Development of an ‘A’ genome-specific sequence characterised amplified region (SCAR) marker in *Musa* L. (bananas and plantains).

Lloyd Mabonga

Vaal
University of Technology
Vanderbijlpark

September 2014

Development of an ‘A’ genome-specific sequence characterised amplified region (SCAR) marker in *Musa* L. (bananas and plantains).

Dissertation submitted in complete fulfillment for the degree Magister Technologiae

in the Department of Biosciences

in the Faculty of Applied and Computer Sciences, Vaal University of Technology

Lloyd Mabonga

210115203

Supervisor: Prof M. Pillay

September 2014

ABSTRACT

Most cultivated bananas and plantains (*Musa* spp. sect. *Eumusa*), originated from two wild diploid species, *Musa acuminata* Colla (AA) and *Musa balbisiana* Colla (BB), which contributed the A and B genomes, respectively. The two genomes confer different traits to a banana plant. Intra- and interspecific hybridization between the wild diploid species, somatic mutations and selection over many thousands of years has given rise to considerable genetic variability in cultivated bananas.

Bananas are classified according to its genome composition and a number of morphological traits are used to identify the genomes of a plant. Morphological classification can be misleading since the morphology of plants can be affected by environmental factors. Molecular techniques to identify the genomes of banana have many advantages. The objective of this study was to develop a SCAR (sequence characterized amplified region) marker from a previously reported A genome-specific RAPD fragment that distinguish the A genome of banana from the B genome. This fragment designated $OPA17_{600}$ was cloned, sequenced and used to design longer 20-mer SCAR primers. Verification of the SCAR primers for its fidelity to the A genome was carried out on a sample of 22 homo-and heterogenomic accessions representing landraces and hybrids of different ploidy and genome combinations. Out of six primers sets that were tested one set (SC3) produced a unique 600 bp in all the A genome A genome containing banana accessions. However, these primers also amplified an 800 bp fragment in all the BB genotypes and some accessions containing the A and B genomes. While previous reports suggested that there was considerable differentiation between the A and B genomes, recent evidence points to the contrary. The presence of the A genome fragment in the B genome genotypes and accessions may be due to recombination between the two genomes, translocations and substitutions. The study concluded that the 600-bp SCAR sequence is conserved across the A genome in *Musa* and can be used to identify the A genome in banana classification and *Musa* breeding programmes.

Key words: Bananas and plantains, RAPD marker, SCAR marker, A genome, genetic relationship

DECLARATION

I hereby give consent for my thesis/dissertation, if accepted, to be available for photocopying and for interlibrary loan, and for the title and summary to be made available to outside organisations.

Signed…….

Date……....

ACKNOWLEDGEMENTS

I hereby wish to express my gratitude to the following individuals who enabled this document to be successfully and timeously completed:

- Prof M. Pillay
- Prof R. Mabuza
- SENEX
- Vaal University of Technology
- Lab Technician
- Mabonga Family

DEDICATION

This thesis is dedicated in loving memories of my late mother Mrs C. E. Mabonga, my father Mr E. Mabonga, my wife Naume, my son Divine Munashe, Mabonga family, relatives and friends.

TABLE OF CONTENTS

CHAPTER 2

LITERATURE REVIEW

CHAPTER 3

RESEARCH METHODOLOGY

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

CHAPTER 1

1. Introduction and overview of the study

This chapter provides a general background on why this study was carried out. It highlights the genome composition of *Musa* (banana and plantain). It presents the role of genomes of *Musa* in banana classification. The chapter demonstrates the advantages of utilizing molecular marker techniques over morphological characteristics in banana classification.

1.1 General background of the study

The classification of banana genotypes is dependent on ploidy, type of genomes and morphological similarities of cultivars to their parental accessions (Cheesman 1948; Simmonds & Shepherd 1955; Oselebe, Tenkouano, Pillay, Obi & Uguru 2006). Four genomes (A, B, S and T) are known to be present in cultivated bananas (Simmonds 1962). The A, B and S genomes are characteristic of species in the section *Eumusa* (Horry, Ortiz, Arnaud, Crouch, Ferris, Jones, Mateo, Picq & Vuylsteke 1997) while the T genome is characteristic of the section *Australimusa* (Cheesman 1948; Carreel 1995; Sharrock 1989). The A and B genomes were derived from the wild, seeded diploid species *Musa acuminata* Colla and *M.balbisiana* Colla, respectively (Cheesman 1948). The S genome is known to be present only in the diploid *M. schizocarpa* whereas the T genome is characteristic of the diploid *M. textiles* (Cheesman 1948). The A and B genomes are found in most edible bananas (Simmonds & Shepherd 1955) while the S and T genomes occur in only a few cultivars (Carreel 1995).

A classification system for banana and plantains was first developed by Simmonds and Shepherd (1955). The classification is based on the relative morphological contribution of the two wild, seeded species (*M. acuminata* and *M. balbisiana*) to the constitution of a cultivar (Purseglove

1972; Stover & Simmonds 1987). The morphological classification system uses a simple numerical method by scoring 15 morphological characters on a score from 1 to 5 (Simmonds & Shepherd 1955). A modified and revised scoring system was developed by Silayoi and Chomchalow (1987) and Singh and Uma (1996). The latter systems incorporate the identification of various groups based on 121 morphological characters using the variability for characters such as pseudostem blotching, pigmentation, duration of flowering and fruition, number of hands and fingers, nature of the peduncle, pedicle length, fruit size, taste and nature of ripe fruit flesh (Robinson 1996). Classification of bananas depending only on a plants morphology is risky because a plants phenotype can change due to environmental conditions. A plant's morphology can change due to environmental factors, and may require evaluation in different environments over many cropping cycles for consistent classification (Oselebe et al. 2006; Pillay et al. 2011). In addition, some characteristics are only expressed at maturity and can only be measured after 18-24 months in field plantations (Pillay et al. 2000, 2011). This hinders classification especially of hybrids (Perrier & Tezenus du Montcel 1990). For example, the morphological classification system failed to distinguish ABB accessions that appeared to be synonyms (Saraswathi, Uma, Vadivel, Durai, Siva, Rajagopal & Sathiamoorthy 2009b).

As a complement to morphological classification, molecular marker characterisation has been adopted to provide greater insights on banana classification (Wongniam, Somana, Swangpol, Seelanan, Chareonsap, Chadchawan & Jenjittikul 2010; Pillay, Tenkouano, Ude & Ortiz 2011). Various molecular marker techniques to classify banana genomes in *Musa* have been reported and these include restriction fragment length polymorphism (RFLP) (Carreel, Gonzalez de Leon, Lagoda, Lanaud, Jenny, Horry & Tezenas du Montcel 2002), polymerase chain reaction- amplified fragment length polymorphism (PCR-RFLP) (Nwakanma, Pillay, Okoli & Tenkouano 2003a, b; Ge, Liu, Wang, Schaal & Chiang 2005) amplified fragment length polymorphism (AFLP) (Ude, Pillay, Nwakanma & Tenkouano 2002a, b; Ude, Pillay, Ogundwin & Tenkouano 2003; Wong, Kiew, Loh, Gan, Set, Lee, Ohn, Lum & Gan 2001a, b; Wong, Kiew, Argent, Set, Lee & Gan 2002), random amplified polymorphic DNA (RAPD) (Pillay, Nwakanma & Tenkouano 2000; Kahangi 2002; Onguso, Kahangi, Ndiritu & Mizutani 2004; Uma, Sudiia, Saraswathi, Manickavasagam, Selvarajan, Durai, Sathiamoorthy & Siva 2004), simple sequence repeat (SSR) or microsatellites (Grapin, Noyer, Carreel, Dambler, Baurens, Lanaaud & Lagoda 1998; Creste, Tulmann, Neto, De

Oliveira Silva & Figueira 2003; Creste, Tulmann, Neto, Vencovsky, De Oliveira Silva & Figueira 2004), genomic in situ hybridisation (GISH) (Osuji, Harrison, Crouch & Heslop-Harrison 1997; D'Hont, Paget-Goy, Escoute & Carreel 2000), isozymes (Espino & Pimentel 1990; Bhat, Bhat & Chandel 1992) and inter-retrotransposon amplified polymorphism (IRAP) (Teo, Tan, Ho, Faridah, Othman, Heslop-Harrison, Kalendar & Schulman 2005). Some of the techniques described above also not problematic for genome identification.

For instance, the GISH technique is time consuming, complicated and requires a high level of skill, and may not be suitable for high-throughput screening of large breeding populations (D'Hont *et al.* 2000). The RAPD marker technique was able to distinguish the A and B genomes (Pillay *et al.* 2000), but showed clusters between AAB clones and AAA group suggesting that these markers do not have enough discriminatory power (Howell, Newbury, Swennen, Whithers & Ford-Lloyd 1994). RAPD fragments show anonymous sequences that may display segregation distortions in mapping experiments (Faure, Noyer, Horry, Bakry, Lanaud & De Leon 1993; Semagn, Bjornstad & Ndjiondjop 2006).The IRAP marker system was able to distinguish the A and B genome using Ty3-gypsy-like retrotransposons probe, but could not differentiate between AAB and ABB genomes (Teo *et al.* 2005). PCR-RFLP markers that are specific for the A and B genomes have been identified (Nwakanma *et al.* 2003a, b; Bhat, Amaravathi, Gautam & Nelayudhan 2004). The relatively high cost and technically demanding nature of the RFLP technique is not appropriate for routine breeding applications (Nwakanma *et al.* 2003a, b). The isozyme technique has been used in banana classification (Espino & Pimentel 1990). The isozyme polymorphism could not differentiate cultivars within the Cavendish subgroup (Jarret & Litz 1986; Espino & Pimentel 1990). AFLP has been used to detect the genetic diversity of both cultivated and wild progenitors of *M. acuminata* (Wong *et al.* 2001b). However, the information content of these banding patterns is restricted, as they must be initially treated as dominant markers (Sotto & Rabara 2000). AFLP assays are also technically demanding and expensive in that they require a number of DNA manipulations and a complex visualization procedure (Sotto & Rabara 2000). They require relatively large amounts of reasonably high DNA quality as the use of poor DNA quality may lead to incomplete digestion which can result in artificial polymorphisms (Ude *et al.* 2003).

The challenges faced in banana classification highlight the need of more reliable techniques for genome identification (Pillay *et al.* 2011). Recently, sequence characterized amplified region (SCAR) marker technique has been successfully used in the classification of plants such as *Lettuca*, *Vicia* and *Triticum* (Paran & Michelmore 1993; Vidal, Gisbert, Talon, Primo-Millo, Lopez-Dias & Garcia-Martinez 2001). A SCAR marker is created from cloned RAPD markers linked to a particular trait of interest (Paran & Michelmore 1993; Horesji, Box & Staub 1999). SCAR primers are longer than RAPDs and have greater reliability (Paran & Michelmore 1993), are generally more allele specific (Horesji *et al.* 1999). Their amplifications are more stable, reliable and more easily reproduced (Ardiel, Grewal, Deberdt & Scoles 2002). The objective of this study is to develop a SCAR marker linked to the A genome in *Musa*.

1.2 Rationale

Bananas and plantains are the fourth most important food in the world today, after rice, wheat and maize (Pearce 2003; Sagi, Remy & Swennen 2007). Their ability to produce fruits all the year round makes it an important cash and food security crop especially in the tropics (Jones 2000). They are an important food security crop (Jones 2000). Bananas supply more than 25% of the carbohydrate requirements for over 70 million people in West and Central Africa (Sagi *et al.* 2007), and has a per capita consumption of approximately 250kg per year. Nutritionally, fresh bananas contain 35% carbohydrates, 6-7% fiber, 1-2% protein and some major elements such as potassium, magnesium, phosphorus, calcium, iron, and vitamins A, B6 and C (Robinson 1996).

Banana production is affected by a wide range of constraints ranging from pests and diseases, drought and low yield (Pillay & Tripathi 2006; Pillay *et al.* 2011). Breeding of new banana cultivars is one way to develop enhanced germplasm than could overcome these production contraints. Other traits of interest to breeders include high yield, fruit quality (finger length, finger curvature and finger pedicel length), flavor, ripening, plant height (stature) and production efficiency (Stover & Simmonds 1987). Several authors (Simmonds 1987; Eckstein, Robinson & Davie 1995; Pillay, Tenkouano & Hartman 2002) have described and emphasized the idea of a banana ideotype cultivar that is disease- and pest resistant, high yielding, photosynthetically efficient, early maturing, displaying minimum delay between consecutive harvests, short stature, strong roots for optimal nutrient uptake and greater resistance to wind damage. Banana breeding

often involves crosses between different genomes producing a variety of genome combinations in the hybrids.

The morphology of many banana cultivars originating from crosses between the A and B genome donors show a bias towards the A or B phenotype (Simmonds & Shepherd 1955; Simmonds 1966). There is wide consensus that certain A or B genome alleles are not fully expressed in the hybrid context, complicating the morphological classification (Vuylsteke, Swennen & Ortiz 1993). It is largely accepted that edibility of mature fruits arose from the A genome, causing parthenocarpy and female sterility in diploid *M. acuminata* (Simmonds 1962). This implies that breeding strategies aiming for high yielding parthenocarpic varieties must incorporate A genome characteristics (Pillay *et al.* 2011). The A genome is believed to harbor fruit characteristics such as sweetness and less acid taste, causing AAA dessert bananas to be sweet and more palatable (Simmonds 1962). *Musa acuminata* has traces of wax on fruits while *M. balbisiana* is often strongly waxed (Pillay *et al.* 2000).

1.3 Problem statement

The classification of banana cultivars remains controversial, in part due to limited DNA information to complement the morphology (Pillay *et al.* 2011). The current classification system has deficiencies and needs modification. A single banana cultivar is sometimes known by different vernacular names showing linguistic diversity in different regions (Amalraj 1992) and multiple genotypes are given a single name (Heslop-Harrison & Schwarzacher 2007). The morphological classification system has failed to fully address the problem of banana classification leading to the adoption of molecular marker characterization (Pillay *et al.* 2011). The morphology of many cultivated banana varieties does not correspond to the proposed genome formulas complicating the classification (Vuylsteke *et al*. 1993). A wide range of morphological variation is a common problem in banana worldwide leading to cultivar confusion (Rossel 1998). Close genetic relationship among the banana cultivars, as well as somatic mutations, complicates the correct classification and characterisation of the clones (Kahangi 2002; Wang, Hu, Sun, Staehelin, Xin & Xie 2012).

Recently, developments in DNA molecular marker technology provided alternative means of addressing the problems of banana classification. Each molecular marker has its advantages and disadvanatages depending on the quality of work that is required (Muralidharan & Wakeland 1993). RAPD is relatively fast, cheap and easy to perform in comparison with other methods such as RFLP, AFLP and microsatellites (Muralidharan & Wakeland 1993). However, RAPD markers have problems of poor reproducibility (Ellsworth, Rittenhouse & Honeycutt 1993) and do not distinguish heterozygous and homozygous alleles (Ellsworth *et al.* 1993; Ning, Xu, Lu, Huang & Ge 2007). Such problems and technical disadvantages have raised questions on the fidelity of the RAPD technique as a genetic marker for banana classification (Creste, Tulmann, Neto, De Oliveira & Figueira 2003). Therefore, RAPD markers are converted into longer primers that can be used to develop allele specific sequence characterized amplified region (SCAR) markers (Ardiel *et al.* 2002).

1.4 Aim

The aim of this study was to develop a SCAR marker from a RAPD fragment that is specific to the A genome of banana.

1.5 Objectives

1. To amplify the DNA of *Musa acuminata* and *M. balbisiana* with the A17 RAPD primer from OPERON technologies.
2. To clone and sequence the RAPD fragment and obtain SCAR primers from the sequence.
3. To test the efficacy of the SCAR primers to identify the A genome in a set of banana accessions with different genome combinations.

1.6 Significance of the study

The global annual production of bananas and plantains (*Musa* spp.) amounts to > 120 Mt (Heslop-Harrison & Schwarzacher 2007), making this species one of the world's most important fruit crops. As well as their prominence as a dessert fruit, they provide a vital source of carbohydrates to many inhabitants of the humid tropics (Vuylsteke 2000; Sagi *et al*. 2007). Improvement of cultivated banana via breeding, unlike other staple crop species, is hampered by the absence of sexual

reproduction and narrow genetic basis (Christelova, Valarik, Hribova, De Langue & Dolezel 2011). Banana production is endangered by a range of pests, diseases and inferior horticultural characteristics affecting both the yield and quality of the fruit (Christelova *et al*. 2011; Nwauzoma, Akagbuo, Uma, Saraswathi & Mustaffa 2011). The development of an A genome-specific SCAR marker would assist in tracing A genome linked traits of interest in breeding programmes that involve interploidy crosses (Pillay *et al.* 2011).

Selection of plants by farmers and *Musa* breeders on the basis of genomic composition would be done at the nursery stage from a few grams of leaf tissue. This would facilitate the design of informed crosses and breeding lines with desired labels (Semagn *et al*. 2006). In addition, the A genome specific SCAR marker would be useful to farmers in gene pyramiding where several identified gene characteristics are combined into one (Pillay, Ogundiwin, Tenkouano & Dolezel 2006).

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

This chapter presents the origin and history of *Musa*. The chapter explains the taxonomical classification in *Musa* that is currently used in phylogenetic analysis and breeding programmes. This chapter unveils the physical characteristics of the genomes in *Musa*, methods used for their identification, their value in phylogenetic analysis and their role in breeding programmes.

2.2 Origin and history of banana

The primary center of origin and domestication of bananas and plantains (*Musa* L.) is considered to lie in the southeast Asian jungles of Malaysia, New Guinea, and the western Pacific regions (Purseglove 1972). According to Robinson (1996) bananas and plantains originated from wild, inedible and seed bearing diploid ancestors that can still be found in the natural forest vegetation in south east Asia. Over many years various inedible diploid subspecies of *M. acuminata* Colla (AA) crossed naturally resulting in the production of numerous intraspecific hybrids (Robinson 1996). Simmonds (1962) added that some of the hybrids were parthenocarpic, female sterile and triploid. Local inhabitants discovered that such plants had edible fruits and the plants could be propagated vegetatively by suckers. In this manner, the superior edible crosses of *M. acuminata* would have been selected, cultivated, propagated and distributed locally as a food crop (Simmonds 1962). The edible triploid bananas in southeast Asia were further selected according to vigour, fruit size and adaptability, and were developed at the expense of the original diploid types which were inferior (Purseglove 1972).

Diploid and triploid selections of *M. acuminata* are believed to have spread throughout the drier monsoon regions of India and The Philippines (Swennen & Ortiz 1997; Valmayor 2000) solely by humans through suckers (Simmonds 1962). These regions also supported the other wild and seeded diploid *M. balbisiana* (BB) (Purseglove 1972). According to Robinson (1996) interspecific hybridization occurred to produce diploid and triploid crosses of *M. acuminata* x *M. balbisiana*. The introduction of *M. balbisiana* genes from the drier monsoon regions into *M. acuminata* clones from the humid tropics of southeast Asia conferred a measure of hardiness, greater disease resistance, improved nutritional value, increased starchness, drought tolerance and provided

hybrids suitable for cooking as opposed to the pure *M. acuminata* cultivars which are sweeter and more suited to dessert use (Robinson 1996; Azhar & Heslop-Harrison 2008).

Bananas are thought to have been introduced into Africa by Arab traders from India (Swennen 1990). Banana first reached Madagascar about AD 500 and thereafter into east Africa, Zaire and West Africa (Simmonds 1962). Plantains arrived much later, and according to Swennen (1990) bananas and plantains were known on the west coast of Africa in the 15th century when the Portuguese arrived. They were then moved inwards by local migrants and later, from Africa spread to other parts of the tropical and subtropical world (Robinson 1996). Bananas and plantains moved from West Africa to Canary Islands, from there to Haiti, and then to the Caribbean and tropical America (Swennen 1990), where most dessert bananas are currently produced for export (Vanhove *et al.* 2012). A great diversity of bananas and plantains now exist in sub-Saharan Africa with various types cultivated in different eco-regions (Swennen & Vuylsteke 1991). Currently, *Musa* species still exist today in secondary loci of genetic diversity in Africa, Latin America, and the Pacific (Simmonds 1987; Robinson 1996). These secondary centers of diversification have enriched the diversity of *Musa* (Lescot 2000).

2.3 The genus *Musa*

The genus *Musa* is in the family *Musaceae*, order *Zingiberales* subclass *Zingiberidae*, class *Liliopsida* and division *Magnoliophyta* (Shepherd & Ferreira 1984). The genus has over 70 species (Horry & Jay 1988a, b) and genus comprises five sections namely, *Australimusa, Callimusa, Rhodochlamys, Eumusa* and *Ingentimusa* (Fig. 1) (Stover & Simmonds 1987; Purseglove 1988). These five sections vary in the basic number of chromosomes (De Langhe 1990; Kamate, Brown, Durand, Bureau, Nay & Trinh 2001). *Callimusa* and *Australimusa* have a basic chromosome number of $x = 10$, while species in *Eumusa* and *Rhodochlamys* have a basic chromosome number of $x = 11$ (Argent 1976). *Ingentimusa* has a single species *M. ingens* with a chromosome number of $2n = 14$. Sections *Callimusa* and *Rhodochlamys* consist of non-parthenocarpic species with no nutritional value and are only important as ornamental crops (Simmonds 1962). *Australimusa* consists of parthenocarpic edible types, collectively known as the Fe'i cultivars (Purseglove 1988).

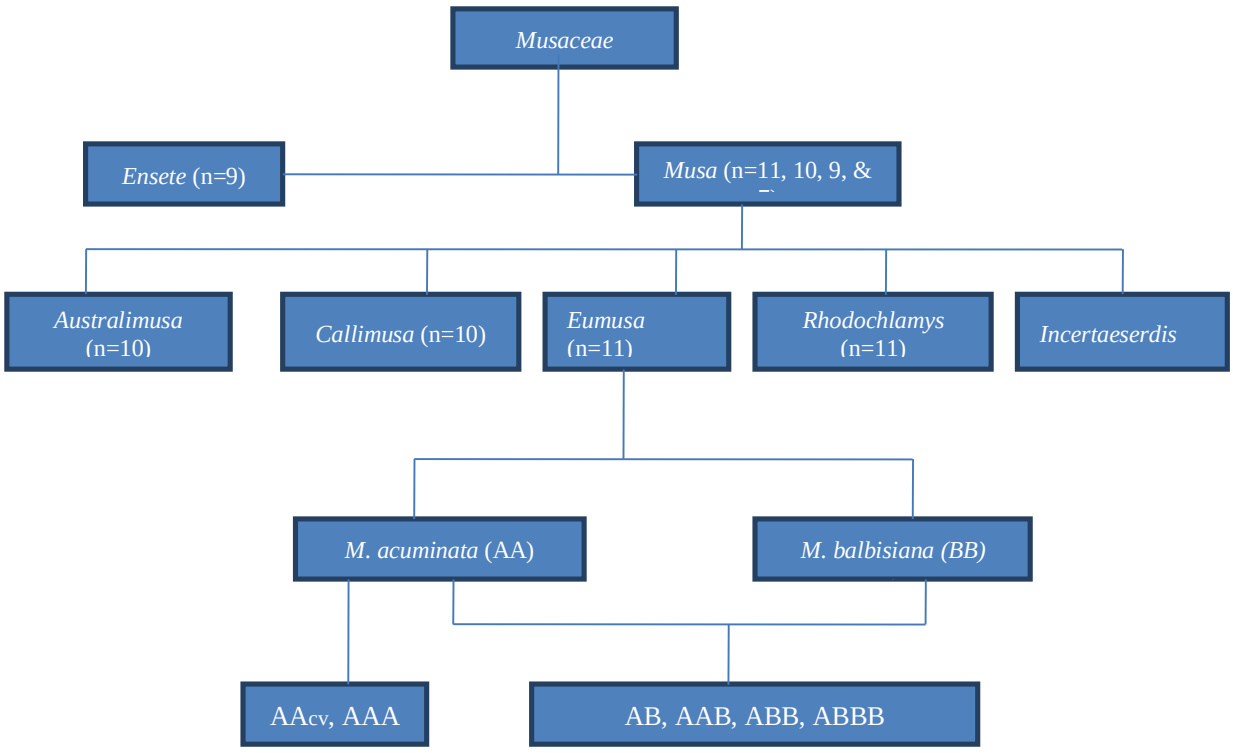


Figure 1: Classification of the family *Musaceae* showing sectional treatment of the genus *Musa* (Simmonds 1962; Simmonds & Shepherd 1955).

According to Simmonds (1962) *Eumusa* is the largest, most widely distributed, highly diversified and the most important section to which all edible bananas belong. Most cultivars in this section are derived from two species *M. acuminata* (AA genome) and *M. balbisiana* (BB genome) (Simmonds 1962). *Musa acuminata* is the most widespread of the *Eumusa* species being found throughout the range of the section, with Malaysia (Simmonds 1962) or Indonesia (Nasution 1991; Horry *et al.* 1997) as the center of diversity.

2.3.1 Genomes in *Musa*

Four genomes A, B, S and T are known to be present in cultivated bananas (Simmonds 1962). According to Horry *et al*. (1997) the A, B and S genomes are characteristic of species in the section *Eumusa*. The A and B genomes were derived from the wild diploid species *M. acuminata* and *M. balbisiana*, respectively (Cheesman 1948). The S genome is known to be present only in the diploid *M. schizocarpa* while the T genome is characteristic of the section *Australimusa* (2*n* = 2*x*

= 20) (Cheesman 1948). While the A and B genomes are found in the great majority of cultivars (Simmonds & Shepherd 1955), the S and T genomes occur in only a few (Carreel 1995). As noted above, breeding programmes in *Musa* are currently more concerned with only the A and B genomes (Arumuganathan & Earle 1991). Cultivated bananas are grouped on the basis of their genomic origins in relation to *M. acuminata* (AA) and *M. balbisiana* (BB) and their ploidy level (Simmonds 1966). A large number of genomic groups exist in banana and include diploids (AA, BB, and AB), triploids (AAA, AAB, and ABB) and tetraploids (AAAA) (Simmonds & Shepherd 1955). Other genomic groups including AAAB, AABB and ABBB from either natural or artificial hybridization are also known to exist (Creste *et al.* 2004; Pillay, Tenkouano, Ude & Ortiz 2004).

2.3.2 Genome attributes

Musa acuminata (A genome) and *M. balbisiana* (B genome) contributed to the genomic constitution of interspecific natural and artificial cultivars (Lebot, Manshardt & Meilleur 1994; Robinson 1996; Amaud & Horry 1997). According to Simmonds (1962) edibility and subsequent domestication of mature fruits came about as a result of mutations causing female sterility and parthenocarpy in diploid *M. acuminata*. Drought resistance and hardiness is contributed by the B genome since *M. balbisiana* clones thrive in areas experiencing pronounced dry seasons alternating with monsoons (Robinson 1996). The organoleptic characteristics such as starchiness and acid taste, causing AAB plantain to be starchier but less sweet and less palatable when raw than the AAA dessert bananas are derived from the B genome (Simmonds 1962). The genes for greater disease and pest resistance are also contributed by the B genome (Lebot, Aradhya, Manshardt & Meilleur 1993; Robinson 1996). *Musa balbisiana* derivatives show greater variability and produce fruits with higher dry matter content, increased nutritional value and Vitamin C (Simmonds 1962). *Musa balbisiana* gives textures and flavors that are not characteristic in *M. acuminata* derived genotypes (Miller, Bertioli, Baurens, Quirino, Ciampi, Santos, Martins & Souza 2009). *Musa acuminata* has traces of wax on fruits while *M. balbisiana* is often strongly waxy (Stover & Simmonds 1987).

Distinguishing genomes is of both practical and theoretical interest for *Musa* breeders (Pillay *et al*. 2011). It provides an effective way to trace useful genes, gene sequences and alien chromatin from the wild relatives (Boonruangrod, Desai, Fluch, Berenyi & Burg 2008; De Langhe, Hribova,

Carpentier, Dolezel & Swennen 2010). Agronomic and horticultural traits that are of interest to banana farmers can be traced in interspecific natural and artificial cultivars in *Musa* (Khayat 2004; Heslop-Harrison & Schwarzacher 2007). Distinguishing genomes would also facilitate development of genome-specific markers that can be used in such programmes like marker-assisted breeding (MAB), marker-assisted gene introgression and marker assisted selection (MAS) (Pillay *et al*. 2011).

Furthermore, distinguishing the A and B genome would provide an objective way of banana classification in *Musa* (Pillay *et al*. 2000). Classification would not rely on subjective scoring of morphological traits but on genome identification (Pillay *et al.* 2011). Classification could be done at any developmental stage of the plant instead of waiting for characteristics to be expressed after 18-24 months of maturity. Identification of genomic constitution in relation to linguistic diversity could be corrected and spurious classification could be verified (Vanhove *et al.* 2012). A SCAR marker would provide a rapid and accurate method to determine a plant's genomic status, especially in breeding programmes that involve interploidy crosses (Pillay *et al.* 2011).

2.4 Genome characterization in *Musa*

The study will only discuss a few techniques that are relevant to the identification and characterisation of genomes in *Musa*.

2.4.1 *In situ* hybridization

Genomic *in situ* hybridization (GISH) has been used to differentiate the chromosomes of the different genomes of *Musa* (Osuji *et al.* 1997; D'Hont *et al.* 2000). The study by Osuji *et al*. (1997) involved accessions containing only the A and B genomes and their combinations while that of D'Hont *et al.* (2000) included accessions with the A, B, S and T genomes and some of their combinations. These studies showed that it was possible to differentiate the chromosomes of the four genomes in banana cultivars and hybrids using fluorochromes. However, both studies reported cross hybridization between the genomes. Greater cross hybridization existed between the A and B genomes than between the A and S genomes (D'Hont, Paget Goy, Jenny, Noyer, Baurens, Lagoda & Carreel 1999). The least cross hybridization appeared between the T genome with the A and B genomes. The intensity of cross hybridization is a reflection of the sequence

homologies and affinities between the genomes (Simmonds & Weatherup 1990). It also reflects the genetic distances between cultivars representing the different genomes, and corresponds with morphological (Simmonds & Weatherup 1990) and molecular analyses (Carreel 1995; Ude *et al*. 2002a, b). The genomic *in situ* hybridization (GISH) results were also useful in settling cases of disputed genomic constitution (D'Hont *et al.* 2000).

2.4.2 Random Amplified Polymorphic DNA (RAPD) markers.

RAPD markers that were specific to nine genotypes of *Musa*, representing AA, AAA, AAB, ABB, and BB genomes was reported by Howell *et al.* (1994). However, cluster analysis showed grouping of AAA with AAB clones, suggesting that these RAPD primers were only specific to the A genome. Pillay *et al.* (2000) also identified RAPD markers linked to A and B genome sequences in *Musa* as shown in Figure 2. The Operon primer A18 amplified three unique RAPD fragments specific to B genome while two primers, designed A17 and D10, produced RAPD fragments specific to the A genome (Pillay *et al*. 2000).

RAPD markers are useful in cultivar analysis with the advantages of simplicity, efficiency, and non-requirement of any previous sequence information (Williams, Kubelik, Livak, Rafalski & Tingey 1990). RAPD markers have been successfully used for cultivar identification, selection and classification in *Musa* (Pillay *et al.* 2000). They are particularly useful for the initial determination of genome composition of breeding populations (Pillay *et al.* 2011). Despite their popularity, they are less informative and present high sequence similarity (Williams *et al.* 1990). RAPD markers have, to some extent, not made plant variety identification an efficient, recordable, and easy exercise as anticipated (Wang, Zhang, Li, Gao, Du & Tan 2007; Demirsoy, Demir, Demirsoy, Kacar & Okumus 2008). Many technical disadvantages associated with RAPDs that include the problem of reproducibility and their inability to distinguish heterozygous and homozygous alleles (Thornmann & Osborn 1992; Muralidharan & Wakeland 1993; Heslop-Harrison & Schwarzacher 2007) have, however, limited its use in modern day traditional genetics (Ercisli, Gadze, Agar, Yildirim & Hizarci 2009; Hasnaoui, Marsa, Ghaffarib, Trific, Melgarejod & Hernandez 2010).

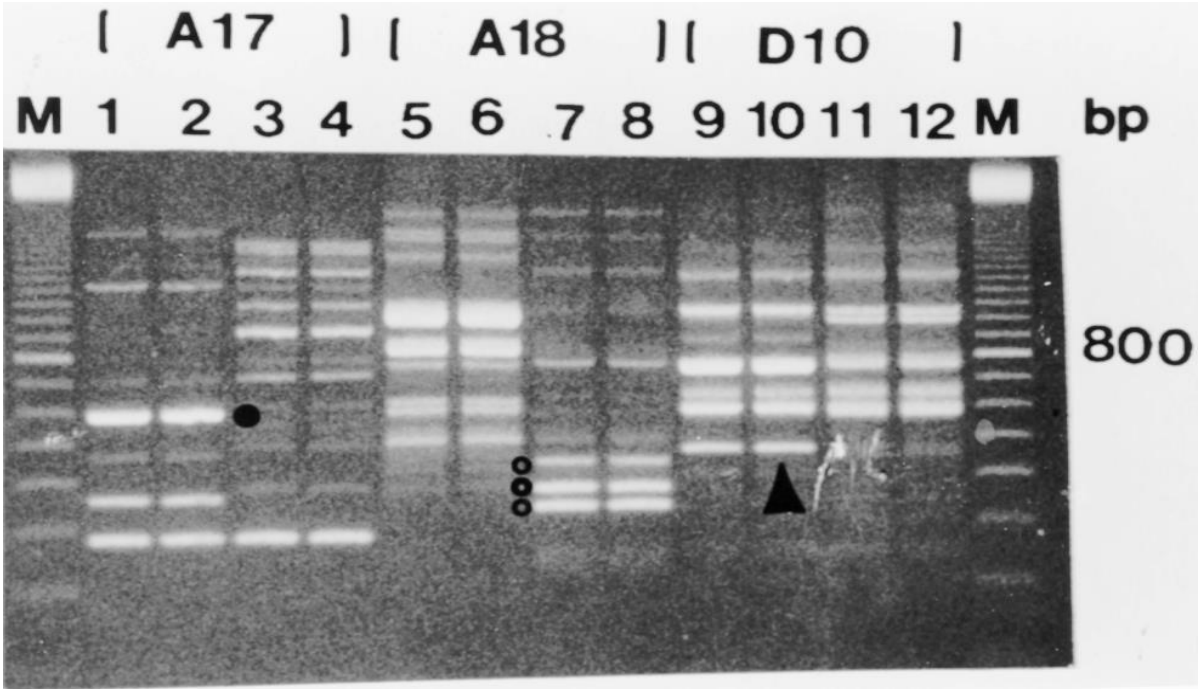


Figure 2: Species-specific RAPD patterns. *Musa acuminata* (lanes 1 and 2, 5 and 6, 9 and 10) and *M. balbisiana* (lanes 3 and 4, 7 and 8, 11 and 12) with primers A17, A18, and D10. Unique bands for A17, A18, and D10 are indicated with a dot (●), open circles (○), and an arrow head (▲), respectively. Lane M represents a 100 bp molecular weight marker (Pillay *et al.* 2000).

2.4.3 Restriction Fragment Length Polymorphism (RFLP).

Polymerase chain reaction (PCR) of the internal transcribed spacer regions of ribosomal RNA genes followed by restriction enzyme digestion has also been reported to provide markers for the A and B genomes in *Musa* (Nwakanma *et al.* 2003a). A 530 bp fragment unique to the A genome and two fragments of 350 bp and 180 bp specific for the B genome were identified. Interspecific cultivars with both A and B genomes possessed all three fragments. A dosage effect was observed for the B genomes since the staining intensity of accessions with two B genomes was approximately twice that of accessions with a single B genome (Nwakanma *et al.* 2003a).

2.4.4 IRAP (Inter-Retrotransposon Amplified Polymorphism)

IRAP is a complementary dominant marker system used to detect polymorphism in retrotransposon insertion (Schulman, Flavell & Ellis 2004; Nair, Teo, Schwarzacher & Heslop-Harisson 2005). Teo *et al.* (2005) used these markers for identification and characterization of banana cultivars and classification of *Musa* genome constitutions. Among the 36 cultivars tested, the B-specific band was absent in the AA and AAA cultivars but present in all interspecific hybrids with the B genome, including the AB, AAB and ABB cultivars. The band intensity was higher in ABB genomes as compared to the AAB genomes (Nair *et al.* 2005). However, four cultivars reported to have the ABB genome showed a pattern similar to AAB, and one cultivar reported to have AAA genome showed a pattern similar to ABB genome, suggesting missampling or misidentification (Teo *et al.* 2005). Balint-Kurti, Clendennen, Dolezelova, Valarik, Dolezel, Beetham and May (2000) were able to distinguish the A and B genome using Ty3-gypsy-like retrotransposons probe. However, when the probe was hybridized on *Hin*dIII digested genomic DNA from eight cultivars of banana with *M. vellutina* as the control, it could not differentiate between AAB and ABB genomes (Balint-Kurti *et al.* 2000).

2.4.5 SCAR (Sequence characterized amplified region) marker

SCARs are longer primers which can be particularly useful to follow the inheritance of marked regions of parental genomes (Paran & Michelmore 1993). SCAR markers detect only a single locus, their amplification is less sensitive to reaction conditions and they can potentially be converted into allele-specific markers (Ardiel *et al* 2002). The locus-specific SCAR markers generate polymorphism of known identity, and are readily considered for questions of phylogeny or genetic relatedness (Fang, Eu & Chung 2011), species identification (Ercisli *et al.* 2009) and fingerprinting studies (Ardiel *et al.* 2002). SCARs are co-dominant markers and have a higher resolving power than RAPDs (Linhart 2000). They distinguish heterozygotes from homozygotes, allowing the determination of genotypes and allele frequencies at loci (Ardiel *et al.* 2002; Ercisli *et al.* 2009). SCAR markers have a higher reproducibility as compared to RAPD markers (Rugienius, Siksnianas, Stanys, Gelvonauskieni, & Bendokas 2006). However, in most cases, SCAR markers rely on the high quality sequencing data from RAPD products for their development (Paran & Michelmore 1993; Linhart 2000). SCAR markers developed from RAPD products have the advantages of cost effectiveness and technical simplicity (Heslop-Harrison & Schwarzacher 2007).

2.5 Conclusion

The development and use of molecular markers for the identification, classification and characterisation of genomes is one of the most significant developments in the field of molecular genetics (Ma & Gustafson 2008). The application of various types of molecular marker techniques in banana classification, and differences in their principles, methodologies, and applications has widened the knowledge of genomes in *Musa* genomics. SCAR markers linked to the A genome in bananas and plantains have not been reported as yet. More effort should be directed at examining genome-specific SCAR markers in *Musa*, which could help solve problems associated with genome characterization and classification (D'Hont, Denoeud, Aury, Baurens, Carreel, Garsmeur, Noel, Bocs, Droc & Rouard 2012; Vanhove *et al.* 2012).

CHAPTER 3

MATERIAL AND METHODS

3.1 Introduction

This chapter outlines how the study was carried out from DNA isolation, quantification and standardization, RAPD analysis, DNA recovery from the agarose gel, cloning, sequencing and SCAR primer development, SCAR primer testing and SCAR marker development.

3.2 DNA extraction

Plant material

Dried leaf material of *Musa* accessions was obtained from the International Transit Centre, Laboratory of Tropical Crop Improvement, Leuven, Belgium.

Solutions required

- Extraction buffer: 100 mM Tris-HCl (pH 8), 50 mM EDTA, 1.4 M NaCl, 2 % CTAB.
- 3M Sodium acetate, β-mercaptoethanol, chloroform isomyl alcohol (24:1), isopropanol, 70 % ethanol, 99 % ethanol, 10 mg/ml RNase.
- 10 X Tris EDTA (TE) buffer: 10 mM Tris-HCl (pH 8), 0.1 mM EDTA.

DNA isolation procedure

Genomic DNA was extracted from 22 samples according to the CTAB procedure by Crouch *et al.* (1998a). Dry leaf tissue (1g) was ground to a fine powder in liquid nitrogen. The leaf powder was transferred into an opaque oak ridge tube containing 20 ml CTAB isolation buffer with 20 µl β-mercaptoethanol with a precooled spatula and mixed thoroughly. The suspension was incubated at 65 ^{o}C for 30 min with intermittent mixing. After cooling the suspension for 2-5 min, 10 ml of chloroform isoamyl alcohol (24:1) was slowly added and the tube contents were mixed thoroughly. The mixture was centrifuged at 6,000 rpm for 10 min. The supernatant was extracted with a micropipette into a new oak tube. The DNA was precipitated by adding 2/3 volume of ice-cold (2 ^{o}C) isopropanol to the supernatant followed by gentle mixing. A strand-like flocculation was observed. After centrifuging the contents at 6,000 rpm for 10 min, the supernatant was decanted and the pellet was rinsed with 70% ethanol. The ethanol was then pipetted off and the tubes tilted downwards to allow the pellet to dry at room temperature. The pellet was re-suspended in 600 µl T.E buffer and left at room temperature to dissolve. To digest RNA, 10 µl RNase (10 mg/ml) was

added and the contents incubated at 37 ºC for 30 min. A volume of 1/10 3 M sodium acetate was added to the mixture followed by 2 volumes of cold 99 % ethanol. The content was mixed and then centrifuged at 6,000 rpm for 5 min. A white precipitate of DNA was observed at the bottom of the tube. The supernatant was decanted, the pellet washed with 70 % ethanol and the pellet dried briefly at room temperature. To dissolve the DNA, 200 µl T.E. buffer was added to the pellet and left for a day to dissolve. The DNA was stored in Eppendorf tubes at -20 ºC before use. The DNA samples were quantified according to the protocol using NanoDrop 2000cUV-Vis spectrophotometer (ThermoScientific, Miami, FL) and diluted to 40 ng/µl using TE buffer. The quality and quantity of the DNA was assessed by agarose gel electrophoresis.

3.3 RAPD assay

Reaction mixtures for RADP analysis consisted of 3 µl DNA, 1.5 µl of 37.5 mM MgCl, 3.0 µl of 2.5 mM each dNTP, 2.0 µl of 2 µM OPA-17 primer purchased from Operon Technologies (Alameda, California), 3.88 µl of nuclease free water, 1.5 µl of 10 x amplification buffer, 0.12 µl of Taq polymerase in a total volume of 15 µl. Amplifications were performed in polyethylene tubes in a Bioer XP Thermal cycler (Bioer, Tokyo, Japan) with the following amplification conditions: an initial 3 min denaturation at 94 ºC followed by 35 cycles of 50 s at 94 ºC, 50 s at 40 ºC, and 1.5 min at 72 ºC, with a final extension step of 7 min at 72 ºC. Approximately 15 µl of the amplification products were separated on 1.2 % agarose gels in 1x TBE buffer. Molecular weight markers included in the gel were the 100 bp and 200 bp ladders purchased from Fermentas (Burlington, Ontario, Canada). The gel was stained in ethidium bromide and photographed under UV light.

3.4 DNA extraction from agarose gel

DNA was extracted from agarose gels with the NucleoSpin® Extract II kit (Macherey-Nagel, Dueren, Germany). A sterile scapel was used to carefully excise the DNA fragment from the agarose gel to minimize the gel volume. The weight of the gel slice was determined before transferring it into a clean tube. About 200 µl buffer NT was added for each 100 mg of agarose gel followed by incubating the sample at 50 ºC for10 min and briefly vortexing every 2-3 min to completely dissolve the gel slices. A Nucleospin® Extract II column was placed into a 2 ml collecting tube before adding the sample. Discarding the flow-through, the sample was centrifuged

at 11,000 x g for 1 min to bind DNA. The sample was further centrifuged at 11,000 x g for 1 min after adding 600 µl of NT3 buffer, to wash the silica membrane. After incubating the Nucleospin® Extract II column for 5 min at 70 ^{o}C, residual NT3 buffer was removed by centrifugation at 11,000 x g for 2 min. The Nucleospin® Extract II column was then placed into a clean 1.5 ml microcentrifuge tube before adding 35 µl DNA elution buffer NE. The sample was then incubated at room temperature for 1 min before centrifuging for 1 min at 11,000 x g.

3.5 Cloning

The pGEM-T vector system cloning kit (Promega) was used to clone the RAPD product following the manufacturer's protocol. For cultivation of bacterial cells harbouring standard high copy plasmids, LB (Luria-Bertani) media was used. The cells were checked for viability, competence and transformation. White colonies were selected (as opposed to blue) indicating possible transformation. The colonies were analysed by PCR to further confirm that the target fragment had been cloned. The white colonies carrying the transformed pGEM-T plasmid were compared to the blue colonies carrying the untransformed pGEM-T plasmid (negative control) and the RAPD products (positive control) using agarose gel electrophoresis. The lack of plasmid insertion was determined by relative migration distance of the samples. The candidate plasmid DNAs were isolated and purified using the NucleoSpin® Plasmid QuickPure kit (Macherey-Nagel, Dueren, Germany) as described by the manufacturer's protocol.

3.6 Sequencing

The cloned RAPD band was sent to Inqaba Biotechnologies (Pvt) Company (Pretoria, South Africa) for sequencing. The cloned RAPD band was sequenced using the ABI 3130XL sequencer (Applied Biosystems, CA). The Geospiza Finch Suite (Geospiza Inc., Washington), a web based sequencing, tracking and retrieval software was used to make DNA sequence generation and data handling simpler, faster and more cost effective.

3.7 SCAR primer designing and synthesis

The SCAR primers were designed using Primer3 (www.simgene.com/Primer3), a primer design and analysis computer software program. The first six primer pairs were selected as the best primer pairs for synthesis. The designed SCAR primer pairs were sent to Inqaba Biotecnologies (Pvt) Company for synthesis.

3.8 SCAR marker development

The six SCAR primer pairs (Table 1) were tested on a sample of eight randomly selected homo- and heterogenomic accessions for screening purposes. The eight accessions included *M. balbisiana* (10852) (BB), 'Kelong Mekintu' (AAB), 'Safet Velchi' (AB_{CV}), *M. acuminata* 'Calcutta 4' (AA), 'Gros Michel' (AAA), *M. textilis* (TT), 'CRBP 39' (AAAB) and *M. schizocarpa* (SS). The successful SCAR primer pair (SC3: CGC TTG TGT GAA TCT CAG GA and TCT TTT CGT TGC TTC TCC GT) was tested on four homogenomic accessions representing the four genomes (A, B, S and T) in *Musa*.

Table 1: SCAR primers and their sequences listed from 5' to 3'.

Designation	SCAR primer sequences	
	Forward	*Reverse*
SC1	GCT TGT ACT GGT GGG CAT AC	CCG CTT GTT AAT TGA GGT GC
SC2	GCT TGT TCC CTC GAC AAG AT	CCG CTT GTA AGA GAT GTG GC
SC3	CGC TTG TGT GAA TCT CAG GA	TCT TTT CGT TGC TTC TCC GT
SC4	AAA GTA TTG CTG GCA CCT GTC	ATT CCT ATG CGC ATT TTT CG
SC5	GGT GAC CGT CTA ATA TCT GAG T	TCA GGT GGG ATT AAG AAC GG
SC6	TTT AAA TCT TCA GGG TGC TGC AGG T	TGG ACT AGA GAG GGC CTG AA

*SC is a prefix used to designate $SCOPA17_{600}$.

The SCAR PCR amplifications were performed in polyethylene tubes in a Bioer XP Thermal cycler (Bioer) with the following amplification conditions: an initial 3min denaturation at 94 ^{o}C followed by 40 cycles of 50 s at 94 ^{o}C, 50 s at 50 ^{o}C, and 1.5 min at 72 ^{o}C, with a final extension step of 7 min at 72 ^{o}C. The thermocycling protocol annealing step was changed to accommodate the longer SCAR primers. Approximately 15 µl of the amplification products were separated on 1.2 % agarose gels in 1x TBE buffer. Molecular weight markers included in the gel were the 100

bp and 200 bp ladders purchased from Fermentas. The gel was stained in ethidium bromide and photographed under UV light.

3.9 SCAR marker validation

The SCAR primers were tested on twenty-four banana accessions shown in Table 2. These plants were selected to represent a wide range of genomic groups that include landraces and synthetic hybrids.

Table 2: List of landraces used to verify and validate the A genome-specific SCAR marker in *Musa* (Bioversity International Transit Centre, Belgium).

ITC code	**Accession name**	**Accession genomes**
ITC0048	'Valery'	AAA
ITC0090	'Tjau Lagada'	AA
ITC 0094	*M. balbisiana* (10852)	BB
ITC0127	'Kamaramasenge'	AB
ITC0200	'Kelong Mekintu'	AAB
ITC0226	'Ntanga 4'	AAB
ITC0245	'Safet Velchi'	AB_{CV}
ITC0247	*M. balbisiana* 'Honduras'	BB
ITC0248	*M. balbisiana* 'Singapuri'	BB
ITC0249	*M. acuminata* 'Calcutta 4'	AA
ITC0346	'Giant Cavendish'	AAA
ITC 0394	'Cardaba'	ABB
ITC0395	'Lidi'	AA
ITC0484	'Gros Michel'	AAA
ITC0513	'Plantain No. 2'	AAB
ITC0539	*M. textilis*	TT
ITC0643	'Cachaco'	ABB
ITC0662	'Khai Thong Ruang'	AAA
ITC0846	*M. schizocarpa*	SS
ITC1120	*M. balbisiana* 'Tani'	BB
ITC1344	'CRBP 39'	AAAB
ITC1418	'FHIA 25'	AAB

CHAPTER 4

RESULTS

4.1 Introduction

The chapter presents the results obtained from the RAPD assay, cloning, sequencing, primer design, primer synthesis and SCAR assay.

4.2 RAPD assay results

The RAPD primer OPA-17 produced a 600 bp fragment ($OPA17_{600}$) only in *M. acuminata* (Fig 3 lanes 1-6 and 8-14). The fragment was absent in *M. balbisiana* (lane 7).

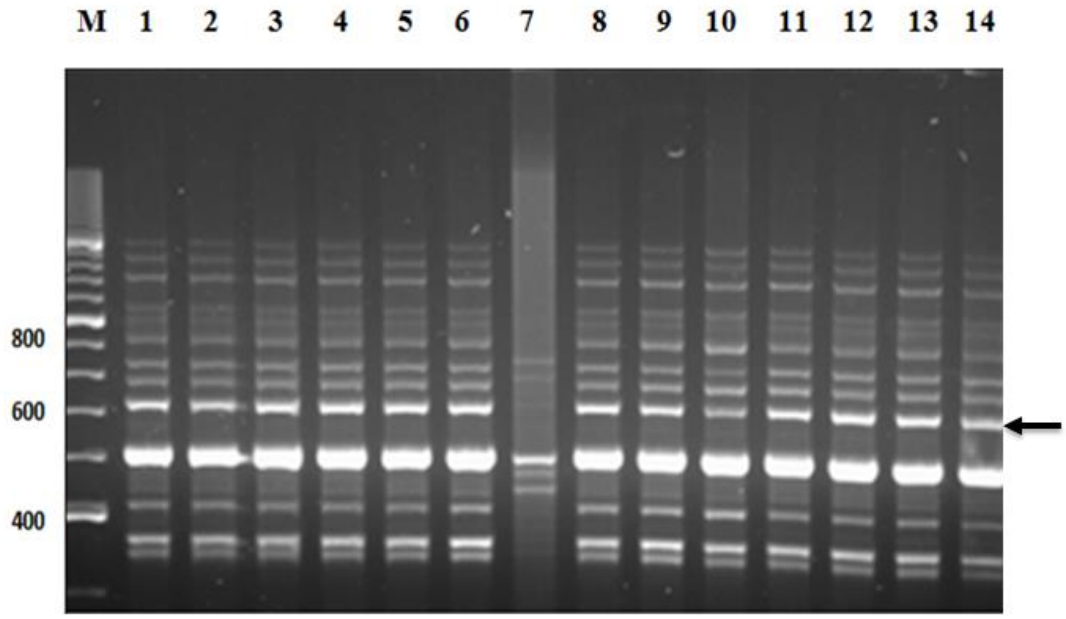


Figure 3: PCR amplification patterns showing the $OPA17_{600}$ (arrow) unique to *M. acuminata* (lanes 1-6 and 8-14) and absent in *M. balbisiana* (lane7). M is the 100 bp molecular marker.

The automatic band detection to confirm the $OPA17_{600}$ using the Quantity One Version 4.6.9 Windows and Macintosh showed that the Operon primer A17 produces a RAPD fragment ($OPA17_{600}$) specific to the A genome in *Musa* (shown in Appendix 1). The results were congruent with the previous investigations noted in Pillay *et al.* (2000).

4.3 Cloning results

The blue-white screening technique was used for the detection of successful ligations in vector-based gene cloning as described by Erlich (1989) and Pollard and Earnshaw (2002). The results are shown in Figure 4.

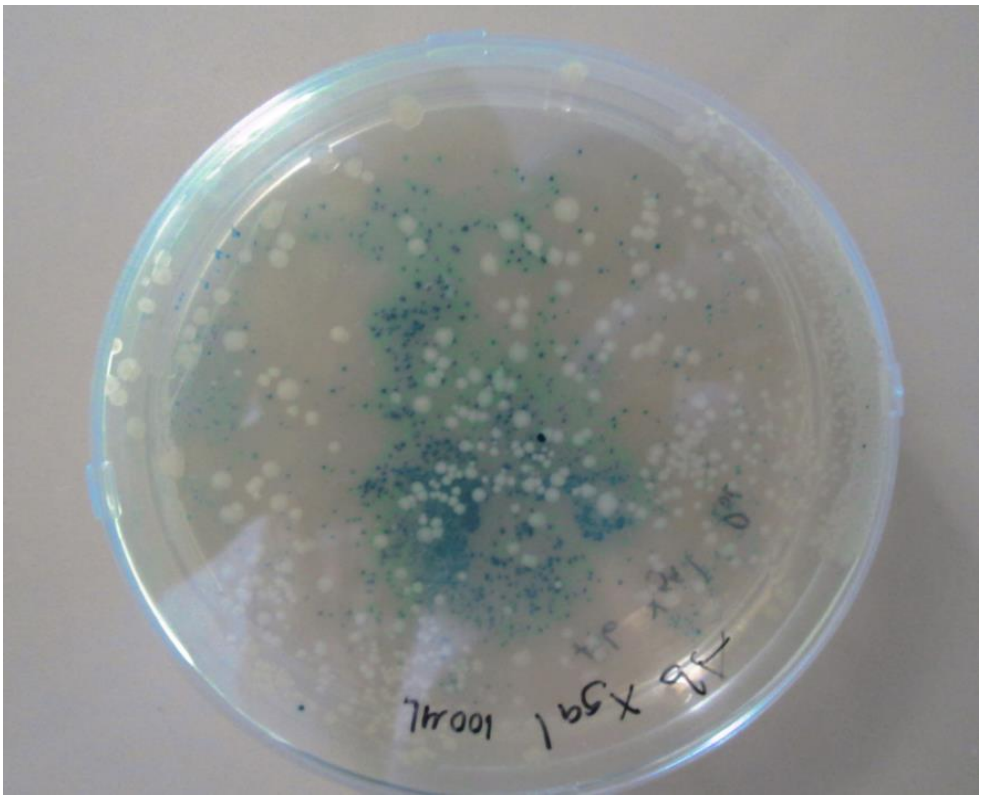


Figure 4: Blue and white colonies on the plate

Blue colonies represent a vector with an uninterrupted *lacZ-α* (therefore no insert), while white colonies, where X-gal is not hydrolysed, indicate the presence of an insert (Pollard & Earnshaw 2002).

4.4 Colony PCR analysis of transformants

White and blue colony PCR analysis of transformants was carried on agarose gel electrophoresis as shown in Figure 5. The results indicated that the selected white colonies (Lane 1, 2 & 4)

migrated the same distance with the positive control (lane 6, $OPA17_{600}$ products) suggesting that the DNA fragment of interest was inserted into the plasmid. The negative control (lane 5, blue colonies) migrated the most; highlighting the lack of the DNA insert. The results obtained in Figure 5 were positive and evidence enough to prepare the bands for sequencing.

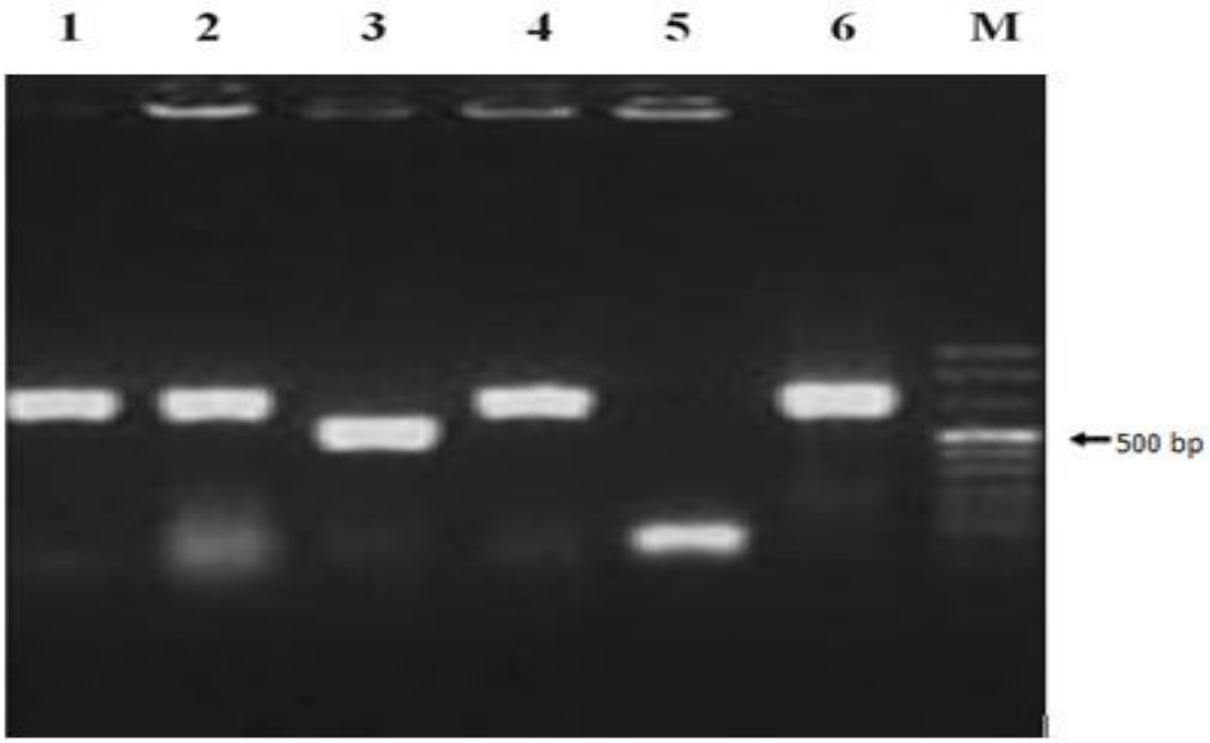


Figure 5: Colony PCR analysis of transformants on agarose gel electrophoresis. Lanes 1-4 represent 4 selected white colonies, lane 5 is the negative control (blue colonies), and lane 6 is the positive control ($OPA17_{600}$ products). M represents the 100 bp DNA ladder.

4.5 SCAR primer synthesis results

The sequence of the DNA fragment corresponding to $OPA17_{600}$ is shown in Figure 6. The highlighted region in bold indicates the sequence of the RAPD primer OPA17. The six SCAR primer pairs (shown in Table 1) were designed according to the sequence of the $OPA17_{600}$ fragment. The SCAR marker nomenclature is according to Paran and Michelmore (1993). SCAR markers have the SC prefix placed before the name of their corresponding RAPD marker with its relative base pair length, (for example, marker $SCOPA17_{600}$ is the SCAR marker derived from

RAPD OPA17$_{600}$) (Paran & Michelmore 1993). The underlined regions shown in Figure 6 indicate the sequence for the selected SCAR primer pair (SC3, hereafter referred to as SCOPA17$_{600}$). Each SCAR primer contained the original ten-bases of the OPA-17 primer at the 5' end and the subsequent internal bases from the other end.

```
1     AGACCGCTTG  TGTGAATCTC  AGGACAGTTT  GTACAGGAGG  TCCACCGAGT
51    GTTTAAGATT  GTTCCTGCTG  AAGTTGGTGG  CAGATTTGGG  ATGGTTAATT
101   AATCTCTGAT  TTCATCCAAC  TCGTCCGAGA  TGTTCTTGAC  CTGTAAGATG
151   ACCGACTTCA  TGTGAGATTC  TCGATACGGT  TCTTTTGATG  CTCGAGATAG
201   AATCTCAGCT  TCATGTGAGA  TTCCCGATAG  GGTTCTCTCG  AGATAGAATC
251   TTGAGATGGG  TTTTATGGTT  AAGAGAGAGA  AGTATCAAAC  CTCCATAGCT
301   ATGCTTGCTT  CCATCTTTTA  ATCGGTCCTT  CCCTACAATC  AGAAACTCTG
351   AGCTTTGTTG  TTTGGTTGCT  TAACGTATAA  GGTAGAAACA  GACCAAAACA
401   AAAAAGGAAG  AACCAACAGA  CAAAGAAACC  CAAAAATAAA  AGATACTTCG
451   ATGACTTAGT  CGAAGAGCAC  AAGATACTAG  AACATACAGC  ATCAAGAATC
501   CGACGGAGAA  GCAACGAAAA  GAACGAAAAA  CCAAAAGGAA  AACATGGAAC
551   GATCCACAAA  GGAGGAAGAA  ATTCGAGGAT  CCAACGCAAA  GATCAGGGAG
601   GAACAAGCGG  TC
```

Figure 6: DNA sequence of the OPA17$_{600}$ fragment.

4.6 SCAR primer screening and selection

The synthesized SCAR primer pairs were tested on a sample of eight randomly selected homo- and heterogenomic accessions for their fidelity. The eight accessions include *M. balbisiana* (BB), 'Kelong Mekintu' (AAB), 'Safet Velchi' (AB$_{CV}$), *M. acuminata* 'Calcutta 4' (AA), 'Gros Michel' (AAA), *M. textilis* (TT), 'CRBP 39' (AAAB) and *M. schizocarpa* (SS). A standard PCR protocol was used for the amplification of each SCAR primer pair. The SCAR primer pair SC3 produced the 600 bp fragment in all the A genome containing banana accessions (Fig. 7).

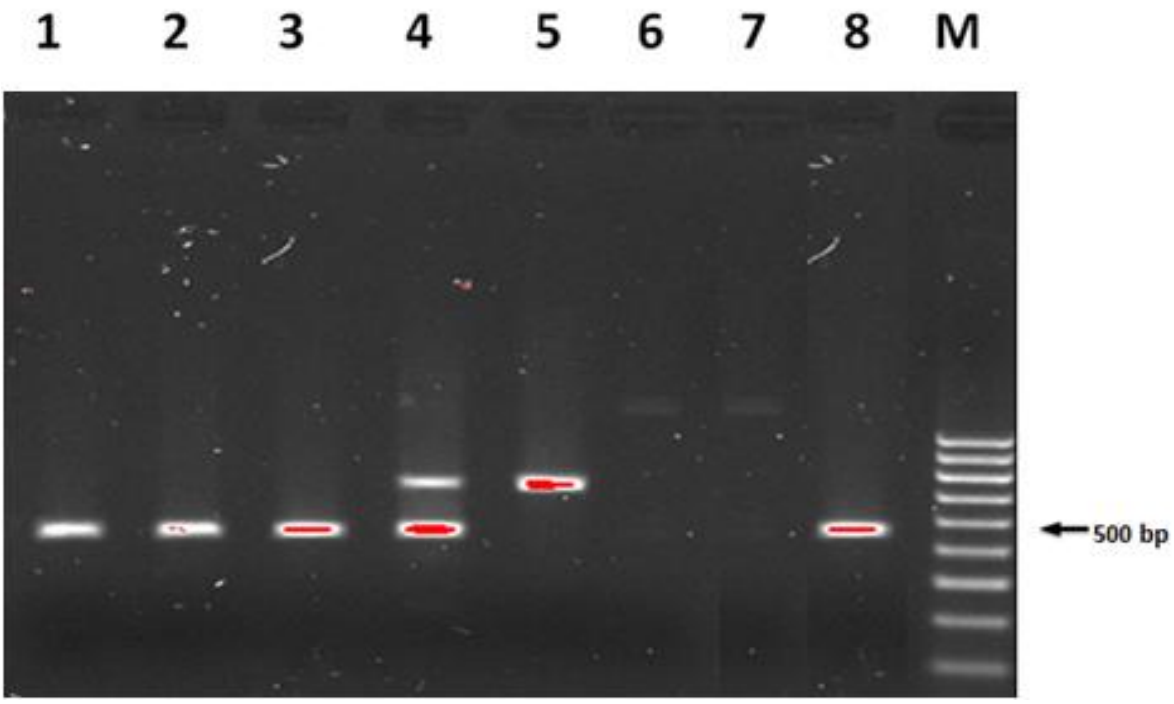


Figure 7: SCAR PCR profile for SC3 SCAR primers. The lanes represent DNA from (1) *M. acuminata* 'Calcutta 4' (AA) (2) 'Gros Michel' (AAA) (3) 'Kelong Mekintu' (AAB) (4) 'Safet Velchi' (AB_{CV}) (5) *M. balbisiana* (BB) (6) *M. schizocarpa* (SS) (7) *M. textilis* (TT) (8) 'CRBP 39' (AAAB). Lane M represents 100 bp molecular marker.

The SCAR PCR profiles of the other five primers are shown in Appendix 2 to 6. The SCAR primer pairs SC1 and SC2 produced single fragments 300 bp and 400 bp, respectively, which were present in all the accessions. The primer pair SC4 produced a same size band in *M. textilis* (TT) and *M. acuminata* (AA) and did not amplify the DNA in the other accessions.

The primer pairs SC5 and SC6 did not amplify the A genome in both homo- and heterogenomic accessions. The primer pair SC3 produced a unique A genome-specific SCAR fragment in all the accessions carrying the A genome, namely 'Kelong Mekintu' (AAB), 'Safet Velchi' (AB_{CV}), *M. acuminata* 'Calcutta 4' (AA), 'Gros Michel' (AAA), 'CRBP 39' (AAAB) (shown in Fig. 7). The SCAR primer pair SC3 was selected for further investigation and verification as a suitable marker for the A genome.

4.7 SCAR marker validation

Further investigations to validate the SCAR marker were carried out on a sample of twenty-two accessions representing species, landraces and hybrids of different ploidy and genome combinations. The $SCOPA17_{600}$ produced a consistent 600 bp banding pattern in 'Valery'(AAA), 'Tjau Lagada' (AA), 'Kamaramasenge' (AB), 'Kelong Mekintu' (AAB), 'Ntanga 4' (AAB), 'Safet Velchi' (AB_{CV}), '*M. acuminata* Calcutta 4' (AA), 'Giant Cavendish' (AAA), 'Cardaba' (ABB), 'Lidi'(AA), 'Gros Michel' (AAA), 'Plantain No. 2' (AAB), 'Cachaco' (ABB), 'Khai Thong Ruang'(AAA), 'CRBP 39' (AAAB) (22) 'FHIA 25' (AAB) (shown in Fig. 8 & Appendix 7). However, the SC3 SCAR primers also produced an 800 bp fragment in some of the accessions harbouring the B genome.

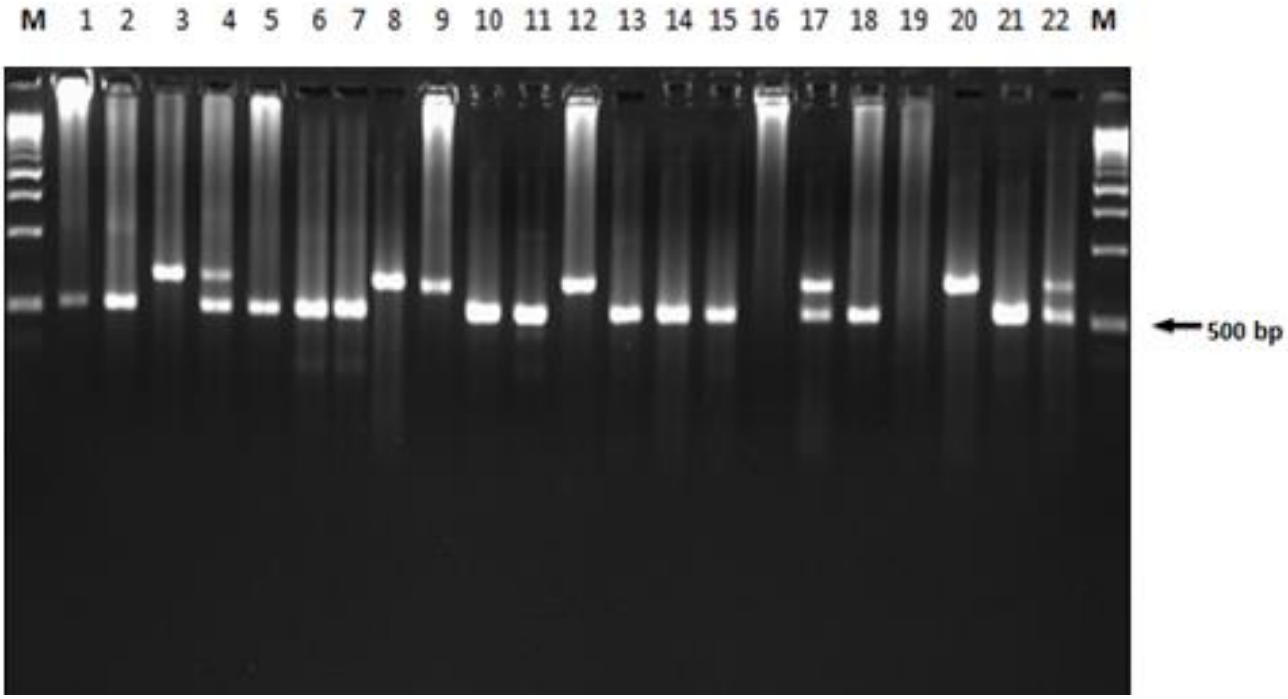


Figure 8: SCAR patterns of *Musa* landraces showing the $SCOPA17_{600}$ (arrow). The lanes represent DNA from (1) 'Valery'(AAA) (2) 'Tjau Lagada' (AA) (3) *M. balbisiana* '(10852)' (BB) (4) 'Kamaramasenge' (AB) (5) 'Kelong Mekintu' (AAB) (6) 'Ntanga 4' (AAB) (7) 'Safet Velchi' (AB_{CV}) (8) *M. balbisiana* 'Honduras' (BB) (9) *M. balbisiana* 'Singapuri' (BB) (10) *M. acuminata* 'Calcutta 4' (AA) (11) 'Giant Cavendish' (AAA) (12) 'Cardaba' (ABB) (13) 'Lidi'(AA) (14) 'Gros Michel' (AAA) (15) 'Plantain No. 2' (AAB) (16) *M. textilis* (TT) (17) 'Cachaco' (ABB)

(18) ‘Khai Thong Ruang’(AAA) (19) *M. schizocarpa* (SS) (20) *M. balbisiana* ‘Tani’ (BB) (21) ‘CRBP 39’ (AAAB) (22) ‘FHIA 25’ (AAB). Lane M represents 500 bp DNA marker.

4.9 Conclusion

The results showed that SC3 primers are not truly specific to the A genome but produced an 800 bp fragment in certain B genome containing fragments.

CHAPTER 5

5.1 Introduction

This chapter is a discussion of the results and findings of this study. The chapter gives a conclusive discussion on the success of the study and the challenges.

5.2 Discussion

The aim of this study was to develop a SCAR marker from a 600 bp RAPD fragment that was found to be specific to the A genome of banana (Pillay *et al*. 2000). The RAPD assay of this study was able to reproduce that 600 bp fragment in the A genome of banana (Fig. 2). This fragment was not present in the B genome containing species *M. balbisiana* (Fig. 2) suggesting that the fragment was specific to the A genome. The fragment was cloned successfully and blue and white colonies were observed (Fig. 3). Colony screening of a random number of white colonies produced the expected size fragments (Fig. 3) in lanes 1, 2 and 4. The odd sized fragment in lane 3 may be due to cloning of an incomplete insert. The fragment in lane 5 was equal to the size of the vector (blue colony).

Sequencing of the RAPD fragment produced a sequence of 601 bp (Fig. 4) that was approximately the size of the RAPD fragment. The Primer3 design produced a number of potential primers from the 601 bp sequence. Of these, 6 primers (Table 1) were selected for further studies on the basis of the GC content, melting temperatures, 3' stability and likelihood of forming primer dimers (Rozen & Skaletsky 2000). The primers were tested for their specificity to the A genome on 8 banana accessions representing different genome combinations (AA, AAA, AAB, AB, BB, SS, TT and AAAB). The primers SC1, SC2 produced a band of about 300 bp and 400 bp respectively in all the 8 accessions (Appendix 2 & 3). Primers SC4 and SC5 produced spurious bands (Appendix 4 & 5) while primers SC6 did not amplify the genomes of the 8 accessions (Appendix 6). One set of primers (SC3) was still specific to the A genome (Fig. 7). The primer did not amplify the genomes of the *M. schizocarpa* (SS) (lane 6) and *M. textilis* (TT) (lane 7). However, in addition to the 500 bp fragment the primers produced a 800 bp band in 'Safet Velchi' (AB) in lane 4. The other anomaly was the presence of only the 800 bp fragment in *M. balbisiana* (lane 5).

The SC3 primers were then tested for their specificity to the A genome on 22 accessions representing different genome combinations. As shown in Figure 6, the SC3 primers produced the

expected 600 bp A genome specific band in the following lanes: (1) 'Valery' (AAA), (2) 'Tjau Lagada' (AA), (4) 'Kamaramasenge' (AB), (5) 'Kelong Mekintu' (AAB), (6) 'Ntanga 4' (AAB), (7) 'Safet Velchi' (AB_{CV}), (10) *M. acuminata* 'Calcutta 4' (AA), (11) 'Giant Cavendish' (AAA), (13) 'Lidi' (AA), (14) 'Gros Michel' (AAA), (15) 'Plantain No. 2' (AAB), (17) 'Cachaco' (ABB), (18) 'Khai Thong Ruang' (AAA), (21) 'CRBP 39' (AAAB), and (22) 'FHIA 25' (AAB). The only accession with an A genome that did not show the A genome specific fragment in Fig.6 was 'Cardaba' (ABB) in lane 12. However, a faint band of 600 bp was observed in 'Cardaba' in a different experiment (lanes 4 and 5) with the SC3 primers (Appendix 6) suggesting that the accessions did carry the 600 bp band. This anomaly may be explained by the presence of several different types of A genomes in the *M. acuminata* complex and the annealing properties of the primers to the different A genomes. There is no experimental evidence for the presence of different A genomes in banana as yet. But the fact that the AAA East African Highland bananas and the AAA dessert bananas have such contrasting fruit characters strongly points to the likelihood of differences in the A genomes of banana. In addition, the SC3 primers produced a 600 bp and 800 bp band in (4) 'Kamaramasenge' (AB), (17) 'Cachaco' (ABB), (22) 'FHIA 25' (AAB) and only a single 800 bp fragment in (3) *M. balbisiana* '10852' (BB), (8) *M. balbisiana* 'Honduras', (9) *M. balbisiana* 'Singapuri', (20) *M. balbisiana* 'Tani'. To summarise the banding patterns observed with primers SC3 the following was observed:

(i) A 600 bp fragment was observed in all the A genome containing accessions

(ii) A 600 bp and 800 bp band was observed in some accessions harbouring the A and B genomes.

(iii) All the BB genome genotypes contained the 800 bp fragment

This study showed that while the 600 bp RAPD fragment was specific only to the A genome, the SC3 primer produced addition bands in some accessions containing the B genome.

If one considers only the 600 bp fragment that was observed in all the A genome containing accessions, the results of this study may point to the fact that the SC3 primers can be considered specific to the A genome of banana. On the other hand the amplification of the 800 bp fragment of the B genome in some of the accessions may imply that there is recombination between the A and B genomes. While the implication in previous studies (Howell *et al*. 1994; Nair et al. 2005; Pillay *et al*. 2000; Nwakanma *et al.* 2003a) alluded marked differentiation of the A and B genomes, the SC3 primers developed from the A genome does not suggest such a differentiation. Evidence

for the lack of complete differentiation of the A and B genomes of banana does exist in the literature (Ortiz & Vuylsteke 1994; Osuji *et al.* 1997; D'Hont *et al.* 2000; Heslop-Harrison & Schwarzacher 2007; De Langhe *et al.* 2010; Jeridi, Bakry, Escoute, Fondi, Carreel, Ferchichi, D'Hont & Rodier-Goud 2011; Jeridi, Perrier, Rodier-Goud, Ferchichi, D'Hont & Bakry 2012; Cizkova, Hribova, Humplikova, Christelova, Suchankova & Dolezel 2013; De Jesus, De Oliveira e Silva, Amorim, Ferreira, De Campos, De Gaspari Silva & Figueira 2013).

The first study that alluded to the lack of differentiation between the A and B genomes was that of Ortiz and Vuylsteke (1994). They reached this conclusion when they found that there was no preferential pairing between the homologous chromosomes of the A genome in the plantain (AAB). FISH (fluorescent in situ hybridization) studies (Osuji *et al*. 1997) showed that there was a high degree of cross-hybridization between the A and B genomes suggesting that the two genomes are incompletely differentiated and share common DNA sequences. D'Hont *et al.* (2000) also showed greater levels of cross hybridization between the T and the A or B genomes. The intensity of the cross hybridization may be a reflection of the sequence homologies and affinities between the genomes (Pillay & Tenkouano 2011).

Sequencing of several pairs of BACs containing homoeologous regions from *M. acuminata* and *M. balbisiana* showed that the A and B genomes are similar and that the gene order between them was largely conserved (Heslop-Harrison & Schwarzacher 2007). De Langhe *et al.* (2010) also suggested that meiosis offers the opportunity of pairing between the A and B chromosomes and formation of gametes not containing complete sets of A and B chromosomes. Cytogenetic analysis of meiotic metaphase configurations of interspecific triploids in *Musa* provided the first evidence that homoeologous chromosome pairing and recombination does occur between the A and B genomes (Jeridi *et al*. 2011). In a subsequent study, Jeridi *et al.* (2012) used cytogenetic evidence of mixed disomic and polysomic inheritance to suggest that there are chromosome exchanges between *M. acuminata* (AA) and *M. balbisiana* (BB). De Jesus *et al.* (2013) used flow cytometry, ITS and SSR to support their hypothesis that recombination does occur between the A and B genomes of banana. Finally, the study by Cizkova, Hribova, Humplikova, Christelova, Suchankova and Dolezel (2013) on the organization of DNA satellites in banana showed that two

satellites derived from *M. acuminata* was widespread in *M. balbisiana*, a number of ABB hybrids and the S genome.

Other mechanisms such as translocations could also account for the presence of the 600 bp A genome band in the B genome. Chromosome pairing at meiosis has revealed that translocations are frequent in banana genomes (Vilarinhos, Carreel, Rodier, Hippolyte, Benabdelmouna, Triaire, Bakry, Courtois & D'Hont 2006). Earlier studies also showed that retroelements class I transposable elements or transposons, are abundant in the *Musa* genome (Baurens, Noyer, Lanaud & Lagoda 1997; Balint-Kurti *et al*. 2000; Teo, Tan, Othman & Schwarzacher 2002). A recent report also showed that transposable elements account for almost half of the *Musa* genome (D'Hont *et al*. 2012).

The final mechanism that could account for the presence of the 800 bp sequence in *M. balbisiana* is chromosome substitution. Evidence for chromosome substitution has been provided by D'Hont *et al.* (2000). FISH analysis of the cultivar 'Pelipita' (ABB) did not show the expected 11 A and 22 B chromosomes but rather 8 A chromosomes and 25 B chromosomes.

While the 600 bp RAPD fragment was specific to the A genome, the SCAR primers (SC3) amplified both the A and B genomes suggesting that the marker was not as specific as expected.

The only other study where RAPD markers for genomes were converted to SCAR was reported in rice (Cheng, Fang, Lin, & Chung 2007). That study also found that a SCAR marker that was supposed to be specific for the BB genome also amplified one AA genome species and copy numbers of the SCAR markers was also present in low numbers in the CC genome.

This discussion has provided recent evidence from the literature that suggests that the A and B genomes of banana may not be as differentiated as previously considered. The possibilities of chromosome exchange between the A and B genomes of banana opens new avenues for breeding of bananas whereby valuable alleles from the two genomes could be combined (Jeridi *et al.* 2012).

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1 Introduction

This chapter presents conclusions drawn from the interpretative discussion of the findings and answers the research objectives, rounding off the dissertation. The implications and recommendations arising from the study are also noted with regard to the field of study.

6.2 Conclusion

Genome composition of banana has played an important role in the classification of bananas (Simmonds & Shepherd 1955). In addition the different genomes of banana harbour genes for different traits. It is accepted that parthenocarpy and female sterility arose in diploid *acuminata* accessions with A genomes. It is also accepted that hardiness to drought and fruit characteristics such as starchiness and acid taste is contributed by the B genome (Pillay *et al*. 2000).

Therefore it is important to identify the genome composition of a banana plant. This becomes more important in a breeding programme that may involve crosses with different genomes such as AA x BB. Such a cross can produce a variety of genotypes with genome combinations such as AB, AAB and ABB if one considers that 2n gametes often produced in banana. Therefore the purpose of this study was to identify the A genome of banana with a SCAR marker that was developed from a previously described RAPD marker (Pillay *et al*. 2000).

Out of the 6 sets of SCAR primers that were designed from the RAPD sequence, only one (SC3) amplified the expected 600 bp band in all the A genome containing accessions. This primer identified an A genome specific sequence in the *Musa* accessions used in this study. This fragment may be used as an A genome marker in *Musa*. This sequence was absent in the S and T genome containing species.

However, the SC3 primer amplified a 800 bp sequence in all the *M. balbisiana* genotypes and three of the eight accessions with mixed genomes (A and B). While this study has provided some reasons for this anomaly further research is required to provide a sound reason for it.

While analysing the meiotic behaviour of AB hybrids and some triploids, Shepherd (1999) concluded that the homology between the A and B genomes was weak. However, new cytogenetic and molecular evidence points to the fact that there is pairing and recombination between the A and B genomes of *Musa* (Osuji *et al.* 1997; D'Hont *et al.* 2000; Heslop-Harrison & Schwarzacher 2007; De Langhe *et al.* 2010; Jeridi *et al*. 2011, 2012; de Jesus *et al.* 2013; Cizkova *et al.* 2013).

6.3 Recommendations

The following recommendations are suggested for further research.

Sequencing of the 600 bp and 800 bp fragments will enable one to determine the homology between the sequences and the sites where the SC3 primers are annealing.

The 600 bp and 800 bp sequences could be used independently as probes in FISH experiments on meiotic or mitotic chromosomes of *M. acuminata* (A genome) and *M. balbisiana* (B genome). This will provide information on the location of these sequences on the A and B genomes. This experiment will also show whether these sequences are unique or repeated in the genomes and whethere they are located on a single or more than one chromosome. Similarly, the 600 bp fragment could be used as a hybridization probe in Southern experiments to ascertain its fidelity for the A genome.

Although GISH experiments were able to distinguish A and B genomes in the past, improvement of this technique will help to identify individual chromosomes. Such techniques will be able to identify intergenomic recombination in *Musa*. According to De Langhe *et al*. (2010) meiotic behaviour of *Musa* chromosomes may not provide unambiguous data on the homology between

the A and B genomes of *Musa*. Perhaps, if meiotic analysis is combined with GISH it may provide valuable information on the homology between the A and B genomes as shown by Jeridi *et al*. (2012).

De Langhe *et al*. (2010) have suggested that the best way to characterise the genomes of banana is to have many genome specific markers. However, very few markers are currently availabe for this type of analysis. It is hopeful that the 500 bp and 700 bp fragments identified in this study may add to this list of markers.

Finally since the species representing the A and B genomes of *Musa* (*M. acuminata* and *M. balbisiana*) are phenotypically different it is assumed that they may contain species specific proteins (De Langhe *et al*. 2010). Such research may provide information on the extent of differentiation/similarity between the A and B genomes of banana.

BIBLIOGRAPHY

AERT, L., SAGI, L. & VOLCKAERT, G. 2004. Gene content and density in banana (*Musa acuminata*) as revealed by genomic sequencing of BAC clones. *Theoretical and Applied Genetics*, 109: 129-139.

AMALRAJ, V.A. 1992. Collecting banana germplasm in Southern India. *Plant genetic Resources, Newsletter*, 88 :64-66.

AMAUD, E. & HORRY, J.P. 1997. Musalogue, a catalogue of *Musa* germplasm, Papua New Guinea Collecting Missions, 1988-1989, INIBAP, Montpellier.

ARDIEL, G.S., GREWAL, P., DEBERDT, B.G. & SCOLES, G.J. 2002. Inheritance of resistance to covered sprout barley and development of a tightly linked SCARS marker. *Theoretical and Applied Genetics*, 104: 457-464.

ARGENT, G.C.G. 1976. The wild bananas of Papua New Guinea. *Notes from the Royal Botanic Garden (Edinburgh)*, 35: 77-114.

ARUMUGANATHAN, K. & EARLE, E.D. 1991. Nuclear DNA content of some important species. *Plant Molecular Biology Reporter*, 9: 208-218.

AZHAR, M. & HESLOP-HARRISON, J.S. 2008. Genomes, diversity and resistance gene analogues in *Musa* species. *Cytogenetic and Genome Research*, 121(1): 59-66.

BALINT-KURTI, P., CLENDENNEN, S.K., DOLEZELOVA, M., VALARIK, M., DOLEZEL, J., BEETHAM, P.R. & MAY, G.D. 2000. Identification and chromosomal localization of the *monkey* retrotransposon in *Musa* spp. *Molecular and General Genetics*, 263: 908-915.

BAURENS, F.C., NOYER, J.L., LANAUD, C. & LAGODA, P.J.L. 1997. Sequence tagged site markets to draft the genomic structure of the banana chloroplast. *Fruits*, 52: 247-259.

BHAT, K.V., BHAT, S.R. & CHANDEL, K.P.S. 1992. Survey of isozyme polymorphisms for clonal identification in *Musa*. *Journal of Horticultural Science*, 67: 501–507.

BHAT, K.V. & JARRET, R.L. 1995. Random amplified polymorphic DNA and genetic diversity in Indian *Musa* germplasm. *Genetic Resources and Crop Evolution*, 42: 107-118.

BHAT, K.V., JARRET, R.L. & RANA, R.S. 1995. DNA profiling of banana and plantain cultivars using random amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP) markers. *Electrophoresis*, 16: 1736-1745.

BHAT, K.V., AMARAVATHI, Y., GAUTAM, P.L. & NELAYUDHAN, K.C. 2004. AFLP characterization and genetic diversity analysis of Indian banana and Plantain cultivars (*Musa* spp.). *Plant Genetic Resources: Characterization and Utilization*, 2: 121-130.

BOONRUANGROD, R., DESAI, D., FLUCH, S., BERENYI, M. & BURG, K. 2008. Identification of cytoplasmic ancestor gene-pools of *Musa acuminata* Colla and *Musa balbisiana* Colla and their hybrids by chloroplast and mitochondrial haplotyping. *Theoretical and Applied Genetics*, 118: 43-55.

CARREEL, F. 1995. Etude de la diversitegenetique des bananiers (genre *Musa*) a l'aide des marqueurs RFLP, PhD Thesis, Institut National Agronomique Paris-Grignon.

CARREEL, F., GONZALEZ DE LEON, D., LAGODA, P., LANAUD, C., JENNY, C., HORRY, J.P. & TEZENAS DU MONTCEL, H. 2002. Ascertaining maternal and paternal lineage within *Musa* by chloroplast and mitochondrial DNA RFLP analyses. *Genome* 45: 679-692.

CHEESMAN, E.E. 1948. Classification of the bananas. ll: The genus *Musa* L. *Kew Bull* 2: 106-117.

CHENG,Y.Y., FANG, S.A., LIN, Y.C. & CHUNG, M.C. 2007. A repetitive sequence specific to Oryza species with BB genome and abundant in *Oryza punctata* Kotschy ex Steud. *Botanical Studies*, 48: 263-272.

CHRISTELOVA, P., VALARIK, M., HRIBOVA, E., DE LANGHE, E. & DOLEZEL, J. 2011. A multi gene sequence-based phylogeny of the Musaceae (banana) family. *BMC Evolutionary Biology*, 11:103.

CIZKOVA, J., HRIBOVA, E., HUMPLIKOVA, L., CHRISTELOVA, P., SUCHANKOVA, P. & DOLEZEL, J. 2013. Molecular Analysis and Genomic Organization of Major DNA Satellites in Banana (*Musa* spp.). *PLoS ONE*, 8(1): e54808. doi:10.1371/journal.pone.0054808.

CRESTE, S., TULMANN, NETO A., DE OLIVEIRA, S.S. & FIGUEIRA, A. 2003. Genetic characterization of banana cultivars (*Musa* spp.) from Brazil using microsatellite markers. *Euphytica* 132: 259-258.

CRESTE, S., TULMANN, NETO A., VENCOVSKY, R., DE OLIVEIRA, S.S. & FIGUEIRA, A. 2004. Genetic diversity of *Musa* diploid and triploid accessions from the Brazilian banana breeding program estimated by microsatellite markers. *Genetic Resources and Crop Evolution*, 51: 723-733.

CROUCH, H.K., CROUCH, J.H., JARRET, R.L., CREGAN, P.B. & ORTIZ, R. 1998a. Segregation at microsatellite loci in haploid and diploid gametes of *Musa*. *Crop Science*, 38: 211-217.

CROUCH, J.H., VUYLSTEKE, D. & 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: 11-22.

CROUCH, J.H. & ORTIZ, R. 2004. Applied genomics in the improvement of crops grown in Africa. *African Journal of Biotechnology*, 3: 489-496.

DE JESUS, N.O., DE OLIVEIRA E SILVA, S., AMORIM, E.P., FERREIRA, C.F., DE CAMPOS, J.M.S., DE GASPARI SILVA, G. & FIGUEIRA, A. 2013. Genetic diversity and population structure of *Musa* accessions in *ex situ* conservation. *BMC Plant Biology*, 13: 41.

DE LANGHE, E. 1990. "Is plantain the oldest fruit crop in the world?" The King Baudouin Award to IITA, Proc. Celebration, Leuven, 6th April 44-47.

DE LANGHE, E., HRIBOVA, E., CARPENTIER, S., DOLEZEL, J. & SWENNEN, R. 2010. Did backcrossing contribute to the origin of hybrid edible bananas? *Annals of Botany*, 106: 849-857.

DERMISOY, L., DEMIR, T., DEMIRSOY, H., KACAR, Y.A. & OKUMUS, A. 2008. Identification of some sweet cherry cultivars grown in Amasya by RAPD markers. *Acta* horticulturae, 795: 147-152.

D'HONT, A., PAGET GOY, A., JENNY, C., NOYER, J. L., BAURENS, F.C., LAGODA, P. & CARREEL, F. 1999. "Investigation of the complex genome structure of cultivated banana (*Musa* spp.) by flow cytometry, genomic DNA in situ hybridization and repeated sequence analysis." International Symposium on the Molecular and Cellular Biology of Banana, 1999-03-22/1999-03-25. Ithaca, New York.

D'HONT, A., PAGET-GOY, A., ESCOUTE, J. & CARREEL, F. 2000. The interspecific genome structure of cultivated bananas, *Musa* spp. revealed by genomic DNA *in situ* hybridization. *Theoretical and Applied Genetics*, 100: 177-18.

D'HONT, A., DENOEUD, F., AURY, J.M., BAURENS, F.C., CARREEL, F., GARSMEUR, O., NOEL, B., BOCS, S., DROC, G. & ROUARD, M. 2012. The banana (*Musa acuminata*) genome and the evolution of monocotyledonous plants. *Nature*, 488: 213–217.

DUBOIS, T. & COYNE, L. 2011. *Integrated pest management of banana*. *In*: M. Pillay and A. Tenkouano (eds). Banana breeding: progress and challenges. CRC Press, Boca Raton FL, USA. pp. 121-144.

ECKSTEIN, K., ROBINSON, F.C. & DAVIE, S.F. 1995. Physiological responses of banana (*Musa* AAA; Cavendish sub group) in the subtropics.III. Gas exchange, growth analysis and source-sink interaction over a complete crop cycle. *Journal of Horticultural Science*, 70: 169-180.

ELLSWORTH, D.L., RITTENHOUSE, K.D. & HONEYCUTT, R.L. 1993. Artifactual variation in random amplified polymorphic DNA banding patterns. *BioTechniques*, 14: 214-217.

ERCISLI, S., GADZE, J., AGAR, G., YILDIRIM, N. & HIZARCI, Y. 2009. Genetic relationships among wild pomegranate (*Punica granatum*) genotypes from Coruh Valley in Turkey. *Genetics and Molecular Research*, 10(1): 459-464.

ERLICH, H.A. 1989. *PCR Technology*: Principles and Applications for DNA Amplification. Macmillan Publishers. University of Michigan. New York.

ESPINO, R.R.C. & PIMENTEL, R.B. 1990. *Electrophoretic analysis of selected isozymes in* BB-*cultivars of Philippine bananas*. *In:* Jarret, R.L. Ed. Proc. Identification of Genetic Diversity in the Genus *Musa*. INIBAP, Parc Scientifique Agropolis, Montpellier, France, pp. 36-40.

FANG, S., EU, T. & CHUNG, M. 2011. Isolation and characterization of genome-specific markers in Oryza species with the BB genome. *Plant science* 181 : 300-308.

FAURE, S., NOYER, J.L., HORRY, J.P., BAKRY, F., LANAUD, C. & DE LEON, D.G. 1993. A molecular marker-based linkage map of diploid bananas (*Musa acuminata*). *Theoretical and Applied Genetics*, 87: 517-526.

GE, X.J., LIU, M.H., WANG, W.K., SCHAAL, B.A. & CHIANG, T.Y. 2005. Population structure of wild bananas, *Musa balbisiana*, in China determined by SSR fingerprinting and cpDNA PCR-RFLP. *Molecular Ecology*, 14: 933-944.

GRAPIN, A., NOYER, J.L., CARREEL, F., DAMBLER, D., BAURENS, F.C., LANAAUD, C. & LAGODA, P.J.L. 1998. Diploid *Musa acuminata* genetic diversity assayed with sequence tagged microsatellite sites. *Electrophoresis*, 19: 1374-1380.

HASNAOUI, N., MARSA, M., GHAFFARIB, S., TRIFIC, M., MELGAREJOD, P. & HERNANDEZD, F. 2010. Seed and juice characterization of pomegranate fruits grown in Tunisia: Comparison between sour and sweet cultivars revealed interesting properties for prospective industrial applications. *Industrial Crops and Products*, 33: 374-381.

HESLOP-HARRISON, J.S. & SCHWARZACHER, T. 2007. Domestication, Genomics and the future for banana. *Annals of Botany,* 100 (5): 1073-1084.

HORESJI, T., BOX, J.M. & STAUB, J.E. 1999. Efficiency of Randomly Amplified Polymorphic DNA to Sequence Characterized Amplified Region Marker Conversion and their Comparative Polymerase Chain Reaction Sensitivity in Cucumber. *Journal of the American Society for Horticultural Science* 124(2): 128-135.

HORRY, J.P., ORTIZ, R., ARNAUD, E., CROUCH. J.H., FERRIS, R.S.B., JONES, D.R., MATEO, N., PICQ, C. & VUYLSTEKE, D. 1997. *Banana and Plantain*, pp 67-81. *In*: D. Fucillo, D., Sears, P.L. & Stapleton P. (Eds), Biodiversity in trust: Conservation and use of plant genetic resources in CGIAR centres, Cambridge University Press, Cambridge, UK.

HORRY, J.P. & JAY, M. 1988a. Distribution of anthocyanins in wild and cultivated banana varieties. *Phytochemistry*, 27: 2667-2672.

HORRY, J.P. & JAY, M. 1988b. *An evolutionary background of bananas as deduced from flavonoids diversification*. *In*: Jarret, R.L. (Eds.). Identification of Genetic Diversity in the genus *Musa*, INIBAP. Montpellier, France, p. 158-165.

HOWELL, E.C., NEWBURY, H.J., SWENNEN, R.L., WHITHERS, L.A. & FORD-LLOYD, B.V. 1994. The use of RAPD markers for identifying and classifying *Musa* germplasm. *Genome*, 37: 328-332.

JARRET, R.L., GAWEL, N., WHITTEMORE, A. & SHARROCK, S. 1993. RFLP-based phylogeny of *Musa* species in Papua New Guinea. *Theoretical and Applied Genetics*, 84: 579-584.

JARRET, R.L. & LITZ, R.E. 1986. Enzyme polymorphism in *Musa acuminata* Colla. *Journal of Heredity*, 77: 183-188.

JAVANSHAH, A., TAJABADIPOUR, A. & MIRZAEI, S. 2007. Identification of a new phenotype (Siah Barg) of pistachio (*Pistacia vera* L.) with shiny-blackish green leaves using RAPD assay. *International Journal of Agriculture and Biology*, 9: 307- 310.

JERIDI, M., BAKRY, F., ESCOUTE, J., FONDI, E., CARREEL, F., FERCHICHI, A., D'HONT, A & RODIER-GOUD, M. 2011. Homoeologous chromosome pairing between the A and B genomes of *Musa* spp. revealed by genomic in situ hybridization. *Annals of Botany*, 108: 975-981.

JERIDI, M., PERRIER, X., RODIER-GOUD, M., FERCHICHI, A., D'HONT, A., BAKRY, F. 2012. Cytogenetic evidence of mixed disomic and polysomic inheritance in an allotetraploid (AABB) *Musa* genotype. *Annals of Botany*, 110(8): 1593-1606.

JONES, D.R. 2000. *History of Banana breeding*. *In*: Jones D.R. (Eds). Diseases of Banana, abaca and enset. CAB International, Wallington, UK.

KAEMMER, D., FISCHER, D., JARRET, R.L., BAURENS, F.C., GRAPIN, A., DAMBIER, D., NOYER, J.L., LANAUD, C., KAHL, G. & LAGODA, P.J.L. 1997. Molecular breeding in the genus *Musa*: a strong case for STMS marker technology. *Euphytica*, 96: 49-63.

KAHANGI, E.M. 2002. *RAPD profiling of some banana* (*Musa* spp.) *varieties selected by small scale farmers in Kennya. In*: Proceedings of the second Horticultural seminar on Sustainable

Horticultural Production in the Tropics. *In*: J.M. Kenya Wesonga, T. Losenge, C. K. Ndungu, K. Ngamau, J. B. M. Njoroge, F. K. Ombwara & S. G. Agong, (Eds.) Jomo Kenyatta University of Agriculture and Technology (JKUAT) Juja, Kenya.

KAMATE, K., BROWN, S., DURAND, P., BUREAU, J., NAY, D.D. & TRINH, T.H. 2001. Nuclear DNA content and base composition in 28 taxa of *Musa*. *Genome*, 44: 622-627.

KHAYAT, E. 2004. *Discovery of the functional genes in the Musa genome*. *In:* Jain M.S & Swennen R. (Eds). Banana improvement: Cellular, Molecular Biology, and Induced mutations. Science Publishing, Plymouth, UK.

LEBOT, V., ARADHYA, K.M., MANSHARDT, R. & MEILLEUR, B. 1993. Genetic relationships among cultivated bananas and plantains from Asia and the Pacific. *Euphytica*, 67: 163-175.

LEBOT, V., MANSHARDT, R. & MEILLEUR, B. 1994. Genetic diversity in Eastern Polynesian cultivated bananas. *Pacific Science*, 48: 16-31.

LESCOT, T. 2000. Importance of plantain and cooking bananas in Africa. Outlets for subtropical zones. *InfoMusa*, 9: 25-28.

LINHART, Y.B. 2000. *Variation in woody plants: molecular markers, evolutionary processes and conservation biology*. *In*: Jain S. M. & Minocha S. C. (Eds). Molecular biology of woody plants. Forestry Sciences, Volume 64. Kluwer Academic Publishers, The Netherlands. pp. 341-374.

LOH, J.P., KIEW, R., SET, O., GAN, L.H. & GAN, Y.Y. 2000. Amplified Fragment Length Polymorphism (AFLP) fingerprinting of 16 banana cultivars (*Musa* spp). *Molecular Phylogenetics and Evolution*, 17: 360-366.

LOPEZ-BUESA, O. 1994. Randomly amplified polymorphic DNA markers as tags for the major virus resistance genes in cucumber. MSc thesis. University of Wisconsin–Madison.

MA, X.F. & GUSTAFSON, J.P. 2008. Allopolyploidization-accommodated genomic sequence changes in triticale. *Annals of Botany*, 101: 825-832.

MILLER, R.N.G., BERTIOLI, D.J., BAURENS, F.C., QUIRINO, B.F., CIAMPI, A.Y., SANTOS, C.M.R., MARTINS, N.F. & SOUZA, Jr., G.J. 2009. Undestanding plant responses to biotic stress: ongoing research in *Musa*. *Acta Horticulturae*, 828: 255-272.

MURALIDHARAN, K. & WAKELAND, E.K. 1993. Concentration of primer and template qualitatively affects products in RAPD-PCR. *BioTechniques*, 14: 362-364.

NAIR, A.S., TEO, C.H., SCHWARZACHER, T. & HESLOP-HARISSON, J.S. 2005. Genome classification of banana cultivars from South India using IRAP markers. Euphytica, 114: 285-290.

NASUTION, R.E. 1991. A taxonomic study of the species *Musa acuminata* Colla with its intraspecific taxa in Indonesia. *Memoirs of Tokyo University of Agriculture*, 23: 1-122.

NEALE, D.B. 1998. *Molecular genetic approaches to measuring and conserving adaptive genetic diversity*. *In*: The proceedings of international symposium on *In Situ* Conservation of Plant Genetic Diversity, pp. 385-390. November 4-8, 1996, Antalya, Turkey, CRIFC, Turkey.

NING, S., XU, L., LU, Y., HUANG, B. & GE, X. 2007. Genome composition and genetic diversity of *Musa* germplasm from China revealed by PCR-RFLP and SSR markers. *Scientia Horticulturae*, 114: 281-28.

NWAKANMA, D.C., PILLAY, M., OKOLI, B.E. & TENKOUANO, A. 2003a. PCR-RFLP of the ribosomal DNA internal transcribed spacers (ITS) provides markers for the A and B genomes in *Musa* L. *Theoretical and Applied Genetics*, 108: 154-159.

NWAKANMA, D.C., PILLAY, M., OKOLI, B.E. & TENKOUANO, A. 2003b. Sectional relationships in the genus *Musa* L. inferred from the PCR-RFLP of organelle DNA sequences. *Theoretical and Applied Genetics*, 107: 850-856.

NWAUZOMA, A., AKAGBUO, B., UMA, S., SARASWATHI, M.S. & MUSTAFFA, M. 2011. Developing markers for Sigatoka leaf spot disease (*Mycosphaerella musicolla* Leach) resistance in banana (*Musa* spp.). *African Journal of Biotechnology*, 10(33): 6213-6219.

ONGUSO, J.M., KAHANGI, E.M., NDIRITU, D.W. & MIZUTANI, F. 2004. Genetic characterization of cultivated bananas and plantains in Kenya by RAPD markers. *Scientia* Horticulturae, 99: 9-20.

ORTIZ, R. & VUYLSTEKE, D. 1994. Inheritance of black sigatoka disease resistance in plantain-banana (*Musa* spp) hybrids. *Theoretical and Applied Genetics*, 89: 146-152.

ORTIZ, R. & VUYLSTEKE, D. 1996. Recent advances in *Musa* genetics, breeding and biotechnology. *Plant Breeding*, 66: 1355-1363.

ORTIZ, R., MADSEN, S. & VUYLSTEKE D. 1998. Classification of African plantain landraces and banana cultivars using a phenotypic distance index of quantitative descriptors. *Theoretical and Applied Genetics*, 96: 904-911.

OSELEBE, H.O., TENKOUANO, A., PILLAY, M., OBI, I.U. & UGURU, M.I. 2006. Ploidy and genome segregation in *Musa* breeding populations assessed by flow cytometry and randomly amplified polymorphic markers. *Journal of Horticultural Sciences*, 131: 780-786.

OSUJI, J.O., HARRISON, G., CROUCH, J.H. & HESLOP-HARRISON, J.S. 1997. Identification of the genomic constitution of *Musa* L. genotypes (bananas, plantains and hybrids) using molecular cytogenetics. *Annals of Botany*, 80: 787-793.

PARAN, L. & MICHELMORE, R.W. 1993. Development of reliable PCR-based marker linked to downy mildew resistance gene in lettuce. *Theoretical and Applied Genetics*, 85: 985-993.

PEARCE, F. 2003. Going bananas. *New Science*, 177: 26-29.

PERRIER, X. & TEZENUS DU MONTCEL, H. 1990. *MUSAID: A computerised determination system*. *In*: R. L Jarret (Eds). Identification of genetic diversity in the genus *Musa*: Proceedings of an international workshop held at Los Banos, Philippines, 5-10 Sept. 1988. International Board of Plant Genetic Resources Publishing, Rome. pp. 76-91.

PILLAY, M., NWAKANMA, D.C. & TENKOUANO, A. 2000. ldentification of RAPD markers linked to A and B genome sequences in *Musa*. *Genome*, 43: 763-767.

PILLAY, M., OGUNDIWIN, E., NWAKANMA, D.C., UDE, G. & TENKOUANO, A. 2001. Analysis of genetic diversity and relationships in East African banana germplasm. *Theoretical and Applied Genetics*, 102: 965–970.

PILLAY, M., TENKOUANO, A. & HARTMAN, J. 2002. "*Bananas and Plantains: Future challenges in Musa breeding*". *In*: Kang, M.S. (Eds.). Crop Improvement, Challenges in the Twenty- First Century. Food Products Press, New York, USA, pp 223–252.

PILLAY, M., TENKOUANO, A., UDE, G. & ORTIZ, R. 2004. *Molecular characterization of genomes in Musa*. *In*: Jain, M.S., Swennen R. (Eds). Banana improvement: cellular, molecular biology, and induced mutations. Science Publishers, Inc. Plymouth, UK.

PILLAY, M., OGUNDIWIN, E., TENKOUANO, A. & 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. & TRIPATHI, L. 2006. *Banana: An overview of breeding and genomics research in Musa*. *In*: Kole, C. (Eds.). Genome mapping and molecular breeding in plants. Vol 4 Fruits and Nuts Springer-Verlag, Heidelberg, Germany. pp. 281-303.

PILLAY, M. & TRIPATHI, L. 2007. *Banana breeding*. *In*: Kang, M.S. & Priyadarshan, P.M. (Eds). Breeding major food staples. Blackwell science, Boston, USA. pp. 393-428.

PILLAY, M., TENKOUANO, A. 2011. *Genomes, cytogenetics and flow cytometry of Musa*. *In*: Pillay, M. & Tenkouano, A. (Eds.). Banana Breeding. Progress and Challenges. CRC Press, Boca, Raton, FL, USA, pp53-70.

PILLAY, M., TENKOUANO, A., UDE, G. & ORTIZ, R. 2011. *Molecular breeding of other vegetatively propagated crops: Lessons for banana. In*: Pillay, M. & Tenkouano, A. (Eds.). Banana breeding. Progress and challenges. CRC Press, Boca Raton FL, USA. pp. 321-350.

POLLARD, T.D. & EARNSHAW, W.C. 2002. *Cell Biology*. Illustrated by Graham T. Johnson. Elsevier Science Publishers, USA.

PURSEGLOVE, J.W. 1988. *Tropical Crops. Monocotyledons*. Longman Publishers, Essex, London, pp. 607.

PURSEGLOVE, J.W. 1972. *Tropical crops – Monocotyledons*. Longman. Publishers, Essex, London. pp. 343-384.

RACHARAK, P. & EIADTHONG, W. 2007. Genetic relationship among subspecies of *Musa acuminata* Colla and A-genome consisting edible cultivated bananas assayed with ISSR markers. *Songklanakarin Journal of Science and Technology*, 29: 1479-1489.

RAFALSKI, J.A. & TINGEY, S.V. 1993. Genetic diagnostics in plant breeding: RAPDs, microsatellites and machines. *Trends in Genetics*, 9: 275-280.

ROBINSON, J. C. 1996. *Bananas and plantains*. CAB international, Wallingford, UK.

ROSSEL, G. 1998. Taxonomic-linguistic study of plantain in Africa, PhD Thesis, Agricultural University, Wageningen, The Netherlands.

ROUX, N., BAURENS, F.C., DOLEZEL, J., HRIBOVA, E., HESLOP-HARRISON, J.S., TOWN, C., SASAKI, T., MATSUMOTO, T., AERT, R., REMY, S., SOUZA, M. & LAGODA, P. 2008. *Genomics of banana and plantain (Musa spp.)*: *Major staple crops in the tropics. In*: Moore, P. & Ming, R. (Eds.). Genomics of Tropical Crop Plants. Plant Genetics and Genomics: Crops and Models Vol 1. Springer, New York. pp. 83-111.

ROZEN, S. & SKALETSKY, H.J. 2000. *Primer3 on the WWW for general users and for biologist programmers*. *In*: Krawetz, S. & Misener, S. (Eds.). Bioinformatics Methods and Protocols: Methods in Molecular Biology. Humana Press, Totowa, NJ, pp. 365-386.

RUGIENIUS, R., SIKSNIANAS, T., STANYS, V., GELVONAUSKIENI, D. & BENDOKAS, V. 2006. Use of RAPD and SCAR markers for identification of Strawberry genotypes carrying red stele (*Phytophtora fragariae*) resistance gene Rpf1. *Agronomy research*, 4: 335-339.

SAGI, L., REMY, S. & SWENNEN, R. 2007. Transgenic and (trans) genomic research in banana (*Musa* spp.). *The African Crop Science*, 8: 592-595.

SARASWATHI, M.S., UMA, S., PRASANYA SELVAM, K., RAMARAJ, S., DURAI, D. & MUSTAFFA, M.M. 2009a. Robustness of IRAP and RAPD marker systems in studying the intra-group diversity of *Musa* Cavendish (AAA) clones. Paper presented at the ISHS/Promusa Banana symposium, September 14-18, 2009. Phoenix city, Guangzhou, China.

SARASWATHI, M.S., UMA, S., VADIVEL, E., DURAI, P., SIVA, S.A., RAJAGOPAL, G. & SATHIAMOORTHY, S. 2009b. Diversity analysis in Indian cooking bananas (ABB) through morpho-taxonomic and molecular characterisation. Paper presented at the ISHS/Promusa Banana symposium, September 14-18, 2009. Phoenix City, Guangzhou, China.

SCHULMAN, A.H., FLAYELL, A.J. & ELLIS, T.H.N. 2004. The application of LTR retrotransposons as molecular markers in plants. *Methods in Molecular Biology*, 260: 145-173.

SEMAGN, K., BJORNSTAD, A. & NDJIONDJOP, M.N. 2006. Progress and prospects of marker assisted backcrossing as a tool in crop breeding programs. *African Journal of Biotechnology*, 5(25): 2588-2603.

SHARROCK, S. 1989. "*Collecting Musa in Papua New Guinea*". *In*: Jarret, R. (Eds.). Identification of Genetic Diversity in the Genus *Musa*. INIBAP, Los Banos, Philippines.

SHEPHERD, K. & FERREIRA, F.R. 1984. The PNG Biological Foundation Banana Collection at Laloki, Port Moresby, Papua New Guinea, South East Asia. *Newsletter* 8: 28-34.

SHEPHERD, K. 1999. *Cytogenetics of the genus Musa*. INIBAP, Montpellier, France. 154 pp.

SILAYOI, B. & CHOMCHALOW, N. 1987. Cytotaxonomic and morphological studies of Thai banana cultivars, *ACIAR Proceedings* 21: 157-160.

SIMMONDS, N.W. & SHEPERD, K. 1955. Taxonomy and origins of cultivated bananas, *The Botanical Journal of the Linnean Society*, *London Botanical*, 55: 302-312.

SIMMONDS, N.W. 1962. *The Evolution of bananas.* Tropical Agriculture Series. Longman, London.

SIMMONDS, N.W. 1966. *Bananas*. 2nd ed. Tropical Agriculture. Series. Longmans, London.

SIMMONDS, N.W. 1976. *Evolution of crop plants*: Scottish breeding station, Pentlandfield, Roslin Midlothian, Scotland. Longman, UK.

SIMMONDS, N.W. 1987. *Classification and breeding of bananas*. *In*: Persley, G. & De Langhe, E. (Eds.). Banana and plantain breeding strategies. Proceedings of the international workshop held at Cairns, Australia, 13-17th October 1986. Proceedings No. 21. ACIAR, Canberra, Australia, pp. 69-73.

SIMMONDS, N.W. & WEATHERUP, S.T.C. 1990. Numerical taxonomy of the wild bananas (*Musa*). *New Phytologist*, 115: 567-571.

SINGH, H.P. & UMA, S. 1996. Banana Cultivation in India. National Research Centre for Banana, Tirichurapali, India.

SOTTO, R.C. & RABARA, R.C. 2000. Morphological diversity of *Musa balbisiana* Colla in the Phillipines. *InfoMusa*, 9: 28-30.

STAUB, J.E. & SERQUEN, F.C. 1996. "Genetic Markers, Map Construction, and Their Application in Plant Breeding". *Horticultural Science*, 31(5): 729-740.

STOVER, R.H. & SIMMONDS, N.W. 1987. *Bananas*. 3rd Ed. Longman Scientific & Technical, Essex, England.

SWENNEN, R. 1990. *Limitations of morphotaxonomy: names and synonyms of plantain in Africa and elsewhere*. *In*: Jarret R.L. (Ed.) Identification of genetic diversity in genus *Musa*, Proc. International Workshop, Los Banos, Phillipines, 5-10th September 1988. INIBAP, Montpellier, France, pp. 172-210.

SWENNEN, R. & VUYLSTEKE, D. 1991. *Bananas in Africa: diversity, uses and prospects for improvement*. *In*: Ng, N.Q., Perrino, P., Attere, F. & Zedan, H. (Eds.). Crop Genetic Resources of Africa, Vol. 2, Proc. Intl. Conf., Ibadan, Nigeria, 17-20th October 1988. The Trinty Press, UK, pp. 151-159.

SWENNEN, R. & ORTIZ, R. 1997. Morphology and growth of plantain and banana. IITA research guide No.66. Training program International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

TENKOUANO, A., CROUCH, J.H., CROUCH, H.K., VUYLSTEKE, D. & 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 and Applied Genetics*, 98: 62-68.

TEO, C.H., TAN, S.H., OTHMAN, Y.R., SCHWARZACHER, T. 2002. The cloning of Ty1-copia-like retrotransposons from 10 varieties of banana (*Musa* spp.). Journal of Biochemistry, Molecular Biology, and Biophysics: JBMBB: *The Official Journal of the Federation of Asian and Oceanian Biochemists and Molecular Biologists (FAOBMB),* 6(3): 193-201.

TEO, C.H., TAN, S.H., HO, C.H., FARIDAH, Q.Z., OTHMAN, Y.R., HESLOP-HARRISON, J.S., KALENDAR, R. & SCHULMAN, A.H. 2005. Genome constitution and classification using retrotransposon based markers in the orphan crop banana. *The Journal of Plant Biology*, 48: 96-105.

TEZENAS DU MONTCEL, H. 1989. "*M. acuminata subspecies banksii*", Identification of genetic diversity in the genus *Musa* (JARRET, R., Ed.), INIBAP, Los Banos, Philippines.

THORNMANN, C.E. & OSBORN, T.C. 1992. *Use of RAPD and RFLP markers for germplasm evaluation*. Applications of RAPD technology to Plant Breeding: Proceedings of the joint Plant Breeding Symposia series, Minneapolis, Minn., *In*: Hoisington, *D*. & McNab, A. (Eds.). Crop science society of America. American society of Horticultural science. American Genetic Society, Minneapolis, Minn, pp. 9-11.

UDE, G., PILLAY, M., NWAKANMA, D. & TENKOUANO, A. 2002a. Analysis of genetic diversity and sectional relationships in *Musa* using AFLP markers. *Theoretical and Applied Genetics*, 104: 1239-1245.

UDE, G., PILLAY, M., NWAKANMA, D. & TENKOUANO, A. 2002b. Genetic diversity in *Musa acuminata* Colla and *M. balbisiana* Colla and some of their natural hybrids using AFLP markers. *Theoretical and Applied Genetics*, 104 (8): 1246-1252.

UDE, G., PILLAY, M., OGUNDWIN, E. & TENKOUANO, A. 2003. Genetic diversity in an African plantain core collection using AFLP and RAPD markers. *Theoretical and Applied Genetics*, 107: 248-255.

UMA, S., SUDIIA, S., SARASWATHI, M.S., MANICKAVASAGAM, M., SELVARAJAN, R., DURAI, P., SATHIAMOORTHY, S. & SIVA, S.A. 2004. Analysis of genetic diversity and phylogenetic relationships among indigenous and exotic Silk (AAB) group of bananas using RAPD markers. *The Journal of Horticultural Science and Biotechnology*, 79: 523-527.

VALMAYOR, R.V., RIVERA, F.N. & LOMULJO, F.M. 1981. Philippine banana cultivar names and synonyms, IPB bulletin No. 3, National plant genetic resources laboratory, Institute of plant breeding, University of Philippines, Los Banos.

VALMAYOR, R.V. 2000. Cooking bananas. Classification, production and utilization in South East Asia. *InfoMusa*, 9: 28-30.

VANHOVE, A., GARCIA, S., SWENNEN, R., PANIS, B. & CARPENTIER, S.C. 2012. Understanding *Musa* drought stress physiology using an autotrophic growth system. *Communications in Agricultural and Applied Biological Sciences*, 77: 89-93.

VIDAL, A.M., GISBERT, C., TALON, M., PRIMO-MILLO, E., LOPEZ-DIAS, I. & GARCIA-MARTINEZ, J.L. 2001. The ectopic overexpression of a citrus gibberellin 20-oxidase enhances the non-13-hydroxylation pathway of gibberellin biosynthesis and induces extremely elongated phenotype in tobacco. *Plant Physiology*, 112: 251-260.

VILARINHOS, A., CARREEL, F., RODIER, M., HIPPOLYTE, I., BENABDELMOUNA, A., TRIAIRE, D., BAKRY, F., COURTOIS, B. & D´HONT, A. 2006. Characterisation of translocations in bananas by FISH of BAC clones anchored to a genetic map. Paper presented at the International Conference Plant and Animal Genome XIV, January 14-18, 2006, San Diego, California.

VUYLSTEKE, D., SWENNEN, R. & ORTIZ, R. 1993. Development and performance ofBlack Sigatoka-resistant tetraploid hybrids of plantain (*Musa* spp., AAB group). *Euphytica*, 65: 33-42.

VUYLSTEKE, D. 2000. Breeding bananas and plantains: from intractability to feasibility. *Acta Horticulturae Sinica*, 540: 149-156.

WANG, Z., ZHANG, Z., LI, H., GAO, X., DU, G. & TAN, C. 2007. Identification of strawberry cultivars by RAPD and SCAR markers. *Acta Horticulturae Sinica,* 34: 591-596.

WANG, W., HU, Y., SUN, D., STAEHELIN, C., XIN, D. & XIE, J. 2012. Identification and evaluation of two diagnostic markers linked to *Fusarium* wilt resistance (race 4) in banana (*Musa* spp.). *Molecular Biology Reports*, 39(1): 451-9.

WEEDEN, N.F., TIMMERMAN, M., HERMMAT, M., KNEEN, B.E. & LODHI, M.A. 1992. *Inheritance and reliability of RAPD markers*. *In*: Proc. Symp. Applications of RAPD technology to plant breeding. Minneapolis, Minn. pp. 12-17.

WILLIAMS, J.G.K., KUBELIK, A.R., LIVAK, K.J., RAFALSKI, J.A. & 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., LOH, J.P., GAN, L.H., SET, O., LEE, S.K., OHN, S., LUM, S. & GAN, Y.Y. 2001a. Sectional placement of three Bornean species of *Musa* (Musaceae) based on amplified fragment length polymorphism (AFLP). *Gardens' Bulletin Singapore,* 53: 237-241.

WONG, C., KIEW, R., LOH, J.P., GAN, L.H., SET, O., LEE, S.K., OHN, S., LUM, S. & GAN, Y.Y. 2001b. Genetic diversity of the wild banana *Musa acuminata* Colla in Malaysia as evidenced by AFLP. *Annals of Botany*, 88: 1017-1025.

WONG, C., KIEW, R., ARGENT, G., SET, O., LEE, S.K. & GAN, Y.Y. 2002. Assessment of the validity of the sections in *Musa* (Musaceae) using AFLP. *Annals of Botany*, 90: 231-238.

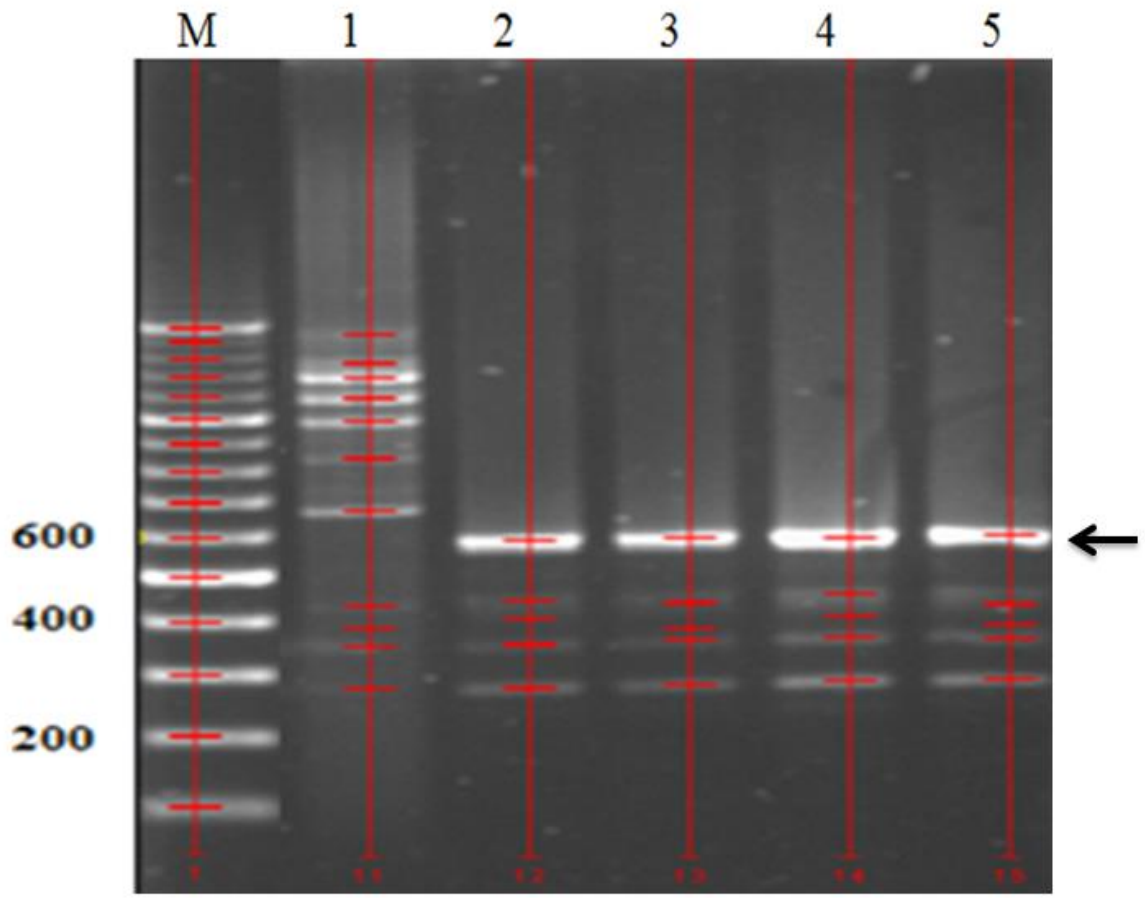
WONGNIAM, T., SOMANA, J., SWANGPOL, S., SEELANAN, T., CHAREONSAP, P., CHADCHAWAN, S. & JENJITTIKUL, T. 2010. Genetic diversity and species-specific PCR-based markers from AFLP analyses of Thai bananas. *Biochemical Systematics and Ecology* 38: 416-427.

www.ncbi.nih.gov/blast accessed on 24/07/13

www.simgene.com/Primer3 accessed on 26/08/13

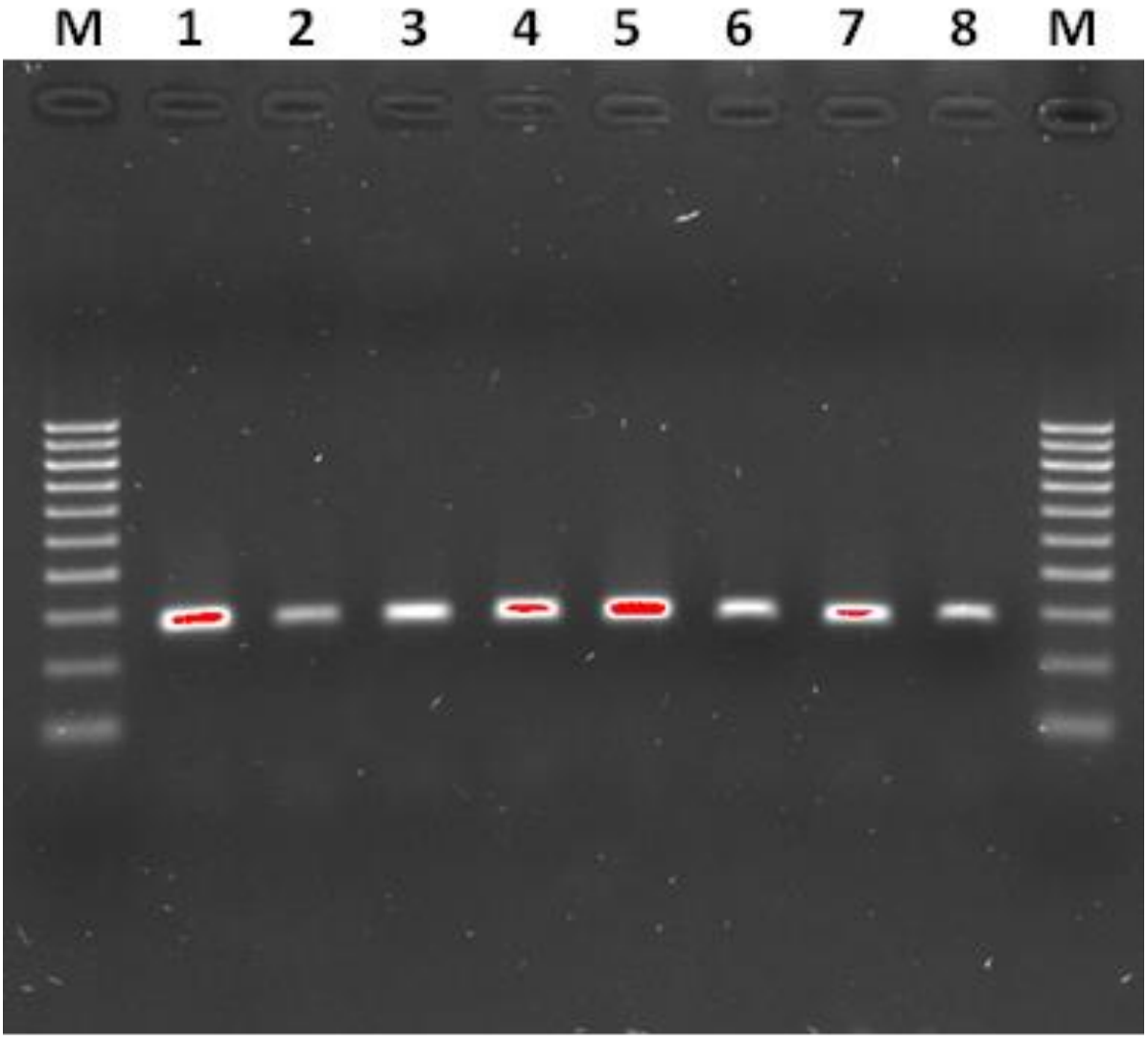
APPENDICES

Appendix 1



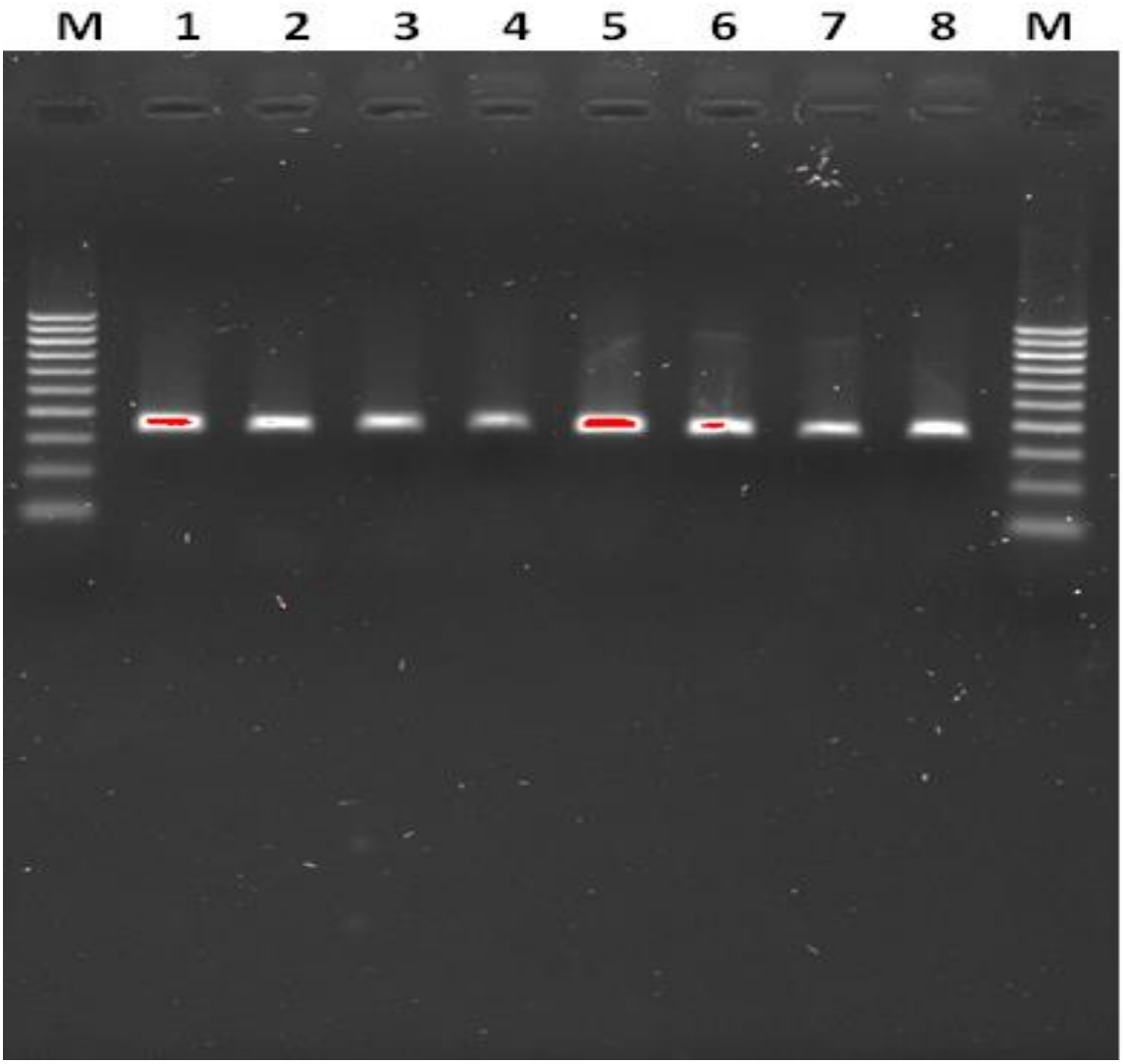
Automatic band detection to confirm the $OPA17_{600}$. Lane 1 represents *M. balbisiana*, lanes 2-5 represent *M. acuminata* and lane M is the 100 bp DNA ladder.

Appendix 2



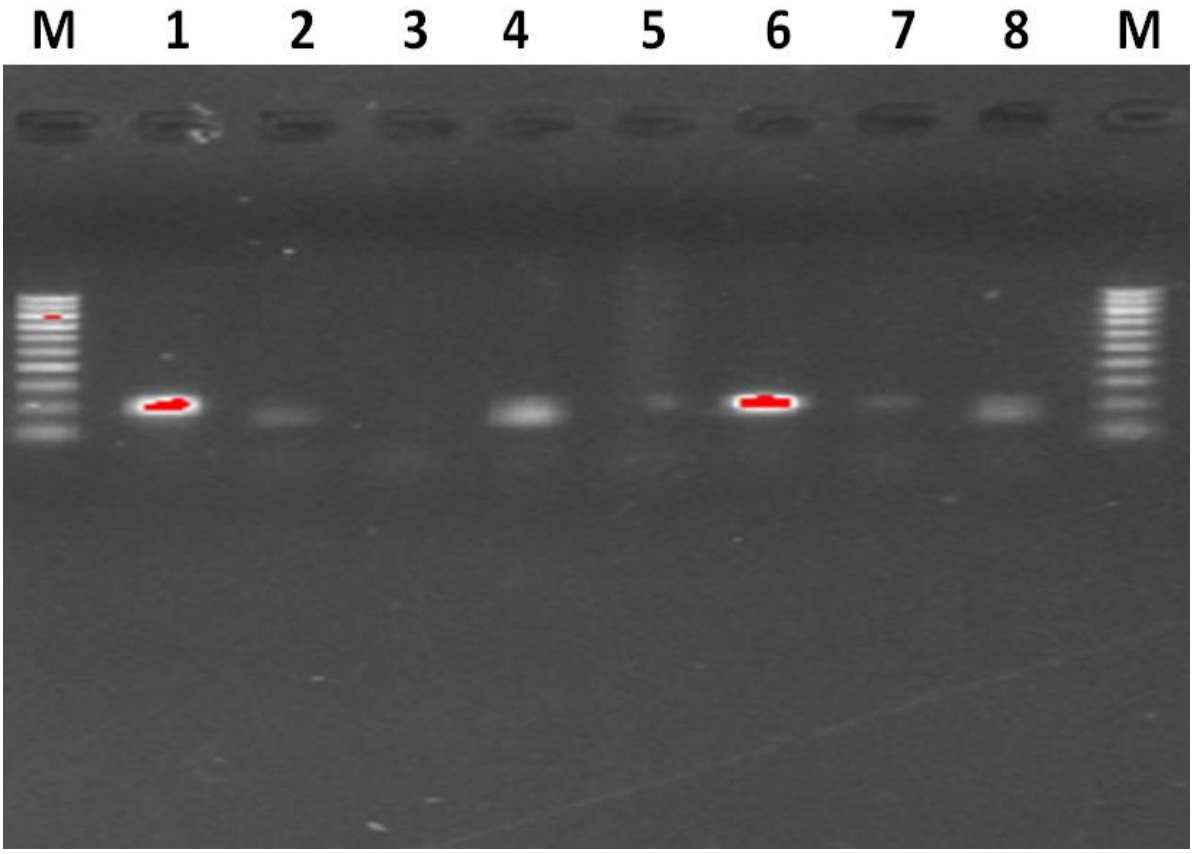
SCAR PCR profile results for SC1 SCAR primers. The lanes represent DNA from (1) *M. acuminata* 'Calcutta 4' (AA) (2) 'Gros Michel' (AAA) (3) 'Kelong Mekintu' (AAB) (4) 'Safet Velchi' (AB_{CV}) (5) *M. balbisiana* (10852) (BB) (6) *M. schizocarpa* (SS) (7) *M. textilis* (TT) (8) 'CRBP 39' (AAAB). Lane M represents 100 bp molecular marker.

Appendix 3



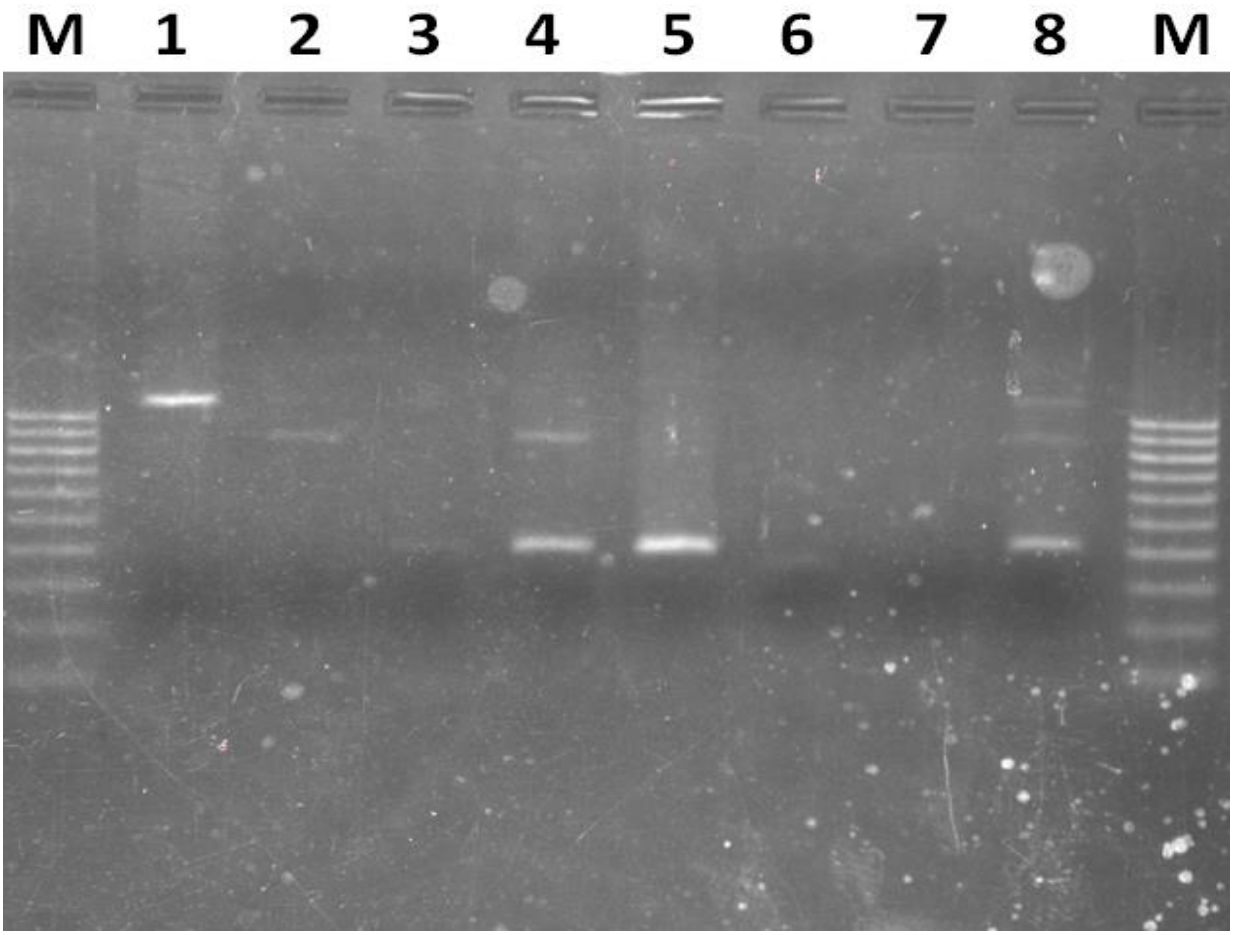
SCAR PCR profile results for SC2 SCAR primers. The lanes represent DNA from (1) *M. acuminata* 'Calcutta 4' (AA) (2) 'Gros Michel' (AAA) (3) 'Kelong Mekintu' (AAB) (4) 'Safet Velchi' (AB_{CV}) (5) *M. balbisiana* (10852) (BB) (6) *M. schizocarpa* (SS) (7) *M. textilis* (TT) (8) 'CRBP 39' (AAAB). Lane M represents 100 bp molecular marker.

Appendix 4



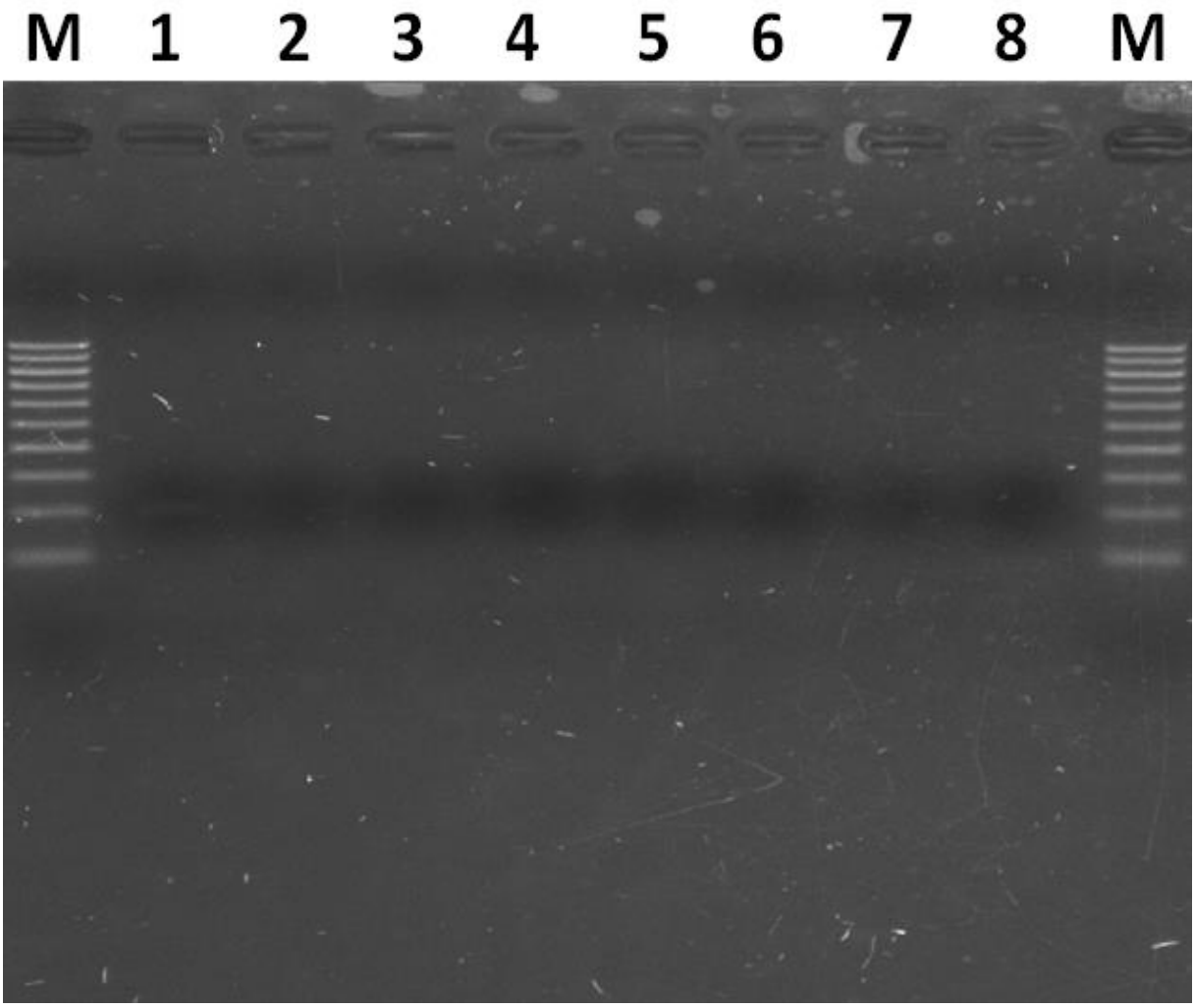
SCAR PCR profile results for SC4 SCAR primers. The lanes represent DNA from (1) *M. acuminata* 'Calcutta 4' (AA) (2) 'Gros Michel' (AAA) (3) 'Kelong Mekintu' (AAB) (4) 'Safet Velchi' (AB_{CV}) (5) *M. balbisiana* (10852) (BB) (6) *M. schizocarpa* (SS) (7) *M. textilis* (TT) (8) 'CRBP 39' (AAAB). Lane M represents 100 bp molecular marker.

Appendix 5



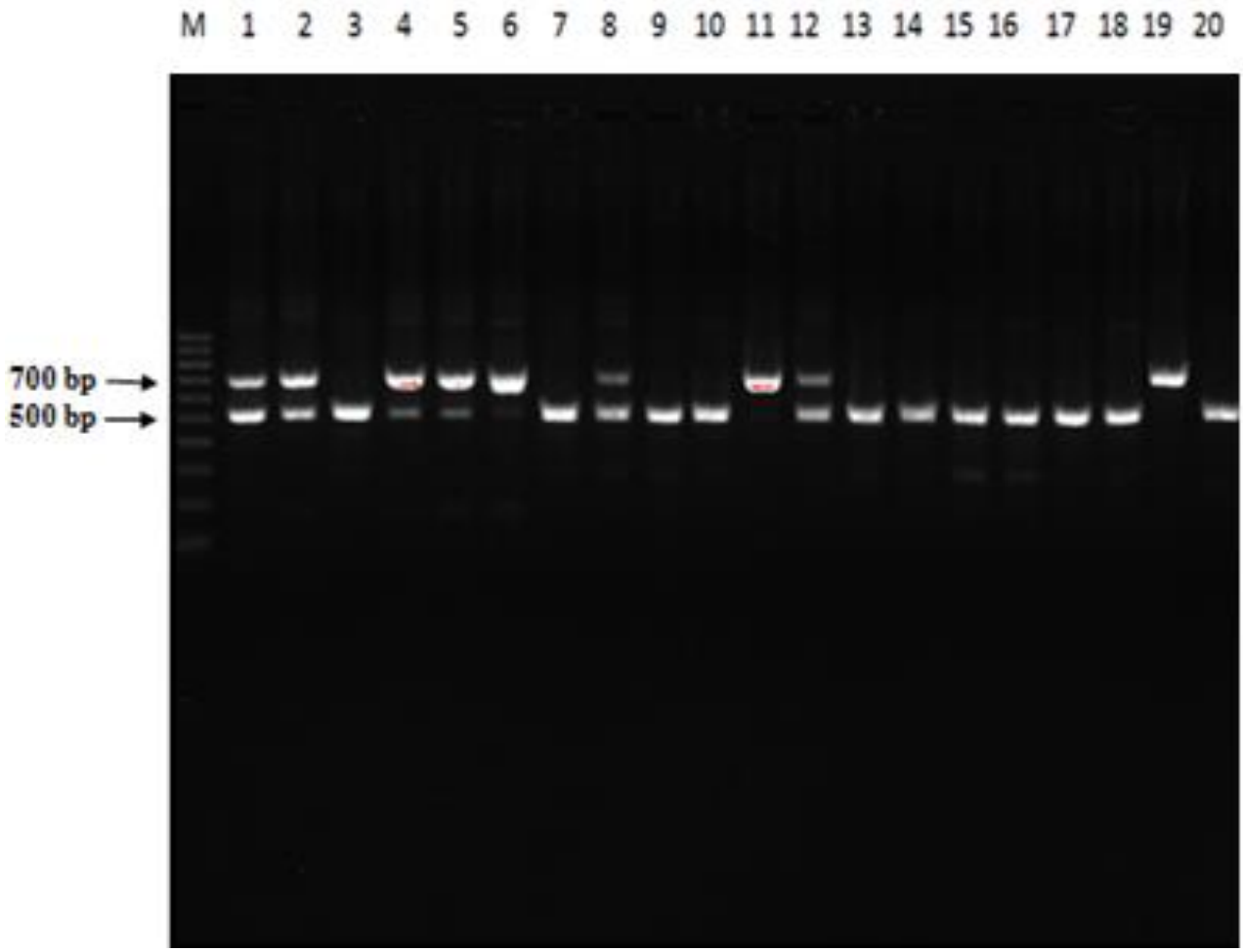
SCAR PCR profile results for SC5 SCAR primers. The lanes represent DNA from (1) *M. acuminata* 'Calcutta 4' (AA) (2) 'Gros Michel' (AAA) (3) 'Kelong Mekintu' (AAB) (4) 'Safet Velchi' (AB_{CV}) (5) *M. balbisiana* (10852) (BB) (6) *M. schizocarpa* (SS) (7) *M. textilis* (TT) (8) 'CRBP 39' (AAAB). Lane M represents 100 bp molecular marker.

Appendix 6



SCAR PCR profile results for SC6 SCAR primers. The lanes represent DNA from (1) *M. acuminata* 'Calcutta 4' (AA) (2) 'Gros Michel' (AAA) (3) 'Kelong Mekintu' (AAB) (4) 'Safet Velchi' (AB_{CV}) (5) *M. balbisiana* (10852) (BB) (6) *M. schizocarpa* (SS) (7) *M. textilis* (TT) (8) 'CRBP 39' (AAAB). Lane M represents 100 bp molecular marker.

Appendix 7



SCAR PCR patterns showing SCOPA17$_{600}$ in *Musa* landraces. The lanes represent DNA from (1) 'FHIA 25' (AAB). (2) 'Cachaco' (ABB) (3) 'Giant Cavendish' (AAA) (4) 'Cardaba' (ABB) (5) 'Cardaba' (ABB) (6) *M. balbisiana* 'Singapuri' (BB) (7) 'Valery' (AAA) (8) 'Safet Velchi' (AB_{CV}) (9) 'Ntanga 4' (AAB) (10) 'Tjau Lagada' (AA) (11) *M. balbisiana* 'Tani' (BB) (12) 'Kamaramasenge' (AB) (13) 'Lidi' (AA) (14) 'CRBP 39' (AAAB) (15) 'Plantain No. 2' (AAB) (16) 'Kelong Mekintu' (AAB) (17) 'Gros Michel' (AAA) (18) 'Khai Thong Ruang' (AAA) (19)'*M. balbisiana*(10852)' (BB) (20) *M. acuminata* 'Calcutta 4' (AA). Lane M represents 500 bp DNA marker.