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SCAR Marker for the A Genome of Bananas (*Musa* spp. L.) Supports Lack of Differentiation between the A and B Genomes

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Abstract

Bananas (*Musa* spp. L.) are grouped on the basis of their genomic origins in relation to *Musa acuminata* (A genome) and *M. balbisiana* (B genome). The two ancestral wild seeded diploid species evolved in vastly different geographical areas and contributed several agronomic traits towards the present genetic composition of cultivated bananas. Most cultivated bananas are triploid (AAA, AAB and ABB), some are diploid (AA, BB and AB) and a few are tetraploids (AAAA, AAAB, AABB and ABBB). Limitations on the correct identification of the A and B genomes in *Musa* have generated need for the development of new and more reliable techniques. Distinguishing the A and the B genome remains practically and theoretically important for banana breeders. The aim of the research was to develop a DNA based A genome specific marker for the identification of the A genome in bananas. A putative marker (600 bp) specific to the A genome was identified by Random Amplified Polymorphic DNA (RAPD) technique. A sequence characterised amplified region (SCAR) marker was developed from the RAPD amplicon. The SCAR primers annealed a 500 bp fragment specific to the A genome in a sample of 22 randomly selected homo- and heterogenomic A genome containing accessions representing different genome combinations. The 500 bp SCAR marker is useful for the identification of the A genome. However an additional 700 bp fragment annealed in all *M. balbisiana* genotypes and in five of the eight heterogenomic accessions, suggesting lack of differentiation between the A and B genome. This study has provided a 500 bp A genome SCAR marker and recent evidence that the A and B genomes of banana may not be as differentiated as previously considered.

Keywords: bananas, plantains, random amplified polymorphic DNA (RAPD), sequence amplified polymorphic (SCAR), A genome, genome differentiation

1. Introduction

Cultivated bananas (*Musa* spp.) are the fourth most important food crop in the world today after rice, wheat and maize (Pearce, 2003; Sagi, Remy, & Swennen, 2007). They are seedless parthenocarpic clones selected by early farmers in Southeast Asia and maintained by vegetative propagation (Pearce, 2003). Four genomes A, B, S and T are known to be present in cultivated bananas (Simmonds, 1962). While the S and T genomes occur in only a few of the cultivars (Carreel, 1995), the A and B genomes are predominant (Simmonds, 1955). Breeding programmes in *Musa* are more concerned with only the A and B genomes (Arumuganathan & Earle, 1991). The A and B genomes are known to originate from two ancestral wild seeded diploid species *Musa acuminata* (A genome) and *M. balbisiana* (B genome) (Cheesman, 1948; Simmonds, 1955). The two species evolved in vastly different geographical areas and contributed several agronomic traits towards the present genetic composition of the various cultivated bananas (Lebot, Manshardt, & Meilleur, 1994; Robinson, 1996; Amaud & Horry, 1997).

Cultivated bananas are grouped on the basis of their genomic origins in relation to the A and B genomes (Simmonds, 1966). A large number of genomic groups exist in banana. Most cultivars are triploid (AAA, AAB, and ABB), some are diploid (AA, BB, and AB) and a few are tetraploids (AAAA, AAAB, AABB and ABBB) (Shepherd, 1999; Creste, Tulmann, Vencovsky, De Oliveira, & Figueira, 2004; Pillay, Tenkouano, Ude, & Ortiz, 2004). Distinguishing the A and B genomes is of both practical and theoretical interest for *Musa* breeders (Miller et al., 2009; Pillay, Tenkouano, Ude, & Ortiz 2011). It provides an effective way to trace useful genes, gene sequences and alien chromatin from the wild relatives (Stover & Simmonds, 1987; 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* (Lebot et al., 1994; 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) (Miller et al., 2009). Furthermore, distinguishing the A and B genome would provide an objective way of banana classification in *Musa* (Pillay, Nwakanma, & Tenkouano, 2000). Classification would not rely on subjective scoring of morphological traits but on genome identification (Miller et al., 2009). 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, Garcia, Swennen, Panis, & Carpentier, 2012).

The applications of various types of molecular marker techniques in banana classification have been reported in *Musa* genomics. However, SCAR markers linked to the A genome in bananas and plantains have not been reported as yet. SCAR markers provide a rapid and a more accurate method to determine a plant's genomic status, especially in breeding programmes that involve interploidy crosses (Miller et al., 2009). The study aims to develop a SCAR marker linked to the identification of the A genome in bananas and plantains from an identified RAPD marker.

2. Method

2.1 RAPD Assay

The plants used in this study (listed in Table 1) were collected from the International Transit Centre, Laboratory of Tropical Crop Improvement, Leuven, Belgium. The plants were selected to represent a wide range of genomic groups that include landraces and synthetic hybrids of known genomic compositions. Genomic DNA was extracted from the samples according to the CTAB procedure (Crouch, Crouch, Jarret, Cregan, & Ortiz, 1998). 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. 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 × 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 ^{o}C followed by 35 cycles of 50 s at 94 ^{o}C, 50 s at 40 ^{o}C, and 1.5 min at 72 ^{o}C, with a final extension step of 7 min at 72 ^{o}C. 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.

2.2 Cloning and SCAR Primer Synthesis

DNA was extracted from agarose gels with the NucleoSpin® Extract II kit (Macherey-Nagel, Dueren, Germany). The pGEM-T vector system cloning kit (Promega, Wisconsin, USA) 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 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., Seattle, WA), a web based sequencing, tracking and retrieval software was used to make DNA sequence generation and data handling simpler, faster and more cost effective. The SCAR primers were designed using Primer3 (www.simgene.com/Primer3) and sent to Inqaba Biotechnologies (Pvt) Company for synthesis.

2.3 SCAR Assay

The synthesised SCAR primer pairs (Table 2) were tested on a sample of eight randomly selected homo- and heterogenomic accessions for screening purposes. Validation tests for the successful SCAR primer pair were conducted on the twenty-two banana accessions listed in Table 1. 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.

Table 1. List of landraces used in the study

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

3. Results

3.1 RAPD Assay Results

The RAPD primer OPA-17 produced a 600 bp fragment (arrow) only in *M. acuminata* (A genome) (lanes 1-6). The fragment was absent in *M. balbisiana* (B genome) (lane 7). The automatic band detection to using the Quantity One Version 4.6.9 Windows and Macintosh showed that the primer OPA-A17 produces a RAPD fragment ($OPA17_{600}$) specific to the A genome in *Musa*. The results were congruent with the previous investigations as noted in Pillay et al. (2000).

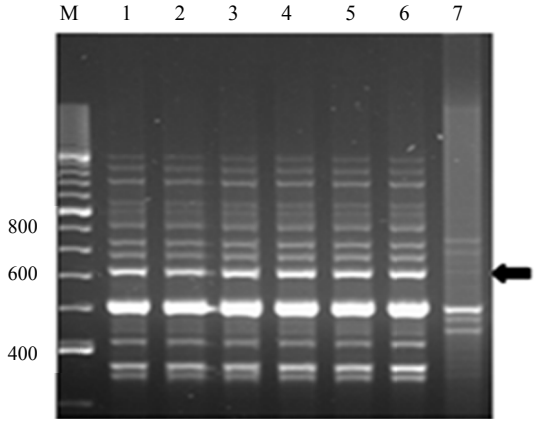

Figure 1. PCR amplification patterns showing the $OPA17_{600}$ (arrow) unique to *M. acuminata* (lanes 1-6) and absent in *M. balbisiana* (lane7). M is the 100 bp molecular marker

3.2 Cloning and Sequencing of RAPD Markers

The $OPA17_{600}$ RAPD fragment was cloned and sequenced (Figure 2). The highlighted region in bold indicates the sequence of the RAPD primer OPA17. The six SCAR primer pairs designed from the $OPA17_{600}$ fragment sequence are shown in Table 2. The SCAR primer pairs 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
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Figure 2. DNA sequence of the $OPA17_{600}$ fragment

3.3 Sequence Data Analysis and SCAR Primer Synthesis

The analysis of the RAPD fragment based on the BLAST sequence analysis tool (www.ncbi.nih.gov/blast) observed that the amplified band has a high homology with the DNA sequences of *M. acuminata* clones. The Primer3 design produced a number of potential primers from the 601 bp sequence. Of these, 6 primers (Table 2) were selected for further studies on the basis of the GC content, melting temperatures, 3' stability and likelihood of forming primer dimers.

Table 2. 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

Note. *SC is a prefix used to designate $SCOPA17_{600}$, a SCAR primer pair designed from the $OPA17_{600}$.

3.4 SCAR Primer Synthesis and Screening

The designed SCAR primer pairs were synthesized and tested for their fidelity on a sample of eight randomly selected homo- and heterogenomic accessions. The best SCAR primer pair (SC3) was selected for further studies on the basis of the GC content, melting temperatures, 3' stability, likelihood of forming primer dimers, quality of the banding profile and A genome specifity (Rozen & Skaletsky, 2000). The underlined regions in Figure 2 indicate the sequence of the selected 20-mer primer pair. The SC3 primers produced a 500 bp frgment specific to the A genome. They did not amplify the genomes of *M. schizocarpa* (SS) (Figure 3, lane 6) and *M. textilis* (TT) (Figure 3, lane 7). However the primers produced an additional 700 bp band in 'Safet Velchi' (AB) (Figure 3, lane 4) and *M. balbisiana* (BB) (lane 5).

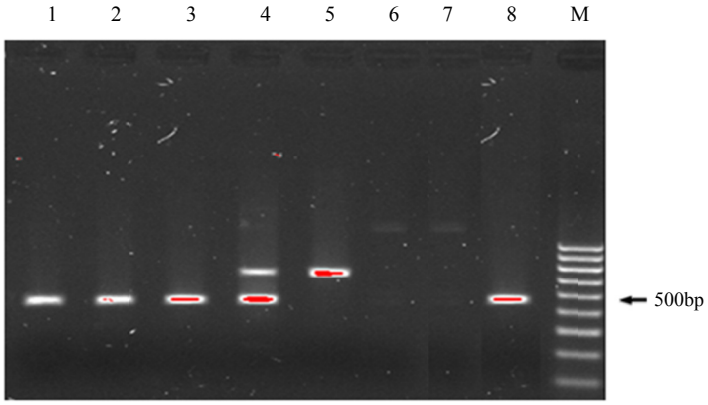

Figure 3. SCAR PCR profile for SC3 SCAR primers. The lanes represent DNA from (1) *M. acuminata* 'Calcutta 4' (AA); (2) 'Gros Michel' (AAA); (3) 'Kelong Mekintu' (AAB); (4) 'Safet Velchi' (AB_{CV}); (5) *M. balbisiana* (BB); (6) *M. schizocarpa* (SS); (7) *M. textilis* (TT); (8) 'CRBP 39' (AAAB)

Note. Lane M represents 100 bp molecular marker.

3.5 SCAR Marker Validation

The SC3 primers were then tested on 22 accessions representing different genome combinations as shown in Figure 4A and Figure 4B. The SC3 primers produced the 500 bp A genome specific band in all accessions carrying the A genome namely; (1) 'Valery' (AAA), (2) 'Tjau Lagada' (AA), (3) 'Kamaramasenge' (AB), (4) 'Kelong Mekintu' (AAB), (5) 'Ntanga 4' (AAB), (6) 'Safet Velchi' (AB_{CV}), (7) *M. acuminata* 'Calcutta 4' (AA), (8) 'Giant Cavendish' (AAA), (9) 'Lidi' (AA), (10) 'Gros Michel' (AAA), (11) 'Plantain No. 2' (AAB), (12) 'Cachaco' (ABB), (13) 'Khai Thong Ruang' (AAA), (14) 'CRBP 39' (AAAB) and (15) 'FHIA 25' (AAB). The only accession with an A genome that did not show the 500 bp A genome specific fragment in Figure 4A was 'Cardaba' (ABB) (Figure 4A, lane 12). However, a faint band of 500 bp was observed in 'Cardaba' (ABB) in a different experiment (Figure 4B, lanes 4 and 5) suggesting that the accessions did carry the 500 bp band. In addition, the SC3 primers produced a 500 bp and 700 bp band in (1) 'Kamaramasenge' (AB), (2) 'Cachaco' (ABB), (3) Safet Velchi' (AB_{CV}) and (4) 'FHIA 25' (AAB), and only a single 700 bp fragment in (3) *M. balbisiana* '10852' (BB), (8) *M. balbisiana* 'Honduras' (BB), (9) *M. balbisiana* 'Singapuri' (BB) and (20) *M. balbisiana* 'Tani' (BB).

To summarise the banding patterns observed with primers SC3 the following was observed:

(i) A 500 bp fragment was observed in all the A genome containing accessions;

(ii) A 500 bp and 700 bp band was observed in some accessions harbouring the A and B genomes, and

(iii) All the BB genome genotypes contained the 700 bp fragment.

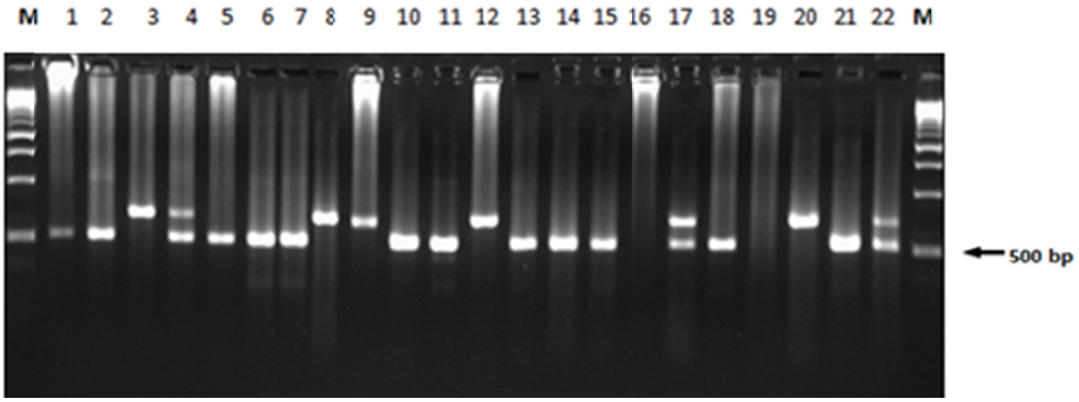

Figure 4A. SCAR PCR profile for SC3 in *Musa* landraces showing the SCOPA17$_{600}$ (arrow). The lanes represent DNA from (1) 'Valery'(AAA); (2) 'Tjau Lagada' (AA); (3) *M. balbisiana* '(10852)' (BB); (4) 'Kamaramasenge' (AB); (5) 'Kelong Mekintu' (AAB); (6) 'Ntanga 4' (AAB); (7) 'Safet Velchi' (AB$_{CV}$); (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)

Note. Lane M represents 500 bp DNA marker.

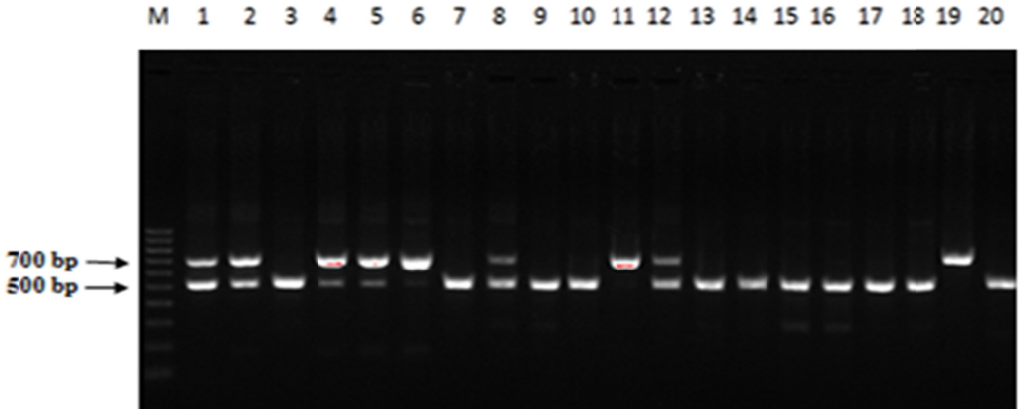

Figure 4B. SCAR PCR patterns of SC3 showing SCOPA17$_{600}$ in *Musa* landraces. The lanes represent DNA from (1) 'FHIA 25' (AAB); (2) 'Cachaco' (ABB); (3) 'Giant Cavendish' (AAA); (4) 'Cardaba' (ABB); (5) 'Cardaba' (ABB); (6) *M. balbisiana* 'Singapuri' (BB); (7) 'Valery' (AAA); (8) 'Safet Velchi' (AB$_{CV}$); (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)

Note. Lane M represents 500 bp DNA marker.

4. Discussion

In this study we were able to reproduce the 600 bp fragment specific to the A genome of banana as observed in Pillay et al. (2000). The fragment was not present in the B, S and T genome containing species *M. balbisiana*, *M. schizocarpa* and *M. textilis* respectively, suggesting that the fragment was specific to the A genome. RAPD analysis can reveal a high degree of polyrnorphism, does not require prior DNA sequence information of the species and is easy to perform. However, they are less informative, present high sequence similarity (Williams et al., 1990), have a problem of reproducibility and are unable to distinguish heterozygous and homozygous alleles (Thornmann & Osborn, 1992; Muralidharan & Wakeland, 1993; Heslop-Harrison & Schwarzacher, 2007). These challenges have, however, limited its use in modern day traditional genetics (Ercisli, Gadze, Agar, Yildirim, & Hizarci, 2009; Hasnaoui et al., 2010). In our RAPD analysis significant problems of reproducibility and high

sequence similarity were observed. Based on the sequence of the OPA_{600} RAPD fragment previously identified in Pillay et al. (2000), we designed and synthesized SCAR primers and tested them on twenty-two homo and heterogenomic *Musa* accessions. The primers identified a 500 bp fragment on all the A genome containing *Musa* accessions used in the study. This sequence was absent in the B, S and T homogenomic accessions *M. balbisiana*, *M. schizocarpa* and *M. textilis* respectively, suggesting that the fragment was specific to the A genome. If one considers the 100% fidelity of the SCAR fragment to identify all A genome containing accessions, the results of this study may point to the fact that the SCAR primers can be considered specific to the A genome in *Musa*.

However the study also observed that the SCAR primers amplified an additional 700 bp sequence in most of the *M. balbisiana* genotypes. Of the nine accessions with mixed genomes (A and B) five amplified the 700 bp sequence while four did not. The findings may imply that there is recombination between the A and B genomes. While the implication in previous studies (Heslop-Harrison & Schwarzacher, 2007; Howell, Newbury, Swennen, Whithers, & Ford-Lloyd, 1994; Nwakanma, Pillay, Okoli, & Tenkouano, 2003; Nair, Teo, & Schwarzacher, Heslop-Harisson, 2005) alluded marked differentiation of the A and B genomes, the SC3 primers developed in this study from the A genome do 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, Harrison, Crouch, & Heslop-Harrison, 1997; D'Hont, Paget-Goy, Escoute, & Carreel, 2000; Khayat, 2004; Boonruangrod, Desai, Fluch, Berenyi, & Burg, 2008; Jeridi et al., 2011, 2012; Cizkova et al., 2013; De Jesus et al., 2013). The first study that alluded to the lack of differentiation between the A and B genomes was that of (Nair, Teo, Schwarzacher, & Heslop-Harisson, 2005). 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 (Ortiz & Vuylsteke, 1994) 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. Osuji, Harrison, Crouch, and Heslop-Harrison (1997) 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 (Khayat, 2004). Boonruangrod et al. (2008) 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 (D'Hont et al., 2000). In a subsequent study, Jeridi et al. (2011) used cytogenetic evidence of mixed disomic and polysomic inheritance to suggest that there are chromosome exchanges between *M. acuminata* (AA) and *M. balbisiana* (BB). Cizkova 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 Jeridi et al. (2012) on the organization of DNA satellites in banana showed that two satellites derived from *M. acuminata* were widespread in *M. balbisiana* and a number of ABB hybrids and the S genome.

Other mechanisms such as translocations could also account for the presence of the 700 bp band in the B genome. Chromosome pairing at meiosis has revealed that translocations are frequent in banana genomes (Vilarinhos et al., 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 700 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). The 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 study 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., 2011).

While this study has provided some reasons for this anomaly further research is required to provide a sound reason for it. Sequencing of the 500 bp and 700 bp fragments will enable one to determine the homology between the sequences and the sites where the SC3 primers are annealing. The 500 bp and 700 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 whether they are located on a single or more than one chromosome. Similarly, the 500 bp fragment could be used as a hybridization probe in Southern blot experiments to ascertain its fidelity for the A genome. Boonruangrod et al. (2008) 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.

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