	pCA/mCCC-s140	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	46	1	30.8	1	*******	[nn:np]
262	pCA/mCCC-t300	<hkxhk>	0	0	0	0	0	0	0	0	8	40	0	0	0	0	0	0	0	5	24	2	*******	[hh:hk:kk]
263	pCA/mCCC-t200	<hkxhk>	0	0	0	0	0	0	0	0	15	36	0	0	0	0	0	0	0	2	17.5	2	******	[hh:hk:kk]
264	pCA/mCCC-t141	<hkxhk>	0	0	0	0	0	0	0	0	15	36	0	0	0	0	0	0	0	2	17.5	2	******	[hh:hk:kk]
265	pCA/mCCT-s248	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19	33	1	3.8	1	*	[nn:np]
266	pCA/mCCT-s202	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	30	22	1	1.2	1	-	[nn:np]
267	pCA/mCCT-s105	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	37	1	9.3	1	****	[nn:np]
268	pCA/mCCT-s104	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	21	31	1	1.9	1	-	[nn:np]
269	pCA/mCCT-s090	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19	33	1	3.8	1	*	[nn:np]
270	pCA/mCCT-t141	<hkxhk>	0	0	0	0	0	0	0	0	5	47	0	0	0	0	0	0	0	1	34.9	2	*******	[hh:hk:kk]
271	pCA/mCGT-s292	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	21	12	0	1	-	[nn:np]
272	pCA/mCGT-s242	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	23	18	12	0.6	1	-	[nn:np]
273	pCA/mCGT-t01	<hkxhk>	0	0	0	0	0	0	0	0	18	23	0	0	0	0	0	0	0	12	16.4	2	******	[hh:hk:kk]
274	pCA/mCTA-s01	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	21	31	1	1.9	1	-	[nn:np]
275	pCA/mCTA-s02	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	26	27	0	0	1	-	[nn:np]
276	pCA/mCTA-t01	<hkxhk>	0	0	0	0	0	0	0	0	12	40	0	0	0	0	0	0	0	1	20.6	2	*******	[hh:hk:kk]
277	pCA/mCTA-t02	<hkxhk>	0	0	0	0	0	0	0	0	13	39	0	0	0	0	0	0	0	1	19.5	2	*******	[hh:hk:kk]
278	pCA/mCTA-t03	<hkxhk>	0	0	0	0	0	0	0	0	12	40	0	0	0	0	0	0	0	1	20.6	2	*******	[hh:hk:kk]
279	pCA/mCTA-t04	<hkxhk>	0	0	0	0	0	0	0	0	9	43	0	0	0	0	0	0	0	1	25.4	2	*******	[hh:hk:kk]
280	pCA/mGAC-s405	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	23	17	13	0.9	1	-	[nn:np]
281	pCA/mGAC-s239	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	27	23	3	0.3	1	-	[nn:np]
282	pCA/mGAC-s177	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	27	23	3	0.3	1	-	[nn:np]
283	pCA/mGAC-s155	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25	25	3	0	1	-	[nn:np]
284	pCA/mGAC-s149	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	22	28	3	0.7	1	-	[nn:np]
285	pCA/mGAC-t340	<hkxhk>	0	0	0	0	0	0	0	0	15	35	0	0	0	0	0	0	0	3	17	2	******	[hh:hk:kk]
286	pCA/mGAC-t232	<hkxhk>	0	0	0	0	0	0	0	0	11	39	0	0	0	0	0	0	0	3	20.5	2	*******	[hh:hk:kk]
287	eAAC/mCA-s425	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	21	20	12	0	1	-	[nn:np]
288	eAAC/mCA-s346	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	26	15	12	3	1	*	[nn:np]

289	eAAC/mCA-s248	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	29	12	7	1	***	[nn:np]
290	eAAC/mCA-s148	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18	23	12	0.6	1	-	[nn:np]
291	eAAC/mCA-t540	<hkxhk>	0	0	0	0	0	0	0	0	16	25	0	0	0	0	0	0	0	12	14.5	2	*****	[hh:hk:kk]
292	eAAC/mCA-t271	<hkxhk>	0	0	0	0	0	0	0	0	15	26	0	0	0	0	0	0	0	12	13.9	2	*****	[hh:hk:kk]
293	eAAC/mCA-t218	<hkxhk>	0	0	0	0	0	0	0	0	17	24	0	0	0	0	0	0	0	12	15.3	2	******	[hh:hk:kk]
294	eAAC/mCA-t197	<hkxhk>	0	0	0	0	0	0	0	0	9	32	0	0	0	0	0	0	0	12	16.9	2	******	[hh:hk:kk]
295	eAAC/mCA-t182	<hkxhk>	0	0	0	0	0	0	0	0	16	25	0	0	0	0	0	0	0	12	14.5	2	*****	[hh:hk:kk]
296	eAAC/mCA-t174	<hkxhk>	0	0	0	0	0	0	0	0	9	32	0	0	0	0	0	0	0	12	16.9	2	******	[hh:hk:kk]
297	eAAC/mCA-t158	<hkxhk>	0	0	0	0	0	0	0	0	8	33	0	0	0	0	0	0	0	12	18.4	2	******	[hh:hk:kk]
298	eAAC/mCA-t156	<hkxhk>	0	0	0	0	0	0	0	0	4	37	0	0	0	0	0	0	0	12	27.3	2	*******	[hh:hk:kk]
299	eAAC/mCA-t140	<hkxhk>	0	0	0	0	0	0	0	0	14	27	0	0	0	0	0	0	0	12	13.7	2	****	[hh:hk:kk]
300	eAAC/mCA-t122	<hkxhk>	0	0	0	0	0	0	0	0	7	34	0	0	0	0	0	0	0	12	20.2	2	*******	[hh:hk:kk]
301	eAAC/mCA-t121	<hkxhk>	0	0	0	0	0	0	0	0	6	35	0	0	0	0	0	0	0	12	22.3	2	*******	[hh:hk:kk]
302	eAAC/mCA-t098	<hkxhk>	0	0	0	0	0	0	0	0	14	27	0	0	0	0	0	0	0	12	13.7	2	****	[hh:hk:kk]
303	eAAC/mAAC-s01	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	32	7	7	1	***	[nn:np]
304	eAAC/mAAC-s02	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	35	2	7.1	1	***	[nn:np]
305	eAAC/mAAC-s03	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	27	24	2	0.2	1	-	[nn:np]
306	eAAC/mAAC-s04	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	27	25	1	0.1	1	-	[nn:np]
307	eAAC/mAAC-s05	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	27	25	1	0.1	1	-	[nn:np]
308	eAAC/mAAC-s06	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	26	26	1	0	1	-	[nn:np]
309	eAAC/mAAC-t01	<hkxhk>	0	0	0	0	0	0	0	0	10	37	0	0	0	0	0	0	0	6	19.8	2	*******	[hh:hk:kk]
310	eAAC/mAAC-t02	<hkxhk>	0	0	0	0	0	0	0	0	13	39	0	0	0	0	0	0	0	1	19.5	2	*******	[hh:hk:kk]
311	eAAC/mAAC-t03	<hkxhk>	0	0	0	0	0	0	0	0	17	35	0	0	0	0	0	0	0	1	17.4	2	******	[hh:hk:kk]
312	eAAC/mAAC-t04	<hkxhk>	0	0	0	0	0	0	0	0	9	43	0	0	0	0	0	0	0	1	25.4	2	*******	[hh:hk:kk]
313	eAAC/mAAC-t05	<hkxhk>	0	0	0	0	0	0	0	0	11	41	0	0	0	0	0	0	0	1	22	2	*******	[hh:hk:kk]
314	eAAC/mAAC-t06	<hkxhk>	0	0	0	0	0	0	0	0	17	35	0	0	0	0	0	0	0	1	17.4	2	******	[hh:hk:kk]
315	eAAC/mAAC-t07	<hkxhk>	0	0	0	0	0	0	0	0	10	42	0	0	0	0	0	0	0	1	23.5	2	*******	[hh:hk:kk]
316	eAAC/mAAC-t08	<hkxhk>	0	0	0	0	0	0	0	0	23	29	0	0	0	0	0	0	0	1	21	2	*******	[hh:hk:kk]
317	eAAC/mACC-s152	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18	23	12	0.6	1	-	[nn:np]
318	eAAC/mACC-s119	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	17	24	12	1.2	1	-	[nn:np]
319	eAAC/mACC-s107	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	23	18	12	0.6	1	-	[nn:np]
320	eAAC/mACC-s101	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	27	12	4.1	1	**	[nn:np]
321	eAAC/mACC-s096	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	17	24	12	1.2	1	-	[nn:np]
322	eAAC/mACC-t450	<hkxhk>	0	0	0	0	0	0	0	0	14	27	0	0	0	0	0	0	0	12	13.7	2	****	[hh:hk:kk]
323	eAAC/mACC-t310	<hkxhk>	0	0	0	0	0	0	0	0	10	31	0	0	0	0	0	0	0	12	15.6	2	******	[hh:hk:kk]
324	eAAC/mACC-t265	<hkxhk>	0	0	0	0	0	0	0	0	16	25	0	0	0	0	0	0	0	12	14.5	2	*****	[hh:hk:kk]
325	eAAC/mACC-t203	<hkxhk>	0	0	0	0	0	0	0	0	8	33	0	0	0	0	0	0	0	12	18.4	2	******	[hh:hk:kk]
326	eAAC/mACC-t128	<hkxhk>	0	0	0	0	0	0	0	0	12	29	0	0	0	0	0	0	0	12	14.1	2	*****	[hh:hk:kk]
327	eAAC/mACT-s220	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25	28	0	0.2	1	-	[nn:np]
328	eAAC/mACT-s182	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	21	32	0	2.3	1	-	[nn:np]
329	eAAC/mACT-s157	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	29	24	0	0.5	1	-	[nn:np]
330	eAAC/mACT-s137	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	31	22	0	1.5	1	-	[nn:np]

331	eAAC/mACT-s123	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	27	26	0	0	1	-	[nn:np]
332	eAAC/mACT-s095	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	35	18	0	5.5	1	**	[nn:np]
333	eAAC/mACT-t384	<hkxhk>	0	0	0	0	0	0	0	0	21	28	0	0	0	0	0	0	0	4	19	2	*******	[hh:hk:kk]
334	eAAC/mACT-t240	<hkxhk>	0	0	0	0	0	0	0	0	24	29	0	0	0	0	0	0	0	0	22.2	2	*******	[hh:hk:kk]
335	eAAC/mACT-t215	<hkxhk>	0	0	0	0	0	0	0	0	23	30	0	0	0	0	0	0	0	0	20.9	2	*******	[hh:hk:kk]
336	eAAC/mACT-t103	<hkxhk>	0	0	0	0	0	0	0	0	15	38	0	0	0	0	0	0	0	0	18.5	2	*******	[hh:hk:kk]
337	eAAC/mAGT-s242	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	22	29	2	1	1	-	[nn:np]
338	eAAC/mAGT-s240	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	48	1	37.2	1	*******	[nn:np]
339	eAAC/mAGT-s194	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	31	2	2.4	1	-	[nn:np]
340	eAAC/mAGT-s163	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	39	12	2	14.3	1	******	[nn:np]
341	eAAC/mAGT-s098	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	22	31	0	1.5	1	-	[nn:np]
342	eAAC/mAGT-t455	<hkxhk>	0	0	0	0	0	0	0	0	23	27	0	0	0	0	0	0	0	3	21.5	2	*******	[hh:hk:kk]
343	eAAC/mAGT-t280	<hkxhk>	0	0	0	0	0	0	0	0	13	38	0	0	0	0	0	0	0	2	18.9	2	*******	[hh:hk:kk]
344	eAAC/mAGT-t254	<hkxhk>	0	0	0	0	0	0	0	0	15	36	0	0	0	0	0	0	0	2	17.5	2	******	[hh:hk:kk]
345	eAAC/mAGT-t245	<hkxhk>	0	0	0	0	0	0	0	0	14	38	0	0	0	0	0	0	0	1	18.6	2	*******	[hh:hk:kk]
346	eAAC/mAGT-t201	<hkxhk>	0	0	0	0	0	0	0	0	21	31	0	0	0	0	0	0	0	1	18.9	2	*******	[hh:hk:kk]
347	eAAC/mAGT-t176	<hkxhk>	0	0	0	0	0	0	0	0	15	36	0	0	0	0	0	0	0	2	17.5	2	******	[hh:hk:kk]
348	eAAC/mAGT-t149	<hkxhk>	0	0	0	0	0	0	0	0	12	39	0	0	0	0	0	0	0	2	19.9	2	*******	[hh:hk:kk]
349	eAAC/mAGT-t133	<hkxhk>	0	0	0	0	0	0	0	0	9	42	0	0	0	0	0	0	0	2	24.5	2	*******	[hh:hk:kk]
350	eAAC/mAGT-t128	<hkxhk>	0	0	0	0	0	0	0	0	9	44	0	0	0	0	0	0	0	0	26.2	2	*******	[hh:hk:kk]
351	eAAC/mAGT-t125	<hkxhk>	0	0	0	0	0	0	0	0	10	42	0	0	0	0	0	0	0	1	23.5	2	*******	[hh:hk:kk]
352	eAAC/mATG-s01	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	37	15	1	9.3	1	****	[nn:np]
353	eAAC/mATG-s02	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	45	0	25.8	1	*******	[nn:np]
354	eAAC/mATG-s03	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	37	0	8.3	1	****	[nn:np]
355	eAAC/mATG-s04	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	31	21	1	1.9	1	-	[nn:np]
356	eAAC/mATG-s05	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25	28	0	0.2	1	-	[nn:np]
357	eAAC/mATG-s06	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	38	0	10	1	****	[nn:np]
358	eAAC/mATG-s07	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	33	0	3.2	1	*	[nn:np]
359	eAAC/mATG-s08	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	26	26	1	0	1	-	[nn:np]
360	eAAC/mATG-s09	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	27	26	0	0	1	-	[nn:np]
361	eAAC/mATG-t01	<hkxhk>	0	0	0	0	0	0	0	0	12	41	0	0	0	0	0	0	0	0	21.3	2	*******	[hh:hk:kk]
362	eAAC/mATG-t02	<hkxhk>	0	0	0	0	0	0	0	0	11	42	0	0	0	0	0	0	0	0	22.7	2	*******	[hh:hk:kk]
363	eAAC/mATG-t03	<hkxhk>	0	0	0	0	0	0	0	0	8	45	0	0	0	0	0	0	0	0	28.3	2	*******	[hh:hk:kk]
364	eAAC/mATG-t04	<hkxhk>	0	0	0	0	0	0	0	0	15	37	0	0	0	0	0	0	0	1	18	2	******	[hh:hk:kk]
365	eAAC/mATG-t05	<hkxhk>	0	0	0	0	0	0	0	0	6	47	0	0	0	0	0	0	0	0	33.1	2	*******	[hh:hk:kk]
366	eAAC/mATG-t06	<hkxhk>	0	0	0	0	0	0	0	0	10	43	0	0	0	0	0	0	0	0	24.3	2	*******	[hh:hk:kk]
367	eAAC/mCAG-s324	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	28	25	0	0.2	1	-	[nn:np]
368	eAAC/mCAG-s238	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	24	29	0	0.5	1	-	[nn:np]
369	eAAC/mCAG-t224	<hkxhk>	0	0	0	0	0	0	0	0	11	42	0	0	0	0	0	0	0	0	22.7	2	*******	[hh:hk:kk]
370	eAAC/mCAG-t196	<hkxhk>	0	0	0	0	0	0	0	0	8	45	0	0	0	0	0	0	0	0	28.3	2	*******	[hh:hk:kk]
371	eAAC/mCAG-t149	<hkxhk>	0	0	0	0	0	0	0	0	8	45	0	0	0	0	0	0	0	0	28.3	2	*******	[hh:hk:kk]
372	eAAC/mCAG-t123	<hkxhk>	0	0	0	0	0	0	0	0	15	38	0	0	0	0	0	0	0	0	18.5	2	*******	[hh:hk:kk]

373	eAAC/mCAG-t107	<hkxhk>	0	0	0	0	0	0	0	0	15	38	0	0	0	0	0	0	0	0	18.5	2	*******	[hh:hk:kk]
374	eAAC/mGAA-s425	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	26	17	10	1.9	1	-	[nn:np]
375	eAAC/mGAA-s420	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	29	10	5.2	1	**	[nn:np]
376	eAAC/mGAA-s112	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	22	22	9	0	1	-	[nn:np]
377	eAAC/mGAA-t495	<hkxhk>	0	0	0	0	0	0	0	0	13	30	0	0	0	0	0	0	0	10	14.6	2	*****	[hh:hk:kk]
378	eAAC/mGAA-t240	<hkxhk>	0	0	0	0	0	0	0	0	9	34	0	0	0	0	0	0	0	10	18.3	2	******	[hh:hk:kk]
379	eAAC/mGAA-t238	<hkxhk>	0	0	0	0	0	0	0	0	13	30	0	0	0	0	0	0	0	10	14.6	2	*****	[hh:hk:kk]
380	eAAC/mGAA-t228	<hkxhk>	0	0	0	0	0	0	0	0	17	26	0	0	0	0	0	0	0	10	15.3	2	******	[hh:hk:kk]
381	eAAC/mGAA-t226	<hkxhk>	0	0	0	0	0	0	0	0	21	22	0	0	0	0	0	0	0	10	20.5	2	*******	[hh:hk:kk]
382	eAAC/mGAA-t224	<hkxhk>	0	0	0	0	0	0	0	0	10	33	0	0	0	0	0	0	0	10	16.9	2	******	[hh:hk:kk]
383	eAAC/mGAA-t200	<hkxhk>	0	0	0	0	0	0	0	0	5	38	0	0	0	0	0	0	0	10	26.5	2	*******	[hh:hk:kk]
384	eAAC/mGAA-t140	<hkxhk>	0	0	0	0	0	0	0	0	12	27	0	0	0	0	0	0	0	14	13.2	2	****	[hh:hk:kk]
385	eAAC/mGAA-t113	<hkxhk>	0	0	0	0	0	0	0	0	17	26	0	0	0	0	0	0	0	10	15.3	2	******	[hh:hk:kk]
386	eAAC/mGAA-t082	<hkxhk>	0	0	0	0	0	0	0	0	6	38	0	0	0	0	0	0	0	9	24.9	2	*******	[hh:hk:kk]
387	eAAC/mGAT-s540	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	17	29	7	3.1	1	*	[nn:np]
388	eAAC/mGAT-s439	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	26	19	8	1.1	1	-	[nn:np]
389	eAAC/mGAT-s360	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	39	10	4	17.2	1	*******	[nn:np]
390	eAAC/mGAT-t468	<hkxhk>	0	0	0	0	0	0	0	0	15	29	0	0	0	0	0	0	0	9	14.7	2	*****	[hh:hk:kk]
391	eAAC/mGAT-t320	<hkxhk>	0	0	0	0	0	0	0	0	10	39	0	0	0	0	0	0	0	4	21.2	2	*******	[hh:hk:kk]
392	eAAC/mGAT-t183	<hkxhk>	0	0	0	0	0	0	0	0	7	46	0	0	0	0	0	0	0	0	30.6	2	*******	[hh:hk:kk]
393	eAAC/mGAT-t163	<hkxhk>	0	0	0	0	0	0	0	0	28	25	0	0	0	0	0	0	0	0	29.8	2	*******	[hh:hk:kk]
394	eAAC/mGAT-t159	<hkxhk>	0	0	0	0	0	0	0	0	26	27	0	0	0	0	0	0	0	0	25.5	2	*******	[hh:hk:kk]
395	eAAC/mGAT-t132	<hkxhk>	0	0	0	0	0	0	0	0	4	49	0	0	0	0	0	0	0	0	38.8	2	*******	[hh:hk:kk]
396	eACA/mAAT-s384	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	19	20	0.8	1	-	[nn:np]
397	eACA/mAAT-s319	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	27	20	13.4	1	******	[nn:np]
398	eACA/mAAT-s268	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	26	20	10.9	1	*****	[nn:np]
399	eACA/mAAT-s228	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	24	20	6.8	1	***	[nn:np]
400	eACA/mAAT-s182	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	22	20	3.7	1	*	[nn:np]
401	eAAC/mAAT-s163	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	21	12	20	2.5	1	-	[nn:np]
402	eACA/mAAT-s148	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	17	20	0	1	-	[nn:np]
403	eACA/mAAT-s094	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	23	20	5.1	1	**	[nn:np]
404	eACA/mAAT-t273	<hkxhk>	0	0	0	0	0	0	0	0	20	13	0	0	0	0	0	0	0	20	25.7	2	*******	[hh:hk:kk]
405	eACA/mAAT-t252	<hkxhk>	0	0	0	0	0	0	0	0	15	18	0	0	0	0	0	0	0	20	13.9	2	*****	[hh:hk:kk]
406	eACA/mAAT-t203	<hkxhk>	0	0	0	0	0	0	0	0	19	14	0	0	0	0	0	0	0	20	22.6	2	*******	[hh:hk:kk]
407	eACA/mACG-s348	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19	33	1	3.8	1	*	[nn:np]
408	eACA/mACG-t249	<hkxhk>	0	0	0	0	0	0	0	0	26	27	0	0	0	0	0	0	0	0	25.5	2	*******	[hh:hk:kk]
409	eACA/mACG-t248	<hkxhk>	0	0	0	0	0	0	0	0	10	42	0	0	0	0	0	0	0	1	23.5	2	*******	[hh:hk:kk]
410	eACA/mATT-s01	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	27	24	2	0.2	1	-	[nn:np]
411	eACA/mATT-s02	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	24	27	2	0.2	1	-	[nn:np]
412	eACA/mATT-s03	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	29	23	1	0.7	1	-	[nn:np]
413	eACA/mATT-s04	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	42	1	19.7	1	*******	[nn:np]
414	eACA/mATT-s05	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25	27	1	0.1	1	-	[nn:np]

415	eACA/mATT-s06	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	35	17	1	6.2	1	**	[nn:np]
416	eACA/mATT-s07	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	29	23	1	0.7	1	-	[nn:np]
417	eACA/mATT-s08	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	17	36	0	6.8	1	***	[nn:np]
418	eACA/mATT-t01	<hkxhk>	0	0	0	0	0	0	0	0	28	23	0	0	0	0	0	0	0	2	31.2	2	*******	[hh:hk:kk]
419	eACA/mATT-t02	<hkxhk>	0	0	0	0	0	0	0	0	27	25	0	0	0	0	0	0	0	1	28.1	2	*******	[hh:hk:kk]
420	eACA/mATT-t03	<hkxhk>	0	0	0	0	0	0	0	0	29	23	0	0	0	0	0	0	0	1	33	2	*******	[hh:hk:kk]
421	eACA/mATT-t04	<hkxhk>	0	0	0	0	0	0	0	0	18	34	0	0	0	0	0	0	0	1	17.4	2	******	[hh:hk:kk]
422	eACA/mATT-t05	<hkxhk>	0	0	0	0	0	0	0	0	15	37	0	0	0	0	0	0	0	1	18	2	******	[hh:hk:kk]
423	eACA/mATT-t06	<hkxhk>	0	0	0	0	0	0	0	0	15	38	0	0	0	0	0	0	0	0	18.5	2	*******	[hh:hk:kk]
424	eACA/mATT-t07	<hkxhk>	0	0	0	0	0	0	0	0	11	42	0	0	0	0	0	0	0	0	22.7	2	*******	[hh:hk:kk]
425	eACA/mATT-t08	<hkxhk>	0	0	0	0	0	0	0	0	11	42	0	0	0	0	0	0	0	0	22.7	2	*******	[hh:hk:kk]
426	eACA/mATT-t09	<hkxhk>	0	0	0	0	0	0	0	0	12	41	0	0	0	0	0	0	0	0	21.3	2	*******	[hh:hk:kk]
427	eACA/mATT-t10	<hkxhk>	0	0	0	0	0	0	0	0	8	45	0	0	0	0	0	0	0	0	28.3	2	*******	[hh:hk:kk]
428	eACA/mCAG-s178	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	34	16	3	6.5	1	**	[nn:np]
429	eACA/mCAG-s130	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13	37	3	11.5	1	*****	[nn:np]
430	eACA/mCAG-s086	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	29	21	3	1.3	1	-	[nn:np]
431	eACA/mCAG-s082	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	29	21	3	1.3	1	-	[nn:np]
432	eACA/mCAG-t460	<hkxhk>	0	0	0	0	0	0	0	0	20	25	0	0	0	0	0	0	0	8	18.3	2	******	[hh:hk:kk]
433	eACA/mCAG-t264	<hkxhk>	0	0	0	0	0	0	0	0	14	35	0	0	0	0	0	0	0	4	17	2	******	[hh:hk:kk]
434	eACA/mCAG-t198	<hkxhk>	0	0	0	0	0	0	0	0	18	32	0	0	0	0	0	0	0	3	16.9	2	******	[hh:hk:kk]
435	eACA/mCAG-t137	<hkxhk>	0	0	0	0	0	0	0	0	8	42	0	0	0	0	0	0	0	3	25.7	2	*******	[hh:hk:kk]
436	eACA/mCAG-t112	<hkxhk>	0	0	0	0	0	0	0	0	17	33	0	0	0	0	0	0	0	3	16.7	2	******	[hh:hk:kk]
437	eACA/mCTG-s01	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	29	23	1	0.7	1	-	[nn:np]
438	eACA/mCTG-t01	<hkxhk>	0	0	0	0	0	0	0	0	20	32	0	0	0	0	0	0	0	1	18.1	2	******	[hh:hk:kk]
439	eACA/mCTG-t02	<hkxhk>	0	0	0	0	0	0	0	0	13	39	0	0	0	0	0	0	0	1	19.5	2	*******	[hh:hk:kk]
440	eACA/mCTG-t03	<hkxhk>	0	0	0	0	0	0	0	0	26	26	0	0	0	0	0	0	0	1	26	2	*******	[hh:hk:kk]
441	eACA/mCTG-t04	<hkxhk>	0	0	0	0	0	0	0	0	13	39	0	0	0	0	0	0	0	1	19.5	2	*******	[hh:hk:kk]
442	P27-1	<hkxhk>	0	0	0	0	0	0	0	0	21	16	0	0	0	0	0	0	0	16	24.5	2	*******	[hh:hk:kk]
443	P27-2	<hkxhk>	0	0	0	0	0	0	0	0	4	33	0	0	0	0	0	0	0	16	23.6	2	*******	[hh:hk:kk]
444	P27-3	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	31	16	16.9	1	*******	[nn:np]
445	P27-4	<hkxhk>	0	0	0	0	0	0	0	0	8	29	0	0	0	0	0	0	0	16	15.4	2	******	[hh:hk:kk]
446	P27-5	<hkxhk>	0	0	0	0	0	0	0	0	26	11	0	0	0	0	0	0	0	16	42.6	2	*******	[hh:hk:kk]
447	P27-6	<hkxhk>	0	0	0	0	0	0	0	0	22	15	0	0	0	0	0	0	0	16	27.5	2	*******	[hh:hk:kk]
448	P21-1	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	20	13	0	1	-	[nn:np]
449	P21-2	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	23	17	13	0.9	1	-	[nn:np]
450	P21-3	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	23	17	13	0.9	1	-	[nn:np]
451	P21-4	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19	21	13	0.1	1	-	[nn:np]
452	P21-5	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25	15	13	2.5	1	-	[nn:np]
453	P21-6	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	28	12	13	6.4	1	**	[nn:np]
454	OPA3-1	<hkxhk>	0	0	0	0	0	0	0	0	25	14	0	0	0	0	0	0	0	14	35.1	2	*******	[hh:hk:kk]
455	OPA3-2	<hkxhk>	0	0	0	0	0	0	0	0	23	16	0	0	0	0	0	0	0	14	28.4	2	*******	[hh:hk:kk]
456	OPA3-3	<hkxhk>	0	0	0	0	0	0	0	0	9	30	0	0	0	0	0	0	0	14	15.5	2	******	[hh:hk:kk]

457	OPA3-4	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	24	15	14	2.1	1	-	[nn:np]
458	OPA3-5	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19	20	14	0	1	-	[nn:np]
459	STMS8fp/rpa	<hkxhk>	0	0	0	0	0	0	0	0	12	29	0	0	0	0	0	0	0	12	14.1	2	*****	[hh:hk:kk]
460	STMS8fp/rpb	<hkxhk>	0	0	0	0	0	0	0	0	8	34	0	0	0	0	0	0	0	11	19.1	2	*******	[hh:hk:kk]
461	AGMI9-93a	<hkxhk>	0	0	0	0	0	0	0	0	2	48	0	0	0	0	0	0	0	3	42.5	2	*******	[hh:hk:kk]
462	AGMI9-93b	<nnxnp>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	29	21	3	1.3	1	-	[nn:np]
463	AGMI10-103a	<hkxhk>	0	0	0	0	0	0	0	0	4	41	0	0	0	0	0	0	0	8	31.1	2	*******	[hh:hk:kk]
464	AGMI10-103b	<hkxhk>	0	0	0	0	0	0	0	0	9	36	0	0	0	0	0	0	0	8	19.8	2	*******	[hh:hk:kk]
465	AGMI35/6a	<hkxhk>	0	0	0	0	0	0	0	0	5	37	0	0	0	0	0	0	0	11	25.6	2	*******	[hh:hk:kk]
466	AGMI35/6b	<hkxhk>	0	0	0	0	0	0	0	0	2	40	0	0	0	0	0	0	0	11	34.6	2	*******	[hh:hk:kk]
467	AGMI95/6a	<hkxhk>	0	0	0	0	0	0	0	0	11	36	0	0	0	0	0	0	0	6	18.4	2	*******	[hh:hk:kk]
468	AGMI95/6b	<hkxhk>	0	0	0	0	0	0	0	0	3	44	0	0	0	0	0	0	0	6	36.1	2	*******	[hh:hk:kk]
469	AGMI101/2a	<hkxhk>	0	0	0	0	0	0	0	0	9	40	0	0	0	0	0	0	0	4	22.9	2	*******	[hh:hk:kk]
470	AGMI101/2b	<hkxhk>	0	0	0	0	0	0	0	0	9	40	0	0	0	0	0	0	0	4	22.9	2	*******	[hh:hk:kk]
471	AGMI105/8b	<hkxhk>	0	0	0	0	0	0	0	0	8	34	0	0	0	0	0	0	0	11	19.1	2	*******	[hh:hk:kk]

References

Afele, J.C. and De Langhe, E. (1991). Increasssing *in vitro* germination of *Musa balbisiana* seed. *Plant Cell, Tissue and Organ Culture*, **27**, 33-36.

Arias, P., Dankers, C., Liu, P. and Pilkauskas, P. (2003). Overview of world banana profuction and trade. In: *The World Banana Economy (1985-2002).* Food and Agriculture Organization of The United Nations, Rome, Italy. Available online at **http://www.fao.org/docrep/007/y5102e00.HTM**

Asif, M.J., Mak C., and Othman R.Y. (2001). *In Vitro* Zygotic embryo culture studies of *Musa acuminata* ssp. *malaccensis* and factors affecting germination and seedling growth. *Plant Cell Tissue and Organ Culture*, **67**(3), 267-270.

Asif, M.J. (2004). *Study of Resistance in Musa acuminata ssp. malaccensis wild seed population to Fusarium oxysporum f. sp. cubense race 4 and further characterization using RAPD markers.* Thesis, (PhD). University of Malaya.

Asif, M.J. and Othman, R.Y. (2005). Characterization of Fusarium Wilt-Resistant and Fusarium Wilt-Susceptible Somaclones of Banana Cultivar Rastali (*Musa* AAB) by Random Amplified DNA and Retrotransposon Markers. *Plant Molecular Biology Reporter*, **23**, 241-249.

Asíns, M.J. (2002). Present and future of quantitative trait locus analysis in plant breeding. *Plant Breeding*, **121**, 281-291.

Bakry, F., Horry, J.P., Teisson, C., Tezenas Du Montcel, H. and Ganry, J. (1990). L'amelioration genetique des bananiers a L'IRPA/CIRAD. Fruits numero special, 25-40.

Bakry, F., Haïcour, R., Horry, J.P., Megia, R. and Rossignol, L. (1993). Applications of Biotechnologies to Banana Breeding. *In*: *Proceeding of the workshop on Biotechnology Applications for Banana and Plantain Improvement*, 27-31 Jan 1992, San José, Costa Rica. Available online at
http://bananas.bioversityinternational.org/files/files/pdf/publications/biotechnology.pdf

Bakry, F., Carreel, F., Caruana, M.L., Côte, F.X., Jenny, C. and Montcel, H.T. (2001). Banana. *In: Charrier, A., Jacquot, M., Harmon, S., Nicolas, D. and Razdan, M.K. (*eds). *Tropical Breeding*. CIRAD Scientific Publishers, Inc. 1-29.

Baldoni, L., Angiolillo, A., Pellegrini, M. and Mencuccini, M. (1999). A linkage genome map for olive as an important tool for marker-assisted selection. *In: Metzidakis, I.T. and Voyiatzi,G.G.* (eds). *Acta Horticulturae*, **474**, 111-115.

Barcaccia G., Albertini, E., Tavoletti, S., Falcinelli, M. and Veronesi, F. (1999). AFLP fingerprinting in *Medicago* spp.: Its development and application in linkage mapping, *Plant Breeding*, **118**, 335-340.

Becker, J., Vos, P., Kuiper, M., Salamini, F. And Heun, M. (1995). Combined mapping of AFLP and RFLP markers in barley. *Molecular and General Genetics*, **249**, 65-73.

Beckman, C.H. (1987). *The Nature of Wilt Disease of Plants*. St Paul, MN, USA: APS Press.

Beckman, C.H. (1990). Host responses to the pathogen. *In*: Ploetz, R.C., *Fusarium wilt of Banana*. APS Press. St. Paul, Minnesota, 93-105.

Bentley, S., Pegg, K.G., Moore, N.Y., Davis, R.D. and Buddenhagen, I.W. (1998). Genetic Variation Among Vegetative Compatibility Groups of *Fusarium oxysporum* f. sp. *cubense* Analyzed by DNA Fingerprinting. *Phytopathology*, **88**, 1283-1293.

Bentley, S., Moore, N.Y., Pegg, K.G., Gerlach, K.S. and Smith, L.J. (1999). Genetic characterization and detection of fusarium wilt. *In*: *International Seminar & Workshop on the Banana Fusarium Wilt Disease*, 18-20 October 1999, Genting Highland Resort, Malaysia, 143-151.

Bert, P.F., Charmet, G., Sourdille, P., Hayward, M.D. and Balfourier, F. (1999). A high-density molecular map for ryegrass (*Lolium perenne*) using AFLP markers. *Theoretical Applied Genetic*, **99**, 445-452.

Bhat, K.V. and Jarret, R.L. (1995). Random amplified polymorphic DNA and genetic diversity in Indian *Musa* germplasm. *Genetic Resources and Crop Evolution*, **42**, 107-118

Billotte, N. Marseillac, N., Risterucci, A.M., Adon, B., Brottier, P., Baurens, F.C., Singh, R., Herrán, A., Asmady, H., Billot, C., Amblard, P., Durand-Gasselin, T., Courtois, B., Asmono, D., Cheah, S.C., Rohde, W., Ritter, E. and Charrier, A. (2005). Microsatellte-based high density linkage map in oil palm (*Elaeis guineensis* Jacq.), *Theoretical Applied Genetics*, **110**, 754-765.

Bohn, M., Friedrich, U. and Melchinger, A.E. (1999). Genetic Similarities among Winter Wheat Cultivars Determined on the Basis of RFLPs, AFLPs, and SSRs and Their Use for Predicting Progeny Variance, *Crop Science*, **39**, 228-237.

Boluarte, T. (1999). *Bulk segregant analysis for anther culture response and leptine content in backcross families of diploid potato.* Thesis (PhD). Virginia Polytechnique Institute and State University.

Brake, V.M. Pegg, K.G., Irwin, J.A.G. and Chaseling, J. (1995). The influence of Temperature, Inoculum levels and race of *Fusarium oxysporum* f. sp. *cubense* on the disease reaction of banana cv. Cavendish. *Australian Journal of Agricultural Research*, **46**, 673-685.

Broman, K.W. (2001). Review of statical methods for QTL mapping in experimental crosses, *Laboratory Animals*, **30**(7), 44-52.

Brown, J.K.M. (1996). The choice of molecular marker methods for population genetic studies of plant pathogens, *New Phytologist*, **133**, 183-195.

Buddenhagen, I.W. (1990). Banana breeding and *Fusarium* wilt. *In*: *Ploetz, R.C.* (ed). *Fusarium Wilt of Banana,* APS Press, St. Paul, 107-113.

Buxton, E. W. (1962). Parasexual recombination in the banana-wilt Fusarium. *Transactions of British Mycological Society*, **45**, 274-279.

Cai, H., Inoue, M., Yuyama, N. and Nakayama, S. (2004). An AFLP-based linkage map of Zoysiagrass (*Zoysia japonica*), *Plant Breeding*, **123**, 543-548.

Carreel, F. (1994). *Etude de la diversité génétique des bananiers (genre Musa) à l'aide des marqueurs RFLP*. Thesis (PhD). Institut National Agronomique Paris-Grignon, Paris, France.

Caruana, M.L. (1992). Principal Virus Diseases of banana. *In*: *Ganry, J.* ed. *The Proceeding of the International Symposium on Genetic Improvement of Bananas for Resistance to Diseases and Pests*, 7-9 September, 1992, CIRAD, Montpellier, France, 99-103.

Cato, S.A., Corbett, G.G. and Richardson, T.E. (1999). Evaluation of AFLP for genetic mapping in *Pinus radiata*, *Molecular Breeding*, **5**, 275-281.

Cervera, M. T., Storme, V., Ivens, B., Gusmão, J., Liu, B.H., Hostyn, V., Slycken, J.V., Montagu, m.V. and Boerjan, W. (2001). Dense Genetic Linkage Maps of Three Populus Species (*Populus deltoids*, *P. nigra* and *P. trichocarpa*) Based on AFLP and Microsatellite Markers, *Genetics*, **158**, 787-809.

Chai, Y.T., Chai, M. and Saad, M. S. (1990). *Pembiakbakaan Tumbuhan*. Dewan Bahasa dan Pustaka.

Chin, H.F. (1996). Germination and Storage of Banana Seeds. *In*: *Frison, E.A., Horry, J.P. and De Waele, D.* (Eds). *Proceeding on the workshop on New Frontiers in Resistance Breeding for Nematodes, Fusarium and Sigatoka*, 2-5 October 1995, Kuala Lumpur Malaysia, 218-227.

Cho, Y.G., Blair, M.W., Panaud, O. and McCouch S.R. (1996). Cloning and mapping of variety specific rice genomic DNA sequences: amplified fragment length polymorphism (AFLP) from silver stained polyacrylamide gels, *Genome*, **9**, 373-378.

Christou, P. (1996). Transformation Technology, *Reviews in Trends in Plant Science*, **1**(12), 423-431.

Churchill, G.A., Giovannoni, J.J. and Tanksley, S.D. (1993). Pooled-sampling makes high-resolution mapping practical with DNA markers. *Proceedings of the National Academic Sciences of the United States of America*, **90**, 16-20.

Collard, B.C.Y., Juhufer, M.Z.Z., Brouwer, J.B. and Pang, E.C.K. (2005). An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts, *Euphytica*, **142**, 169-196.

Creste, S. Neto, T.A., and Fiqueira, A. (2001). Detection of Single Sequence Repeat Polymorphisms in Denaturing Polyacrylamide Sequencing Gels by Silver Staining, *Plant Molecular Biology Reporter*, **19**, 299-306.

Creste, S. Neto, T. A., Silva, S.O. and Fiqueira, A. (2003). Genetic characterization of banana cultivars (*Musa* spp.) from Brazil using microsatellite markers, *Euphytica*, **132**, 259-268.

Crouch, H.K. Crouch, J.H., Jarret, R.L., Cregan, P.B. and Ortiz, R. (1998a). Segregation of microsatellite loci from haploid and diploid gametes in *Musa*, *Crop Science*, **38**, 211-217.

Crouch, J.H., Vuylsteke, D. and Ortiz, R. (1998b). Perspectives on the application of biotechnology to assist the genetic enhancement of plantain and banana (*Musa* spp.). *Electronic Journal of Biotechnology*, **1** (1), 11-22.

Crouch, J.H., Ortiz, R., Crouch, H.K., Ford-Lloyd, B.V, Howell, E.C. Newbury, H.J. and Jarret, R.L. (1998c). Utilization of molecular genetic techniques in support of plantain and banana improvement, *Acta Horticulturae*, **540**, 185-191.

Crouch, J.H., Crouch, H.K, Constandt, H., Van Gysel, A., Breyne, P., Van Montagu, M. Jarret, R.L. and Ortiz, R. (1999a). Comparison of PCR-based molecular marker analysis of *Musa* breeding populations, *Molecular Breeding*, **5**, 233-244.

Crouch, J.H., Ortiz, R., Crouch, H.K., Tenkouano, A. (1999b). VNTR-based diversity analysis of 2x and 4x full-sib Musa hybrids, *Electronic Journal of Biotechnology*, **2**, 130-139.

Daniells, J. (2006). New banana cultivars with market potential. *In*: *Proceeding of the XVII Reunião international da Associaçã para a Cooperaçã0 nas Pesquisas sobre Banana no Caribe e na América Tropical, Joinville*, 15-20 October 2006, Santa Catarina, Brasil, 284-288.

Daniells, J., Jenny, C., Karamura, D. and Tomekpe, K. (2001). Musalogue: A catalogue of *Musa* germplasm. Diversity in the genus *Musa. In: E. Arnaud & S.Sharrock*, (eds). INIBAP, Montpellier, France. Available online at http://bananas.bioversityinternational.org/file/files/pdf/publications/musalogue2.pdf

Davis, R. (2005). *Fusarium wilt (Panama disease) of banana.* Secretariat of the Pacific Community, Suva, Fiji Islands. Pest Advisory Leaflet No. 42.

Dekkers, J.C.M. (2004). Commercial application of marker- and gene-assisted selection in livestock: Strategies and lessons, *Journal of Animal Science*, **82**, E313-328.

De Langhe, E. (1992). Genetic Improvement of Banana and Plantain: the New Era. *In*: *Ganry, J.* ed. *The Proceeding of the International Symposium on Genetic Improvement of Bananas for Resistance to Diseases and Pests*, 7-9 September, 1992, CIRAD, Montpellier, France, 1-9.

De Langhe, E. (1995). *Banana and Plantain: The Earliest Fruit Crops?* INIBAP, Montpellier, France. INIBAP Annual Report 1995, 6-9. Available online at http://bananas.bioversityinternational.org/file/files/pdf/publications/ar95_en.pdf

De Léon, D.G. and Fauré, S. (1993). Genetic Mapping of the Banana Diploid Genome: toward an integrated approach to the study of the *Musa* genome and the use of molecular marker technologies in *Musa* breeding. *In*: *The Proceeding of the*

workshop on Biotechnology Applications for Banana and Plantain Improvement, 27-31 Jan 1992, San José, Costa Rica. Available online at http://bananas.bioversityinternational.org/files/files/pdf/publications/biotechnology.pdf

Doyle, J.J., and J. L. Doyle (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue, *Phytochem Bulletin*, **19**, 11-15.

Dreher, K., Morris, M., Khairallah, M., Ribaut, J-M., Pandey, S. and Srinivasan, G. (2000). Is Marker-Assisted Selection Cost-Effective Compared to Conventional Plant Breeding Methods? The Case of Quality Protein Maize. *In*: *The Fourth Annual Conference of the International Consortium on Agricultural Biotechnological Research (ICABR), `The Economic of Agricultural Biotechnology'* 24-28 August, 2000, Ravello, Italy, 1-25. Available online at http://www.cimmyt.org/research/economics/map/research_results/other_tech/marker_assisted.pdf

Ellis, R.P., McNicol, J.W., Baird, E., Booth, A., Lawrence, P., Thomas, B. and Powell, W. (1997). The use of AFLPs to examine genetic relatedness in barley, *Molecular Breeding*, **3**, 359 – 369.

Engelborghs, I., Swennen, R. and van Campenhout, S. (1998). The potential of AFLP to detect genetic differences and somaclonal variants in *Musa* spp., *Infomusa*, **7**, 3-6.

Escalant, J.V. and Teisson, C. (1987). Comportement in vitro de l'embryon isle du bananier (*Musa* spp.), *Fruits*, **42**, 333-342.

Escalant, J.E. and Panis, B. (2002). Biotechnologies toward the genetic improvement in *Musa*. *In*: *Acorbat. Memorias XV reunión. Realizada en Cartagena de Indias, Colombia*, 27 de octubre al 02 noviembre 2002. Medellin (COL): Asociación de Banananeros de Colombia AUGURA, 2002, 68-85. Available online at http://musalit.inibap.org/pdf/IN030008_en.pdf

Escalant, J.E., Sharrock, S. and Frison, E. (2002). *The genetic improvement of Musa using conventional breeding, and modern tools of molecular and cellular biology.* INIBAP Newsletter. December 2002. Available online at http://www.promusa.org/research/conventional-modern.pdf

Escalant, J.E. and Jain, S.M. (2004). Banana improvement with cellular and molecular biology, and induced mutations: Future and perspectives. *In*: *Jain, S.M. and Swennen, R.* (eds.) *Banana Improvement: Cellular, Molecular Biology and Mutagenesis Approaches.* Science publishers, New Hampshire, USA, 359-368. Available online at http://www.fao.org/docrep/007/ae216e/ae216e0w.htm

Escheverria, P.S., Dale, J., Khanna, H., Smith, M. and Collet, C. (2004). Potential resistance gene against ***Fusarium*** wilt race 4. *In*: *Picq, C. and Vezina, A. (eds). The 1st International Congress on* Musa*: Harnessing research to improve livelihoods.* 6 – 9 July 2004 Penang, Malaysia., 33

FAO (1998). Available online at http://apps.fao.org/lim 500/nph-wrap.pl?Production.Crops.Primary& Domain=SUA

Farooq, S. and Azam, F. (2002). Molecular Markers in Plant Breeding-II. Some Pre-requisites for Use, *Pakistan Journal of Biological Sciences*, **5**(10), 1141-1147.

Faturoti, B.O., Madukwe, M.C., Tenkouano, A. and Agwu, A.E. (2007). A review of policy acts and initiatives in plantain and banana innovation system in Nigeria, *African Journal of Biotechnology*, **6**(20), 2297-2302.

Fauré, S., Noyer, J.L., Horry, J.P., Bakry, F., Lanaud, C., De León, D. G. (1993). A molecular marker-based linkage map of diploid bananas, (*Musa acuminata*), *Theoretical Applied Genetics*, **87**, 517-526.

Fawcett, R. (1921). *The Banana: Its Cultivation, Distribution and Commercial Uses.* London Duckworth and Company, UK.

Ferreira C.F., Silva, S.O., Sobrinho, N.P.D., Darnascena, S.C.S., Oliveira Alves, S. A. and Pereira, O. (2004). Molecular Characterization of Banana (AA) Diploids with Contrasting Levels of black and Yellow Sigatoka Resistance, *American Journal of Applied Sciences*, **1**(4), 276-278.

Ford, R., Roux, K.L., Itman, C., Brouwer, J.B. and Taylor, P.W.J. (2002). Diversity analysis and genotyping in *Pisum* with sequence tagged microsatellite site (STMS) primers, *Euphytica*, **124**, 397-405.

Gallego, F.J., Calles, B. and Benito, C (1998). Molecular markers linked to the aluminium tolerance gene *Alt*1 in rye (*Secale cereale* L.), *Theoretical Applied Genetics*, **97**, 1104-1109.

Ganry, J. (1992). The STD Project for the Improvement of Bananas for Local and Export Markets. *In*: *Ganry, J.* (ed). *The Proceeding of the International Symposium on Genetic Improvement of Bananas for Resistance to Diseases and Pests*, 7-9 September, 1992, CIRAD, Montpellier, France, 11-19.

Gawel, N.J. and Jarret, R.L. (1991). Cytoplasmic genetic diversity in banana and plantain, *Euphytica*, **52**, 19-23.

Grapin, A., Noyer, J.L., Dambier, D., Baurens, F.C., Lanaud, C. and Lagoda, P.J.L. (1998). Diploid *Musa acuminata* genetic diversity assayed with sequence-tagged microsatellite sites, *Electrophoresis*, **19,** 1374-1380.

Grimm, D. (2008). Plant Genomics: A Bunch of Trouble, *Science*, **322**, 1046-1047. Available online at www.sciencemagorg

Groenewald, S., Van den Berg, N., Marasas, W.F.O. and Viljoen, A. (2006). The application of high-throughput AFLPs in assessing genetic diversity in *Fusarium oxysporum* f. sp. *cubense*, *Mycological Research*, **110**, 297-305.

Gupta, P.K. and Varshney, R.K. (2000). The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat, *Euphytica*, **113**, 163-185.

Gupta, P.K. (2002). Molecular markers and QTL analysis in crop plants, *Current Science*, **83**(2), 113-114.

Gupta, P.K. and Roy, J.K. (2002). Molecular markers in crop improvement: Present status and future needs in India, *Plant Cell, Tissue and Organ Culture*, **70**, 229-234.

Haines, R. (1994). *Biotechnology in forest tree improvement with special reference to developing countries.* FAO Forestry Paper, 118.

Hajjar, R. and Hodgkin, T. (2007). The use of wild relatives in crop improvement: A survey of development over the last 20 years, *Euphytica*, **156**, 1-13.

Hayes, A.J., Ma, G., Buss, G.R. and Saghai, M.A. (2000). Molecular marker Mapping of *RSV*4, a gene conferring resistant to all known strains of soybean mosaic virus, *Crop Science*, **40**, 1434-1437.

Hemmat, M., Weeden, N.F., Manganaris, A.G., Lawson, D.M. (1994). Molecular marker linkage map for apple, *Journal of Heredity*, **85,** 4-11.

Heslop-Harrison, J.S. and Schwarzacher, T. (2007). Domestication, Genomics and the Future for Banana, *Annals of Botany*, **100**, 1073-1084.

Ho, Y.W. (1999). The development of Pisang Mutiara – a Fusarium wilt tolerant Rastali. *In: Wahab, Z., Mahmaud, T.M.M., Siti Khalijah, D., Nor'Aini Mohd, F. and Mahmood, M.* (eds). *Proceedings of the First National Banana Seminar*, 23-25 November, 1998, Awana Genting, Pahang, Malaysia, 144-147.

Hori, K., Kobayashi, T., Shimizu, A., Sato, k., Takeda, K. and Kawasaki, S. (2003) Efficient construction of high-density linkage map and its application to QTL analysis in barley, *Theoretical Applied Genetics*, **107**, 806-813.

Horry, J.P., Bakry, F. and Ganry, J. (1993). Creation of varieties through hybridization of diploids. *In*: *Ganry, J.* (Ed.) *Breeding Banana and Plantain for Resistance to Diseases and Pests.* CIRAD-FLHOR. Montpellier, 293-299.

Howell, E.C., Newbury, H.J., Swennen, R.L., Whithers, L.A. and Ford-Lloyd, B.V. (1994). The use of RAPD for identifying and classifying *Musa* germplasm, *Genome*, **37**, 328-332

Humphrey, J.E. (1896). The development of seeds in Scitamineae, *American Journal of Botany*, **10**, 1-40.

Hwang, S.C. (1999). Recent development on Fusarium R&D of banana in Taiwan. *In*: *International Seminar & Workshop on the Banana Fusarium Wilt Disease*, 18-20 October 1999, Genting Highland Resort, Malaysia, 39-49.

Hwang, S.C. and Ko, W.H. (2004). Cavendish Banana Cultivars Resistant to Fusarium Wilt Acquired through Somaclonal Variation in Taiwan, *Plant Disease*, **88**(6), 580-588.

Jain, S.M. (2004). Banana Improvement with Cellular Biology and Biotechnology Including Mutation Techniques for Creation of New Useful Banana Genotypes. *In*: *Jain, S.M. and Swennen, R.* (eds). *Banana Improvement: Cellular, Molecular Biology, and Induced Mutations.* Science Publishers, Inc., Enfield, NH, USA, 1-2.

Jansen, J., de Jong, A.G. and van Ooijen, J.W. (2001). Constructing dense genetic linkage maps, *Theoretical Applied Genetics*, **102**, 1113-1122.

Jamaluddin, S.H., Nik Masdek, N.H. and Ibrahim, O. (1999). Preliminary survey of banana fusarium wilt disease in Malaysia: Implication on production and the industry. *In*: *International Seminar & Workshop on the Banana Fusarium Wilt Disease*, 18-20 October 1999, Genting Highland Resort, Malaysia, 76-85.

Jarret, R.L., Bhat, K.V., Cregan, P., Ortiz, R. and Vuylsteke, D. (1994). Isolation of microsatellite DNA markers in *Musa*, *Infomusa*, **3**, 3-4.

Javed, M.A., Chai, M. & Othman, R.Y. (2004). Study of resistance of *Musa acuminata* to *Fusarium oxysporum* using RAPD markers, *Biologia Plantarum*, **48**(4), 93-99.

Jones, D.R. (2000). *Diseases of Banana, Abaca and Ensete.* CABI International Publishing.

Jones, D.R. (1995). The characterization of isolates of *Fusarium oxysporum* f. sp. *cubense* from Asia, *Infomusa*, **4**(2), 3-4.

Jones, C.J., Edwards, K.J., Castaglione, S., Winfield, M.O., Sala, F., van de Wiel, C., Bredemeijer, G., Vosman, B., Matthes, M., Daly, A., Brettschneider, R., Bettini, P., Buiatti, M., Maestri, E., Malcevschi, M., Marmiroli, N., Aert, R., Volckaert, G., Rueda, J., Linacero, R., Vazquez, A. and Karp, A. (1997). Reproducibility testing of RAPD, AFLP and SSR markers in plants by a network of European laboratories. *Molecular Breeding*, **3**, 381-390.

Kaemmer, D., Afza, R., Weising, K., Kahl, G. and Novak, F.J. (1992). Oligonucleotide and amplification fingerprinting of wild species and cultivars of banana (*Musa* spp.), *Bio/Technology*, **10**, 1030-1035.

Kaemmer, D., Fisher, D., Jarret, R.L, Baurens, F.C., Grapin, A., Dambier, D., Noyer, J.L, Lanaud, C. and Lagoda, P.J.L. (1997). Molecular breeding in the genus *Musa*: a strong case for STMS marker technology, *Euphytica*, **96**, 49-63.

Kangire, A. Rutherford, M.A. and Gold, C.S. (1999). Distribution of fusarium wilt and the populations of *Fusarium oxysporum* f. sp. *cubense* on bananas in Uganda. *In*: *International Seminar & Workshop on the Banana Fusarium Wilt Disease*, 18-20 October 1999, Genting Highland Resort, Malaysia, 152-161.

Karamura, D.A. (1999). *Numerical taxonomicstudies of the East African Highland Banana (Musa AAA – East Africa) in Uganda.* Thesis (PhD). University of Reading.

Karp, A., and Edwards, J. (1997). DNA markers: a global overview. *In*: G. *Caetano-Anolles and P. M. Gresshoff* (eds). *DNA markers- protocols, applications, and overviews.* Wiley-Liss, Inc., New York, N.Y, 1-13.

Kearsey, M.J. (1998). The principles of QTL analysis (a minimal mathematics approach), *Journal of Experimental Botany*, **49**(327), 1619-1623.

Kearsey, M.J. & Farquhar, A.G.L. (1998). QTL analysis in plants: where are we now? *Heredity*, **80**, 137-142.

Keim, P., Schupp, J.M., Travis, S.E., Clayton, K., Zhu, T., Shi, L., Ferreira, A. and Webb, D.M. (1997). A high-density soybean genetic map based on AFLP markers, *Crop Science*, **37**, 537-543.

Kema, G.H.J., Goodwin, S.B., Hamza, S., Verstappen, E.C.P., Cavaletto, J.R., Van der Lee, T.A.J., de Weerdt, M., Bonants, P.J.M. and Waalwijk, C. (2002). A Combined Amplified Fragment Length Polymorphism and Randomly Amplified Polymorphism DNA Genetic Linkage Map of *Mycosphaerella graminicola*, the Septoria Tritici Leaf Blotch Pathogen of Wheat, *Genetics*, **161**, 1497-1505.

Khayat, E., Duvdevani, A., Lahav, E., and Ballesteros, B.A. (2004). Somaclonal variation in banana (*Musa acuminata* cv. Grande Naine). Genetic mechanism, frequency and application as a tool for selection. *In*: *Jain, S.M. and Swennen, R.* (eds.) *Banana Improvement: Cellular, Molecular Biology and Induced Mutations.* Science publishers, Inc. Enfield, NH, USA. Available online at http://www.fao.org/docrep/007/ae216e/ae216e0b.htm#bm11

Kijas, J.M.H., Thomas, M.R., Foeler, J.C.S. and Roose, M.L. (1997). Integration of trinucleotide microsatellites into a linkage map of *Citrus*, *Theoretical Applied Genetics*, **94**, 701-706.

Knapp, S.J., Bridges, W.C. and Liu, B.H. (1992). Mapping quantitative trait loci using non-simultaneous and simultaneous estimators and hypothesis tests. *In: Plant Genome: Methods for Genetic and Physical Mapping.* Kluwer Academic Publisher, Netherland , 209-237.

Koenig, R.L., Ploetz., R.C. and Kistler, H.C. (1995). *Fusarium oxysporum* f. sp. *cubense* Consists of a Small Number of Divergent and Globally Distributed Clonal Lineages, *Phytopathology*, **87**(9), 915-923.

Korzun, V. (2003). Molecular Markers and Their Application in Cereals Breeding. *In*: *International Workshop on Marker Assisted Selection: A Fast Track to Increase Genetic Gain in Plant and Animal Breeding?* , 17-18 October 2003, Turin, Italy, 18-22. Available online at http://www.fao.org/biotech/docs/Korzun.pdf

Kriegner, A., Cervantes, J.C., Burg, K., Mwanga, R.O.M. and Zhang, D. (2003). A genetic linkage map of sweetpotato [*Ipomea batatas* (L.) Lam.] based on AFLP markers, *Molecular Breeding*, **11**, 169-185.

Ky, C.L., Barre, P., Lorieux, M., Trouslot, P., Akaffou, S., Louarn, J., Charrier, A., Hamon, S. and Noirot, M. (2000). Interspecific genetic linkage map, segregation distortion and genetic conversion in coffee (*Coffea* sp.), *Theoretical Applied Genetics*, **101,** 669-676.

Kyazma (2004). *Solutions in Statistical Genomics*. Kyazma. Wageningen, Netherlands.

Lagoda, P.J.L., Noyer, J.L, Dambier, D., Baurens, F.C. and Lanaud, C. (1995). Abundance and distribution of SSR (simple sequence repeats) in the Musaceae family: microsatellite markers to map the banana genome. *In*: *Proceedings of the FAO/IAEA symposium on Induced Mutations and Molecular Techniques for Crop Improvement*, 19-23 June 1995, Vienna, Austria 287-295.

Lagoda, P.J.L., Noyer, J.L., Dambier, D., Baurens, F.C., Grapin, A. and Lanaud, C. (1998). Sequence tagged microsatellite site (STMS) markers in the Musaceae, *Molecular Ecology*, **7**, 657-666.

Lallias, D. (2007). *Genetic linkage mapping in the blue mussel Mytilus edulis and the European flat oyster Ostrea edulis, and the search for Quantitative Trait Loci of resistance to a disease in O. edulis.* Thesis, (PhD). University of Wales, Bangor.

Lambrides, C.J., Godwin, I.D., Lawn, R.J. and Imre, B.C. (2004). Segregation Distortion for Seed Testa Color in Mungbean (*Vigna radiate* L. Wilcek), *Journal of Heredity*, **95**(6), 532-535.

Lander, E.S. and Botstein, D. (1989). Mapping Mendelian Factors Underlying Quantitative Traits Using RFLP Linkage Maps, *Genetics*, **121**, 183-199.

Lanteri, S. and Barcaccia, G. (2005). Molecular Markers Based Analysis for Crop Germplasm Preservation. *In: The Proceeding of The Role of Biotechnology for The Characterization and Conservation of Crop, Forestry, Animal and Fishery Genetic Resources*, 5-7 March 2005, Turin, Italy, 55-63. Available online at http://www.fao.org/biotech/docs/lanteri.pdf

Li, Y.H., Han, Z.H. and Xu, X. (2004). Segregation patterns of AFLP markers in F_1 hybrids of a cross between tetraploid and diploid species in the genus *Malus*, *Plant Breeding*, **123**, 316-320.

Lin, Y.H., Chang, J.Y., Liu, E.T., Huang, J.W. and Chang, F.L. (2008). Development of a molecular marker for specific detection of *Fusarium oxysporum* f. sp. *cubense* race 4, *European Journal of Plant Pathology*, DOI 10.1007/s10658-008-9372-4

Loh, J.P., Kiew, R., Set, O., Gan, L.H. and Gan, Y.Y. (2000). Amplified Fragment Length Polymorphism Fingerprinting of 16 Banana Cultivars (*Musa* cvs.), *Molecular Phylogenetics and Evolution*, **17**(3), 360-366.

Lyttle, T.W. (1991). Segregation distorters, *Annual Review Genetics*, **25**, 511-557.

Mak, C., Ho, Y.W., Liew, Y.P., Ibrahim, R. (1996). Novaria - a new banana mutant, *Infomusa*, **5**(1), 35-36.

Mak, C., Ho, Y.W., Liew, K.W. and Asif, M.J. (2004). Biotechnology and *in vitro* mutagenesis for banana improvement. *In*: *Mohan Jain S., Swennen R.* (ed.). *Banana Improvement: Cellular, Molecular Biology, and Induced Mutations.* Science Publishers Inc., Enfield, NH, USA. Available online at http://www.fao.org/docrep/007/ae216e/ae216e08.htm#bm08

Melchinger, A.E. (1990). Use of molecular markers in breeding for oligogenic disease resistance, *Plant Breeding*, **104**, 1-9

Melchinger, A.E., Graner, A., Singh, M. and Messmer, M.M. (1994). Relationships among European barley germplsm: 1. Genetic diversity among winter and spring cultivars revealed by RFLPs, *Crop Science*, **34**, 1191-1199.

Melchinger, A.E., Utz, H.F. and Schön, C.C. (1998). Quantitative Trait Locus (QTL) Mapping Using Different Tester and Independent Population Samples in Maize Reveals Low Power of QTL Detection and Large Bias in Estimates of QTL Effects, *Genetics*, **149**, 383-403.

Michelmore, R.W. Paran, I., Kesseli, R.V. (1991). Identification of markers linked to disease resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations, *Proceedings of the National Academic Sciences of the United States of America*, **88**, 9828-9832.

MOA (2008). *Agriculture Statistical Handbook 2008*. Ministry of Agriculture and Agrobased Industries. Available online at http://www.agribdc.gov.my/c/document_library/get_file?p_l_id=10402&folderId=25349&name=DLFE-1001.pdf

Molina, A.B (2006). Transcontinental diseases that are potential threat to the banana industry in Latin America. *In*: *In*: *Proceeding of the XVII Reunião international da Associaçã para a Cooperaçã0 nas Pesquisas sobre Banana no Caribe e na América Tropical, Joinville,* 15-20 October 2006, Santa Catarina, Brasil, 24-31.

Montaldo, H.H. and Herrera, C.A. (1998). Use of molecular markers and major genes in the genetic improvement of livestock, *Electronic Journal of Biotechnology*, **1**, 83-178.

Montcel, H.T., Carreel, F. and Bakry, F. (1996). Improve the Diploids: The Key for Banana Breeding *In*: *Frison, E.A., Horry, J.P. and De Waele, D.* (Eds). *Proceeding on the workshop on New Frontiers in Resistance Breeding for Nematodes, Fusarium and Sigatoka,* 2-5 October 1995, Kuala Lumpur Malaysia, 119- 128.

Moore, N.Y., Pegg, K.G., Bentley, S. and Smith, L.J. (1999). Fusarium wilt of banana: Global problems and perspectives. *In*: *International Seminar & Workshop on the Banana Fusarium Wilt Disease,* 18-20 October 1999, Genting Highland Resort, Malaysia, 11-30.

Morán, J. F. A. (2006). Banana hybrids develop by FHIA. *In*: *Proceeding of the XVII Reunião international da Associaçã para a Cooperaçã0 nas Pesquisas sobre Banana no Caribe e na América Tropical, Joinville,* 15-20 October 2006, Santa Catarina, Brasil, 173-177p.

Mohamed, A.A., Mak, C., Liew, K.W. and Ho, Y.W. (1999). Early evaluation of banana plantsat nursery stage for fusarium wilt tolerance. *In*: *International Seminar & Workshop on the Banana Fusarium Wilt Disease,* 18-20 October 1999, Genting Highland Resort, Malaysia, 194-200.

Negi, M.S., Devic, M., Delseny, M. and Lakshmikumaran, M. (2000). Identification of AFLP fragments linked to seed coat colour in *Brassica juncea* and conversion to SCAR marker for rapid selection, *Theoretical Applied Genetics*, **101**, 146-152.

Negi, M.S., Sabharwal, V., Bhat, S.R. and Lakshmikumaran, M. (2004). Utility of AFLP markers for the assessment of genetic diversity within *Brassica nigra* germplasm, *Plant Breeding*, **123**(1), 13-16.

Novak, F.J. (1992). *Musa* (Bananas and Plantains) in Biotechnology of perennial fruit crops. *In: Hammerschlag, F.A. and Litz., R.* (eds). Wallingford, Oxon: CAB International, UK, 449-481.

Ong, K.P. (1996). Fusarium Wilt of Cavendish Banana in Commercial Farm in Malaysia. *In*: *Frison, E.A., Horry, J.P. and De Waele, D.* (Eds). *Proceeding on the workshop on New Frontiers in Resistance Breeding for Nematodes, Fusarium and Sigatoka*, 2-5 October 1995, Kuala Lumpur Malaysia, 211-217.

Ortiz, R. and Vuylsteke, D. (1996). Recent advances in *Musa* genetics, breeding and biotechnology, *Plant Breeding Abstracts*, **66**, 1355-1363

Panis, B., Wauwe, A.V. and Swennen, R. (1993). Plant regeneration through direct somatic embryogenesis from protoplasts of banana (*Musa* app.), *Plant Cell Reports*, **12**, 403-407.

Paterson, A.H., Tanksley, S.D. and Sorrells, M.E. (1991). DNA Markers In Plant Improvement. *In: Advances in Agronomy*, Academic Press, Inc., Volume 46, 40-90.

Pegg, G.F. (1985). Life in a Black Hole: The Micro-Environment of the Vascular Pathogen, *Transactions of the British Mycological Society*, **85**, 1-20.

Pegg, K.G., Moore, N.Y. and Bently, S. (1996). Fusarium wilt of banana in Australia, *Australian Journal of Agricultural Research*, **47**, 637-650.

Pillay, M. (2005). *Hungry for improvement*. INIBAP Annual Report 2005, 9 – 11.

Pillay, M., Ogundiwin, E., Nwakanma, D.C., Ude, G. and Tenkouano, A. (2001). Analysis of genetic diversity and relationships in East African banana germplasm, *Theoretical Applied Genetics*, **102**, 965-970.

Pillay, M., Ogundiwin, E., Tenkouano, A. and Dolezel, J. (2006). Ploidy and genome composition of *Musa* germplasm at the International Institute of Tropical Agriculture (IITA), *African Journal of Biotechnology*, **5**(13), 1224-1232.

Pillay, M. and Tripathi, L. (2007). *Banana in Genome Mapping and Molecular Breeding in Plants*. Springer-Verlag Berlin Heidelbergh. Volume 4, 281-301.

Pillay, M., Tenkouano, A. and Hartman, J. (2002) Bananas and Plantains: Future Challenges in Musa Breeding Musa germplasm. *In: Kang, M.S.* (ed). *Crop Improvement: Challenges in the Twenty-First Century*, The Haworth Press, Inc., 223-244.

Pinochet, J. (1988). Comments on the difficulty in breeding bananas and plantains for resistance to nematodes, *Revue de Nematologie*, **11**, 3-5.

Ploetz, R.C., and Correll, J.C. (1988). Vegetative Compatibility Among Races of *Fusarium oxysporum* f. sp. *cubense*, *Plant Disease*, **72**, 325-328

Ploetz, R.C. (1993a). Fusarium Wilt (Panama Disease). *In*: *Ganry, J.* ed. *The Proceeding of the International Symposium on Genetic Improvement of Bananas for*

Resistance to Diseases and Pests, 7-9 September, 1992, CIRAD, Montpellier, France, 148-158.

Ploetz, R.C. (1993b). A brief Introduction to Studies on Variability in *Fusarium oxysporum* f. sp. *cubense*. *In: Valmayor, R.V., Hwang, S.C., Ploetz, R.C., Lee, S.W. and Roa, V.N.* (eds). *The Proceedings of International Symposium on Recent Developments in Banana Cultivation Technology*, 14-18 December, 1992, Chiuju, Pingtung, Taiwan, 214-219.

Ploetz, R.C. (1994). Panama disease: Return of the first banana menace, *International Journal of Pest Management*, **40**, 326-336.

Ploetz, R.C. (1999). The phylogenies and reproductive strategies of globally dispersed populations of *Fusarium oxysporum* f. sp. *cubense*. *In*: *Molina, A.B., Nik Masdek, N.K. Liew, K.W.* (Ed.s). *In*: *International Seminar & Workshop on the Banana Fusarium Wilt Disease*, 18-20 October 1999, Genting Highland Resort, Malaysia, 133-146.

Ploetz, R.C. (2000). Panama Disease: A Classic and Destructive Disease of Banana, *Plant Health Progress*, Doi:10.1094/PHP-2000-1204-01-HM.

Ploetz, R.C. and Pegg, K.G. (2000). Fusarium wilt. *In: Jones, D.R.*. (ed.) *Diseases of Banana, Abacá and Enset.* CABI Publishing, Wallingford, UK, 143-159

Ploetz, R.C. (2003) *Diseases of Tropical Fruit Crops*. CABI Publishing, Wallingford, UK.

Ploetz, R.C. (2005). Panama Disease: An Old Nemesis Rears its Ugly head. Part 2: The Cavendish Era and Beyond, *The American Phytopathological Society*, APSnet, Feature Story: October 2005.

Ploetz, R.C. (2006). Fusarium Wilt of Banana Is Caused by Several Pathogens Referred to as *Fusarium oxysporum* f. sp. *cubense*, *Phytopathology*, **96**(6), 653-656.

Ploetz, R.C., Kepler, A.K., Daniells, J. and Nelson, S.C. (2007). Banana and plantain-an overview with emphasis on Pacific island cultivars, ver.1. *In*: *Elevitch, C.R.* (ed.) *Species Profiles for Pacific Island Agroforestry*. Permanent Agriculture Resources (PAR), Hõlualoa, Hawai`i. Avaiable online at http://www.agroforestry.net/tti/Banana-plantain-overview.pdf

Puhalla, J.C. (1985). Classification of strains of Fusarium oxysporum on the basis of vegetative compatibility, *Canadian Journal Botany*, **63**, 179-183

Purseglove, J.W. (1988). *Tropical Crops: Monocotyledons*. English Language Book Society/Longman.

Rabiei, B., Valizadeh, M., Ghareyazie, B., Moghaddam, M. and Ali, A.J. (2004). Identification of QTLs for rice grain size and shape of Iranian cultivars using SSR markers, *Euphytica*, **137**, 325-332.

RISBAP (2007). Fusarium wilt threatens livelihoods of banana farmers in Southern China, *RISBAP Bulletion*, **11**(2), 1-2.

Ritter , E., Gebhardt, C., Salamini, F. (1990). Esrimation of recombination frequencies and construction of RFLP linkage maps in plants from crosses between heterozygous parents, *Genetics,* **125**, 645-654.

Robinson, J.C. (1996). *Bananas and Plantains*. CAB International, Wallingford, UK.

Rout, G.R., Samantaray, S. and Das, P. (2000). Biotechnology of the Banana: A Review of Recent Progress, *Plant Biology,* **2**, 512-524.

Rowe, P.R. (1987). Banana breeding in Honduras. *In*: *Persly, G.J. and De Langhe E.A.* (eds). *Banana and plantain breeding strategies.* ACIAR Proceedings, no 21, Canberra, Australia. ACIAR, 74-77.

Rowe, P. (1998a). A banana breeder's response to `The Global Programme for Musa Improvement (PROMUSA)', *Infomusa,* **7**(1), 1-4.

Rowe, P. (1998b). Breeding bananas and plantains resistant to diseases and pests. In*: Rosales, F.E., Tripon, S.C. and Cerna, J.* (eds). *Proceedings of a workshop on Organic/environmentally friendly banana production. EARTH,* 27-29 July 1998, Guacimo, Costa Rica.

Rowe, P.R., Rosales, F.E. (1993). Banana and plantains. *In*: *Janick, J., Moore, N.* (eds). *Advances in Fruit Breeding.* 2nd ed. Timber Press, Portland, 167-211.

Rowe, P. and Rosales, F.E. (1996a). Bananas and plantains. *In*: Janick, J. and Moore, J. (eds). *Fruit Breeding. Vol 1: Tree and Tropical Fruits.* New York, John Wiley, 167-211

Rowe, P. and Rosales, F.E. (1996b). Current Approaches and Future Opportunities for Improving Major *Musa* (ABB) Types present in the Asian/Pacific Region: Saba, Pisang Awak, Bluggoe. *In*: *Frison, E.A., Horry, J.P. and De Waele, D.* (Eds). *Proceeding on the workshop on New Frontiers in Resistance Breeding for Nematodes, Fusarium and Sigatoka,* 2-5 October 1995, Kuala Lumpur Malaysia, 129- 141.

Roy, A., Frascaria, N., Mackay, J. and Bonsquet, J. (1992). Segregating random amplified polymorphic DNAs (RAPDs) in *Betulla alleghanionsis*. *Theoretical and Applied Genetics*. **85**: 173-180.

Rutherford, M.A. (1999). Fusarium wilt banana in East Africa. *In*: *International Seminar & Workshop on the Banana Fusarium Wilt Disease,* 18-20 October 1999, Genting Highland Resort, Malaysia, 86-94.

Saal, B. and Wricke, G. (2002). Clustering of amplified fragment length polymorphism markers in a linkage map of rye, *Plant Breeding,* **121**, 117-123.

Sagi, L., May, G.D., Remy, S. and Swennen, R. (1998). Recent Development in Biotechnological Research on Bananas (*Musa* spp.), *Biotechnology and genetic Engineering Reviews,* **15**, 313-327.

Saghai, M.A., Yang, G.P. Zhang, Q., Gravois, K.A. (1997). Correlation between molecular marker distance and hybrid performance in US southern long grain rice, *Crop Science*, **37**, 145-150.

Santos, C.M.R., Martins, N.F., Hörberg, H.M., de Almeida, E.R.P., Coelho, M.C.F., Togawa, R.C., da Silva, F.R., Caetano, A.R., Miller, R.N.G. and Souza Jr, M.T. (2005). Analysis of expressed sequence tags from *Musa acuminata* ssp. *burmannicoides*, var. *Calcutta 4* (*AA*) leaves submitted to temperature stresses, *Theoretical Applied Genetics*, **110**, 1517-1522.

Sasson, A. (1997). Importance of tropical and subtropical horticulture, future prospect of biotechnology in tropical and subtropical horticulture species, *Acta Horticulturae*, **460**, 12-26.

Sax, K. (1923). The association of size differences with seed coat pattern and pigmentation in *Phaseolus vulgaris*, *Genetics*, **8**, 552-560.

Schmidt, R. and West, J. (1995). Physical map and organization of *Arabidopsis thaliana* Chromosome 4, *Science*, **270**, 480-483.

Seefelder, S., Ehrmaier, H., Schweizer, G. and Seigner, E. (2000). Male and female genetic linkage map of hops *Humulus lupulus*, *Plant Breeding*, **119**, 249-255.

Sharma, P.C., Winter, P., Bunger, T., Huttel, B., Weigand, F., Weising, K. and Kahl, G. (1995). Abundance of di-, tri-, and tetranucleotide tandem repeats in chickpea (*Cicer arietinum* L.), *Theoritical Applied Genetics*, **90**, 90-96.

Sharrock, S. (1995). Collecting the *Musa* gene pool in Papua New Guinea. *In: Guarino, L., Ramanatha, R. V. and Reid, R.* (eds*). Collecting Plant Genetic Diversity, Technical Guidelines.* CAB International, UK, 647-658.

Shillingford, C.A & Edmunds, J.E. (2006). The role of science and technology in small-scale banana farming-A Caribbean perspective. *In*: *Proceeding of the XVII Reunião international da Associaçã para a Cooperaçã0 nas Pesquisas sobre Banana no Caribe e na América Tropical, Joinville*, 15-20 October 2006, Santa Catarina, Brasil, 164-172.

Silva, E.S. de O. de da Silva K.I.M. Borges, M.F. and de Olieveira, R.P. (1999). Pollination and culture of banana embryos, *Infomusa*, **8**, 24-26.

Silva, E.S. de O., Souza-Jr., M.T., Alves, E.J., Silveeira, J.R.S. and Lima, M.B. (2001). Banana breeding program at Embrapa, *Crop Breeding and Applied Biotechnology*, **1**(4), 399-436.

Simmonds, N.W. (1955). Wild Bananas in Malays, *Malayan Nature Journal*, **10**, 1-8.

Simmonds, N.W. (1959). Experiments on the Germination of Banana Seeds, *Journal of Tropical Agriculture (Trinidad)*, **36**(4), 259-272.

Simmonds, N. W. (1962). *Evolution of the Bananas.* London, Longmans.

Simmonds, N.W. and Shepherd, K. (1955). The taxonomy and origins of cultivated bananas, *Journal of the Linnean Society of London*, **55**, 302-312.

Smith, S.N. (2007). An Overview of Ecological and Habitat Aspects in the Genus *Fusarium* with Special Emphasis on the Soil-Borne Pathogenic Forms, *Plant Pathology Bulletin*, **16**, 97-120.

Snowdon, R.J. and Friedt, W. (2004). Molecular markers in *Brassica* oilseed breeding: current status and future possibilities, *Plant Breeding*, **123**, 1-8.

Staub, J.E. and Serquen, F. (1996). Genetic markers, map construction and their application in plant breeding, *HortScience*, **31**, 729-741.

Stam, P (1993). Construction of intergrated genetic linkage maps by means of a new computer package: JOINMAP, *The Plant Journal*, **3**(5), 739-744.

Stover, R.H. and Buddenhagen, I.W. (1986). Banana breeding: polyploidy, disease resistance and productivity, *Fruits*, **41**, 175-190.

Stover, R.H. and Simmonds, N.W. (1987). *Bananas.* 3rd edition. Tropical Agriculture Series, Longman Scientific & Technical, Singapore.

Strosse, H., Schoofs, H., Panis, B., Andre, E., Reyneirs, K. and Swennen, R. (2004). Development of embryogenic cell suspensions from shoot meristematic tissue in bananas and plantains (*Musa* spp.), *Plant Science*, **170**, 104-112.

Swennen, R. (2005). Banking on the future. *In*: *The symposium on the Conservation and Use of Musa Biodiversity for Improving Livelihoods*, 18 October 2005, Leuven, Belgium,. INIBAP Annual Report 2005, 4-6.

Tanksley, S., and McCouch, S. (1997). Seed banks and molecular maps: unlocking genetic potential from the wild, *Science*, **277**, 1063 – 1066.

Tenkouano, A., Crouch, J.H., Crouch, H.K., Vuylsteke, D. and Ortiz,R. (1999). Comparison of DNA marker and pedigree-based methods of genetic analysis of plantain and banana (*Musa* spp.) clones. I. estimation of genetic relationships. *Theoretical Applied Genetics.* **98**: 62-68.

Tautz, D. and Renz, M. (1984). Simple sequences are ubiquitous repetitive components of eukaryotic genomes, *Nucliec Acids Research*, **12**, 4127-4138.

Ubi, B.E., Fujimori, M., Mano, Y. and Komatsu, T. (2004) A genetic linkage map of rhodesgrass based on an F_1 pseudo-testcross population, *Plant Breeding* **123**, 247–253.

Ude, G., Pillay, M., Nwakanma, D. and Tenkouano, A. (2002). Genetic Diversity in *Musa acuminata* Colla and *Musa balbisiana* Colla and some their natural hybrids using AFLP Markers, *Theoretical Applied Genetics*, **104**, 1246-1252.

Uzun, B., Lee, D., Donini, P. and Cağirgan, M.I. (2003). Identification of a molecular marker linked to the closed capsule mutant trait in sesame using AFLP, *Plant Breeding*, **122**, 95-97.

Vakili, N.G. (1965). Fusarium wilt resistance in seedlings and mature plants of *Musa* species, *Phytopathology*, **55**, 135-140.

Valmayor, R.V., Jamaluddin, S.H., Silayoi, B., Kusumo, S., Danh, L.D., Pascua, O.C. and Espino, R.R.C. (2000). *Banana Cultivar Names and Synonyms in Southeast Asia in Wide Choice of Banana Cultivars Available in Local Markets of Southeast Asia.* INIBAP, Monttpellier, France, 1-24. Available online at http://www.bananas.bioversityinternational.org/files/files/pdf/publications/synonyms.pdf

Van Eck, H.J., Van der Voort, J.R., Draaistra, J., Van, Zandvoort, P., Van Enckevort, E., Segers, B., Peleman, J. Jacobsen, E., Helder, J. and Bakker, J. (1995). The inheritance and chromosomal localization of AFLP markers in non-inbred potato offspring, *Molecular Breeding*, **1**, 397-410.

Virk, P.S., Ford-Lloyd, B.V. and Newbury, H.J. (1998). Mapping AFLP markers associated with subspecific differentiation of *Oryza sativa* (rice) and an investigation of segregation distortion, *Heredity*, **81**, 613-620.

Vos, P., Hogers, R., Bleeker, M., Reijans, M. & Van de Lee, T. (1995). AFLP: a new technique for DNA fingerprinting, *Nucleic Acid Research*, **23**, 4407-4414.

Vuylsteke, D. (1989). *Shoot-tip culture for the propagation, conservation and exchange of Musa Germplasm. Practical Manuals for Handling Crop Germplasm in vitro 2.* International Board for Plant Genetic Resources, Rome, Italy.

Vuylsteke, D., Swennen, R. and De Langhe, E. (1990). Tissue culture technology for the improvement of African plantains. *In*: *Fullerton, R.A.* and *Stover, R. H.* (eds). *Sigatoka Leaf Spot Diseases of Bananas.* Montpellier, INIBAP, 316-337.

Vuylsteke, D. and Swennen, R. (1993). Genetic Improvement of Plantains: the potential of conventional approaches and the interface with *in-vitro* culture and biotechnology. *In*: *Proceeding of the workshop on Biotechnology Applications for Banana and Plantain Improvement*, 27-31 Jan 1992, San José, Costa Rica. Available online at http://bananas.bioversityinternational.org/files/files/pdf/publications/biotechnology.pdf

Vuylsteke, D., Swennen, R. and Ortiz, R. (1993a). Development and performance of black-sigatoka resistant tetraploids hybrids of plantain (*Musa* spp., AAB group), *Euphytica*, **65**, 33-42.

Vuyslteke, D., Ortiz, R. and Swennen, R. (1993b). Genetic Improvement of Plantains at the International Institute of Tropical Agriculture (IITA), *InfoMusa*, **2**(1), 10-12

Vuylsteke, D., Ortiz, R., Ferris, R.S.B. and Crouch, J.H. (1997). Plantain improvement, *Plant Breeding Reviews*, **14**, 267-320

Vuylsteke, M. ,Mank, R., Antonise, R., Bastiaans, E., Senior, M. L., Stuber, C.W., Melchinger, A.E., Lübberstedt, T., Xia, X.C., Stam, P., Zabeau, M. amd Kuiper, M. (1999). Two high-density AFLP® linkage maps of *Zea mays* L.: analysis of distribution of AFLP markers, *Theoretical Applied Genetics*, **99,** 921-935.

Weising, K., Kaemmer, D., Weigand, F., Epplen, J.T. and Kahl, G. (1992). Oligonucleotide fingerprinting reveals various probe-dependent levels of informativeness in chickpea (*Cicer arietinum*), *Genome*, **35**, 436-442.

Weising, K., Winter, P., Huttel, B. and Kahl, G. (1998). Microsatellite Markers for Molecular Breeding, *Journal of Crop Production*, **1**(1), 113-143.

Weising, K., Nybon, H., Wolff, K. & Meyer, W. (1995). *DNA Fingerprinting in Plant and Fungi.* CRC Press, Boca Raton.

Weller, J.I. (1992). Statistical methodologies for mapping and analysis of QTL. *In*: *Plant Genome: Methods for Genetic and Physical Mapping.* Kluwer Academic Publisher, Netherland, 181-207.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A. and Tingey, S.V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers, *Nucleic Acids Research*, **18**, 6531-6535.

Wong, C., Kiew, R., Ardent, G., Set, O., Lee, S.K., and Gan, Y.Y. (2002). Assessment of the Validity of the Sections in *Musa* (Musaceae) using AFLP, *Annals of Botany*, **90**, 231-238.

Wright, S. (1951). The genetic structure of population, *Annals of Eugenetics*, **15**,323-354.

Young, N.D. (1996). QTL Mapping and Quantitative Disease Resistance in Plants, *Annual Review of Phytopathology*, **34**, 479-501.

Young, N.D. (1999). A cautiously optimistic vision for marker-assisted breeding, *Molecular Breeding*, **5**, 505-510.

Yu, K.F. and Pauls, K.P. (1992). Segregation of random amplified polymorphic DNA markers and strategies for molecular mapping in tetraploid alfalfa, *Genome*, **36**, 844-851.

Yuan, L., Duble, C.M., Muminovic, J., Melchinger, A.E. and Lübberstedt (2004). Targetted BSA mapping of *Scmv1* and *Scmv2* conferring resistance to SCMV using *Pst*I/*Mse*I compared with *Eco*RI/*Mse*I AFLP markers, *Plant Breeding*, **123**, 434-437.