Genetic Diversity in Bananas and Plantains (*Musa* spp.)

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1. Introduction

Bananas and plantains belong to the family *Musaceae* and are cultivated throughout the humid tropics and sub-tropics. This crop is perennial with a faster relative growth rate compared to other fruit crops, while producing fruit all year round. Because of their nutritional value, bananas and plantains are considered the fourth most important crop worldwide after rice, wheat and corn. In many countries of Africa, bananas are considered an important part of the diet; the population in Uganda consumes per capita an average of 191 kg per year [1]. This crop also represents an important source of income for many rural families that work directly or indirectly in this industry. The edible *Musa* spp. originate from two wild species, *Musa acuminata* Colla and *M. balbisiana* Colla, with the A and B genomes, respectively, as well as their hybrids and polyploids.

The genus *Musa* is of great importance worldwide due to the commercial and nutritional value of cultivated varieties. Morphological data have suggested that *Musa* is diverse, with well-defined characteristics giving a number of indicators of the genome constitution. However, phenotyping for many physiological characteristics, including biotic and abiotic stress tolerance, particularly under controlled, contained and reproducible conditions, is difficult because of the size of the plants and their long life cycle.

DNA marker technologies have been widely used in banana genetics and diversity analysis, e.g., in taxonomy, cultivar true-to-type assessment and genetic linkage map development. Currently, proteomic analysis is giving rise to new trends in genetic diversity and plant system biology analyses. These approaches will yield detailed insights into the *Musa* genome and...
provide important genetic data for Musa breeders. In this chapter, we discuss the contribution of different DNA and protein-based markers to understanding the genetic diversity of the Musaceae family.

2. Musa genome

The Musaceae family genome is complex, and there are four genomes present, corresponding to the genetic constitutions of the four wild Musa species, i.e., M. acuminata (A-genome, 2n=2x=22), M. balbisiana (B-genome, 2n=2x=22), M. schizocarpa (S genome, 2n=2x=22) and M. textillis (T genome, 2n=2x=20). Modern bananas and plantain varieties are polyploids, and have a complex genetic structure; because most cultivated bananas are parthenocarpic (generally seedless) they have to be vegetatively propagated [2]. The traits of inflorescence in some accessions of Musa spp. are presented in Figure 1.

With the advent of modern DNA sequencing technologies and powerful bioinformatics tools, the sequencing and assembly of genomes for economically important crops and their relatives is becoming more common [3]. Knowing and understanding the genetic make-up of these crops represents a great opportunity to not only elucidate the function of genes of interest, but also to detect regions in the genome that could present polymorphism associated with agronomic traits [4]. This genetic variability is extremely valuable in plant breeding programmes where the selection of individuals with desirable characteristics is carried out.

Significant progress has being made towards gaining a better understanding of the Musa genome. Recently, [5] published the first draft of a 523-megabase M. acuminata genome and showed 36,542 protein-coding gene models. Transposable elements accounted for almost 50% of the genome. More recently, a draft of the M. balbisiana genome sequence was published [6]. The data in that study showed a great divergence between the A and B genome, which is useful in terms of increasing genetic diversity. The M. balbisiana genome was shown to be 79% smaller than that of M. acuminata, but with a highly similar number of predicted functional gene sequences: 36,638. Genomic information sheds light on polymorphisms that can be used in plant breeding programmes, such as the use of single nucleotide polymorphism (SNPs) where there is a high degree of heterozygosity between the A and B genome of one in every 55.9 base pairs. SNP polymorphisms have been successfully used with Musa spp. for mapping the genes phytoene synthase and lycopene β-cyclase, both of which are involved in β-carotene biosynthesis. Genomic information will bring about a strong and significant improvement in the use of DNA-based molecular markers, and make banana and plantain breeding programmes more efficient in improving the crop’s traits.

A large dataset of genomic information is publicly available through different databases, such as The Banana Genome Hub (http://banana-genome.cirad.fr/home), resources from the Global Musa Genomics Consortium (GMGC; http://musagenomics.org/), and the National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov/), among many others.
Figure 1. Musa accessions in a germplasm collection at Yucatan, Mexico. A: Musa coccinea, B: M. velutina, C: M. laterita, D: M. beccarii, E: M. textiles, F: M. acuminata, G: M. balbisiana, H: dessert banana (AAA), I: plantain (AAB), J: tetraploid hybrid (AAAB) and K: cooking banana (ABB) with two inflorescences. (Accessions as described in references [7, 8].)

3. DNA markers

In Musa breeding programmes, the use of morphological and cytogenetic markers has played an important role in identifying genes that control discrete traits with simple Mendelian inheritance, and has helped to estimate genome size and diversity [9]. However, a more detailed insight into the genomic polymorphism associated with agronomic traits is required.

Molecular DNA-based markers are powerful tools for gaining insights into individual genetic characteristics, and for determining allele frequency. DNA markers were developed first for humans, then applied in plants, and subsequently for the analysis of the banana genome (Figure 2). This feature allows plant breeders to select only those individuals with desirable characteristics and significantly reduce the selection time. In this chapter, we discuss the development and applications of molecular marker technology to improve some of the commercially available Musa cultivars and to assess their genetic diversity. The more important advantages and disadvantages of some DNA markers are presented in Table 1.

3.1. Restriction Fragment Length Polymorphism (RFLP)

RFLP’s markers are widely used to detect variations in DNA fragment length banding patterns of electrophoresed restriction digests of DNA samples [10]. These variations are mainly due
to the presence of a restriction enzyme cleavage site at one site in the genome of one individual, and the absence of the site in another individual. It can also detect changes in fragment size due to insertions or deletions between the restriction fragments. RFLP is a codominant marker, meaning that it is able to distinguish between homozygotes and heterozygotes. RFLP is robust, easily transferred between laboratories, and requires no prior sequence information for its use.

RFLPs have been found to be useful in _Musa_ for constructing genetic linkage maps, characterizing germplasm, phylogenetic analysis [11-15] and analysis of variation in the chloroplast genome [16, 17], and most recently have been linked to polymorphisms in resistance gene analogues [18]. These markers were useful for detecting genetic variations in Indian wild _Musa balbisiana_ populations associated with morphotaxonomic characterization clustering of most of the test types of bananas. However, they fail for some specific clusters [19], suggesting the need for more specific types of markers.

Figure 2. Schematic representation describing the development of DNA marker techniques and the first report on studies of the genetic diversity in _Musa_ species.

RFLPs make locus-specific estimations of conserved synteny possible; however, some of the disadvantages are that it is expensive to develop, requires large amounts of DNA, is not possible to automate, unlike other DNA markers [AFLP, diversity array technology (DArT), or variable number tandem repeats (VNTR)], needs a suitable probe library, may require
radioactive labelling, is laborious and time consuming. The relatively high cost and technically demanding nature of this technique make it inappropriate for routine breeding applications [20]. The use of more specific, PCR-based types of markers overcomes most of the disadvantages associated with RFLPs.

3.2. Random Amplified Polymorphic DNA (RAPD)

With the development of the polymerase chain reaction (PCR) technique, amplifying specific regions of an individual genome became possible, and identifying polymorphisms is made more precise by detecting small nucleotide changes compared to RFLPs. Random amplified polymorphic DNA (RAPD) depends on the PCR and is used as a very fast way to obtain information about genetic variation with a relatively low cost [21]. Some other characteristics are the fact that no prior knowledge of the genome sequence is required; low amounts of DNA template are used; and the advantage of technical simplicity. RAPD assays have proven to be powerful and efficient means of assisting introgression and backcross breeding [22]. But reproducibility is sometimes limited, and reliability depends on the skills of the operator, which is a dominant feature of the marker system.

RAPD has been widely used to distinguish diverse Musa germplasms [19, 23-26], for identification of duplications among accessions in tissue culture germplasm banks and somaclonal variation [27-29], and differentiation of irradiated banana genotypes [30, 31]. In addition, a molecular linkage map has also been developed using a variety of marker systems including RAPD [32]. Specific RAPD markers for the A and B genome of Musa have been identified [33, 34] and full-sib hybrids in plantain breeding populations [35]. These reports clearly demonstrate the potential value of this technique for germplasm characterization and cultivar identification, but give little insight into the value of the assay for molecular breeding. Despite the criticism of the technique, it is still being reported in recent publications [36-38].

Kaemmer [23] was first to report the use of RAPDs for fingerprinting of wild species and cultivars of banana (Musa spp.) by using simple sequence repeats (SSRs) as primers labelled with $\alpha^{32}$P. Fingerprinting analysis detected enough genetic variation to help discriminate between most of the 15 banana clones tested. In this study, polymorphic bands were unique to most of the clones tested and helped to distinguish between most of the wild type M. balbisiana (BB) and the M. acuminata (AAA) clones, as well as their hybrids for plantains (AAB) and cooking bananas (ABB); however, this technique generally fails to explain some of the variation within the species. Later, [24] used RAPD markers by adopting a set of nine primers that were shorter (N<sub>10</sub>) than the SSRs (N<sub>16</sub>) reported by [23], but the authors were able to find enough polymorphism that was unique to each of the nine genotypes representing the AA, AAA, AAB, ABB and BB genomes, and multivariate analysis showed a strong correlation between the polymorphism obtained and the morphological characters used to classify each of the groups.

The use of RAPDs was reported to identify 57 cultivars by using 60 10-mer random primers, where only 49 primers gave consistent results, and the primer OPC-15 ($^5\text{GACGGATCAG}^3$) helped to distinguish 55 of the cultivars by producing 24 bands of all tested primers [25]. These markers failed to properly characterize the clones that Gros Michel and Venkel had previously
thought belonged to the *Acuminata* group (AAA). However, chloroplast polymorphism was shown to be identical to *M. balbisiana*. This showed the potential of using RAPD markers for proper cultivar identification and germplasm classification. Similar applications, but with a variant, were reported later by [17], who used a single primer (5’-TATAGTAC-CAAGTGGTGGGG-3’) designed from a human *Alu* sequence. This sequence is a member of well-conserved short interspersed nuclear elements in the primate’s genome. Its use in fingerprinting the *Musa* genome produced bands that ranged between 300 bp and 3 kb, indicating that *Alu* related sequences are inverted repeats that are relatively close to each other.

### 3.3. Variable Number Tandem Repeats (VNTR)

VNTR are generated by highly specific PCR amplification and, therefore, should not suffer from the reproducibility problems experienced with RAPD analysis. VNTR are regions of short, tandemly repeated DNA motifs (generally less than or equal to 4 bp), with an overall length in the order of tens of base pairs [39]. VNTR have been reported to be highly abundant and randomly dispersed throughout the genomes of many plant species. Variation in the number of times the motif is repeated is thought to arise through slippage errors during DNA replication.

Furthermore, the isolation of VNTR is becoming increasingly routine with the availability of automated DNA sequencing facilities, along with improved techniques for the construction of genomic libraries enriched for VNTR and improved techniques for the screening of appropriate clones [40], bacterial artificial chromosome (BAC) end-sequences and, recently, the availability of genome sequencing facilities attributed to the discovery of VNTRs [41].

The development and utilization of VNTR in *Musa* research was first reported by [35, 40, 42] and [35]. VNTR have been considered optimum markers in other systems due to their abundance, polymorphism and reliability. VNTR analysis has been shown to detect a high level of polymorphism between individuals of *Musa* breeding populations [35], and used for development of a linkage map [18]. Nevertheless, several hundred VNTR markers have been generated in *Musa* [40, 42, 43]. New VNTR loci were discovered in the *M. acuminata* Calcutta 4 BAC end-sequence [41] from five fully sequenced consensi datasets, with validation for polymorphism conducted on genotypes contrasting in host plant resistance to Sigatoka disease [44]. Recently, [45] used a transcriptome database to design primers that were able to distinguish 32 VNTR and 119 target region amplified polymorphism (TRAP) alleles in 14 diploid *Musa* accessions.

### 3.4. Inter Simple Sequence Repeats (ISSR)

The ISSR technique developed by [46] does not require the knowledge of flanking sequences and has wide applications for all organisms, regardless of the availability of information about their genome sequence. They have also proved to be simple, fast, cost effective and versatile sets of markers for repeatable amplification of DNA sequences using single primers. As for the disadvantages, the homology of the bands is uncertain, and because they are dominant...
markers, they do not allow the calculation of certain parameters, requiring that heterozygous be distinguished from homozygous dominance.

ISSR and RAPD were used in determine genetic stability of three economically important micropropagated banana (Musa spp.) cultivars. The results showed that ISSR detected more polymorphism than RAPD [27]. Similarly, ISSR were used for detection of genetic uniformity of micropropagated plantlets [47] and for screening in vitro mutagenesis and variance [37]. Another study reported the use of ISSR to assess the genetic diversity and classification of 27 wild banana accessions collected in Guangxi, China. The results showed that the collected germplasm was derived from diverse origins and evolutionary paths of banana in Guangxi [48]. ISSR were employed for molecular assessment of genetic identity and genetic stability in banana cultivars [49]. Recently, ISSR were used to analyse the pattern of genetic variation and differentiation in 32 individuals along with two reference samples of wild Musa, which corresponded to three populations across the biodiversity-rich hot-spot of the southern Western Ghats of India [50].

3.5. Sequence-Related Amplified Polymorphism (SRAP)

The sequence-related amplified polymorphism (SRAP) technique is a simple and efficient marker system that can be adapted for a variety of purposes, including map construction, gene tagging, genomic and cDNA fingerprinting, and map-based cloning. It has several advantages over other molecular marker systems, such as simplicity, reasonable throughput rate, disclosure of numerous codominant markers, ease of isolation of polymorphic bands for sequencing, and most importantly, the targeting of open reading frames (ORFs) [51].

The SRAP marker has been adopted recently for the assessment of genetic diversity and relationships in Musa. In this regard, the study of Thailand’s wild landraces and cultivars of M. acuminata (A genome), M. balbisiana (B genome) and plantains using SRAP and RAPD has shown that the former marker was more efficient for detecting differences among closer cultivars in the same group, and the BB banana accessions were clustered separately from the AA banana accessions [52].

In another study, SRAP and AFLP were used to study 40 Musa accessions in a core collection being established in Mexico, which includes commercial cultivars and wild species of interest for genetic enhancement [7]. In addition to its practical simplicity, SRAP exhibited approximately three times more specific and unique bands than AFLP. Furthermore, SRAP was demonstrated to be a proficient tool for discriminating among M. acuminata, M. balbisiana and M. schizocarpa in the Musa section, as well as between plantains and cooking bananas within triploid cultivars. The six dessert banana cultivars used clustered according to their subgroup, i.e., Cavendish, Ibota and Gros Michel. Moreover, unique and specific bands were clearly recognized for each of seven subspecies of the acuminata complex, i.e., microcarpa, malaccensis, zebrina, banksii, truncata and burmanica-burmanicoides [7].

Moreover, the study of genetic relationships among some banana cultivars from China analysed by SRAP showed a correlation between the cultivars and their region of origin; the cultivars closely clustered into two major clusters according to their genome composition.
Likewise, the genetic data generated by the SRAP marker were reliable in respect to the morphology and agronomic trait classification, indicating the efficiency of SRAP for estimating genetic similarity among banana cultivars and providing a scientific basis for banana genetic and breeding research [53]. More recently, the fluorescently labelled SRAP molecular marker system was used to characterize the genetic variability within 71 accessions of a core collection, including wild species and cultivars of different subgroups [8], which complements previous work from the same collection [7].

The fluorescent SRAP marker information shows that *M. acuminata* subspecies *errans* was gathered with *banksii* in one cluster, while *malaccensis* was separated in a single sub-cluster. This study also found that Pisang Batu, which is known as diploid BB, was clustered with AB hybrids, i.e., Safet Velchi, Kunnan and Kamaramasenge, which supports the previous finding [54], and that Pisang Batu possesses a nucleotide polymorphism pattern of AA. Thus, the study suggested that Pisang Batu is a mislabelled accession of AAB or AB. Moreover, the SRAP marker system was found to be useful in identifying closely related accessions in the genus *Musa*, and facilitated the recognition of duplicates to be eliminated and clarified from uncertainties or mislabelled banana accessions introduced to the collection.

### 3.6. Amplified Fragment Length Polymorphism (AFLP)

AFLP is a DNA marker based on PCR amplification of selected restricted fragments obtained from the digestion of total genomic DNA or cDNA [55, 56]. It is a robust and reliable molecular technique recently employed in many systematic plant studies. AFLP banding patterns should be treated initially as dominant markers; this makes the information content limited. However, AFLP patterns can be detected as codominant markers in a segregating population when the analysis is applied to large populations [57].

The nature of an individual, whether a homozygote or heterozygote, could be distinguished using software developed on the basis of band intensity. Moreover, AFLP results in a binary band presence–absence matrix profile. In that case, two factors may affect band detection and analysis: the first is that identical bands may correspond to different fragments (homoplasy); the second is that different fragments appear as a single band (collision). An estimation method was reported for solving the effect of these factors [58], in which AFLP was demonstrated as a sampling procedure of fragments, with lengths sampled from a distribution. This study focused on estimation of pairwise genetic similarity, defined as average fraction of common fragments. Levels of polymorphism in *Musa* were shown to be high when analysed using AFLP, indicating that the technique was effective for genetic diversity analysis [59-62]. In this regard, three subspecies were suspected in the *acuminata* complex based on AFLP analysis, dominated by the subspecies *microcarpa*, *malaccensis* and *burmannica* [63].

The relationship between *M. acuminata* and *M. balbisiana* and their relatedness to cultivated bananas has been reported more clearly using AFLP [64]. Furthermore, several primers were selected from the AFLP results that can be easily used to identify A and B genomes within cultivars using a simple PCR. Additionally, AFLP markers were reported as a powerful tool for evaluating genetic polymorphisms and relationships in *Musa*. They also serve for discriminating amongst species with, A, B, S and T genomes within *Musa* species, as well as between
plantains and cooking bananas [7]. Compared to other DNA markers, AFLP was shown to be a more powerful tool than RAPD for assaying genetic polymorphisms, genetic relationships and cultivar identification among the West African plantain [65]. However, SRAP markers were more informative than AFLP in giving a higher number of unique bands specific for certain genotypes [7]. AFLP markers were appropriate for demonstrating 10 markers co-segregating with the presence or absence of banana streak badnavirus infection in *Musa* hybrids [66].

![Figure 3](image.png)

**Figure 3.** AFLP polymorphism of parental (P, bulk of 25 plants) and somatic embryogenesis regenerated *M. acuminata* AAA, cv. Williams triploid plants (R, bulk of 50), unique bands (arrows). The AFLP primer combinations as described by [69].

On the other hand, the AFLP marker technique was shown to be a good tool for detection of genetic variation in banana organogenesis and somatic embryogenesis-derived plants [67-69]. AFLP techniques have some disadvantages compared to other PCR-based markers, in that
they are technically challenging, time consuming and relatively expensive, while requiring a number of DNA processes including digestion, ligation and amplification, as well as a complex staining system. Additionally, a relatively large amount of high-quality DNA is necessary for complete digestion, which is required to reduce the presence of fake polymorphisms. However, microsatellite markers and AFLP analysis is considered to be one of the most suitable tools for marker-assisted breeding in *Musa*. DNA markers associated with fruit parthenocarpy, dwarfism and apical dominance in banana and plantain are being identified using AFLP and SSR techniques [58, 31].

4. Single Nucleotide Polymorphism (SNP)

Single nucleotide variations in the genome sequence of individuals of a population or species are known as single nucleotide polymorphisms (SNPs). The development of this technique in humans demonstrated improvements in sequencing technology and availability of an increasing number of SNP sequences [70]; this development has made direct analysis of genetic variation at the DNA sequence level possible in genomes from different organisms [71]. Modern high-throughput DNA sequencing technologies and bioinformatics tools have led to the discovery that SNPs constitute the most abundant molecular markers in the plant genomes, which has revolutionized the pace and precision of plant genetic analysis, and the discovery that SNPs are widely distributed throughout genomes, although their occurrence and distribution varies among species [72].

4.1. Diversity Arrays Technology (DArT)

DArTs are attractive approaches to detecting large numbers of genome-specific single nucleotide polymorphism (SNP) markers [73] and EcoTILLING. In principle, DArT is a DNA hybridization-based genotyping technology, which enables low-cost whole-genome profiling of crops without prior sequence information. DArT reduces the complexity of a representative sample (such as pooled DNA representing the diversity of *Musa*) using the principle that the genomic “representation” contains two types of fragments: constant fragments, found in any “representation” prepared from a DNA sample from an individual belonging to a given cultivar or species, and variable (polymorphic) fragments called molecular markers, found in some but not all of the “representations”. DArT markers are biallelic and may be dominant (present or absent) or codominant (two doses vs. one dose or absent). However, this technology has disadvantages when compared to other molecular markers, in that it depends on the availability of the array, a microarray printer and scanner, and computer infrastructure to analyse, store and manage the data produced. Despite these disadvantages, the markers are sequence-ready and, therefore, if sequenced they can be developed for a PCR analysis using standard electrophoresis.

Sequenced DArT markers have been used with *Musa* in several studies. In this regard, approximately 1,500 DArT markers have been developed using a wide array (“metagenome”) of *Musa* accessions [74]. These can be attached to BAC contigs, and can simplify the construction
of high-quality physical maps of the banana genome, which is a critical step in a sequencing
project. On the other hand, the carotenoid content and genetic variability of some banana
accessions were evaluated from the *Musa* germplasm collection held at Embrapa Cassava and
Tropical Fruits, Brazil [75]. Forty-two samples were analysed, including diploids, triploids and
tetraploids. The molecular analysis performed using 653 DArT markers showed that DArT
was an efficient tool and revealed wide-ranging genetic variability in the collected accessions.
Furthermore, DArT markers were used to analyse a panel of 168 *Musa* genotypes; thus, the
genomic origin of the markers can help to resolve the pedigree of valuable genotypes of
unknown origin. A total of 836 markers were identified and used for genotyping. Ten percent
of them were specific to the A genome and enabled the targeting of this genome portion in
relatedness analysis among diverse ploidy constitutions.

DArT revealed genetic relationships among *Musa* genotypes consistent with those provided
by the other marker technologies, but at a significantly higher resolution and speed [76].
Likewise, DArTs were used for the *Musa* framework map that was developed at CIRAD;
additionally, 380 of these markers have been used in the construction of the BORLI map, also
at CIRAD [18]. In another study, DArT analysis was used to verify the genome constitution
of 24 Philippine *Musa* cultivars. Results of the molecular data showed that some DArT markers
were specific for the B genome; subsequently, these markers can identify cultivars with B
genome regardless of the presence of the A genome. Hence, these markers can be used to
establish genome identity of the *Musa* cultivars. Moreover, BB and BBB accessions were
separated from AA/AAA and AAB cultivars in a dendrogram based on DArT data [77].
Recently, a molecular marker-based genetic linkage map of two related diploid banana
populations with a complex pedigree was achieved [78], using 121 DArT markers accompa-
nied with allele-specific-polymerase chain reactions (AS-PCR) and simple sequence repeats
(SSR). The linkage analysis indicated the likely presence of structural rearrangements.

4.2. (Eco) Targeting Induced Local Lesions in Genomes (EcoTILLING)

EcoTILLING is a high-throughput method for the discovery and characterization of SNPs and
small insertions/deletions (indels) in genomes [53, 79, 80]. It is an adaptation of the enzymatic
mismatch cleavage and fluorescence detection methods originally developed for the targeting
induced local lesions in genomes (TILLING) reverse-genetic strategy [79, 81]. The technique
was first described for *Arabidopsis* ecotypes (it was therefore named EcoTILLING). It is has
been used in many organisms due to its accuracy, low-cost and high-throughput routine, and
it also serves for the discovery and assessment of genetic diversity. About 700–1,600 bp gene
target regions are amplified using gene-specific primers that are fluorescently labelled for
EcoTILLING using enzymatic mismatch cleavage. After PCR, samples are denatured and
annealed, and heteroduplexed molecules are created through the hybridization of polymor-
phic amplicons. Mismatched regions in otherwise double-stranded duplex are then cleaved
using a crude extract of celery juice containing the single-strand specific nuclease CEL I.
Cleaved products are resolved by denaturing polyacrylamide gel electrophoresis (PAGE) and
observed by fluorescence detection.
The EcoTILLING method was used for the discovery and characterization of nucleotide polymorphisms in *Musa*, including diploid and polyploid accessions. Over 800 novel alleles were discovered in 80 accessions, indicating that EcoTILLING is a robust and accurate platform for the discovery of polymorphisms in homologous gene targets. The method proved to be valid in identifying two SNPs that might be deleterious for the function of an important gene in phototropism. Using a principle component analysis, it was shown that evaluation of heterozygous SNPs alone was sufficient to discriminate hybrids from non-hybrids and triploid *acuminata* plants from diploids. Thus, a rapid SNP assessment can in some cases replace flow cytometric methods used to differentiate ploidy.

Furthermore, differentiation between *acuminata* and *balbisiana* diploids was adequate to uncover an accidental miss-assignment of an AA type as a BB type by the stock centre. Moreover, a high level of nucleotide diversity in *Musa* accessions was revealed by evaluating the heterozygous polymorphism and haplotype blocks. The authors concluded that EcoTILLING was an accurate and efficient method for the detection and classification of nucleotide polymorphisms in diploid and polyploid banana. It is highly scalable and many applications can be considered, from simple measurements of heterozygosity as a selection criterion in breeding programmes to more nuanced studies of chromosomal inheritance and functional genomics analysis. This strategy can be used to develop hypotheses for inheritance patterns of nucleotide polymorphisms within and between genome types [53].

More recently, single nucleotide polymorphism (SNP) studies for marker discovery of the use of beta carotene (provitamin A) in plantains [82], and SNPs found in the partial sequence of the gene encoding the large sub-units of ADP-glucose pyrophosphorylase, a key enzyme related to starch metabolism, in banana and plantains [83], give important information for new approaches to investigating the wide range of banana germplasm biodiversity and incorporating the information in banana and plantain breeding.

5. Molecular cytogenetic

Using cytogenetics, chromosome studies of humans and several plant species have been going on for more than a century, helping to establish the typical number of chromosomes for each of the species and to assign a chromosome number according to their size and centromere position. Chromosome size and banding pattern helped to identify subchromosomal regions and were associated with some phenotypic characteristics. Chromosome number has also been important for identifying individuals among species. Even though cytogenetic tools have been improved to obtain high-resolution banding patterns for identifying deletions, insertions or translocations [84], it remains a challenge to elucidate the origins of the chromosomes that are involved in chromosome rearrangements. Molecular cytogenetics is adding a set of powerful tools to those already available for studying genome organization, evolution and recombination. This technology can help to identify small changes at the level of the gene, for which several techniques have been developed.
5.1. Fluorescent in situ Hybridization (FISH) to chromosomes

Fluorescent in situ hybridization (FISH) allows hybridization sites to be visualized directly and, moreover, several probes can be simultaneously detected with different fluorochromes, allowing the physical order of the chromosomes to be determined. FISH was used on mitotic chromosomes to localize the physical sites of 18S-5.8S-25S and 5S rRNA genes in Musa [85, 86]. A single major intercalary site was observed on the short arm of the nucleolar organizing chromosome in both A and B genomes. Diploid, triploid and tetraploid genotypes showed two, three and four sites, respectively. Heterogeneous Musa lines showed different intensity of signals that indicate variation in the number of copies of these genes. In the case of 5S rDNA, eight subterminal sites were observed in Calcutta 4 (AA), while Butohan 2 (BB) had six sites. Triploid lines showed six to nine major sites of 5S rDNA of widely varying intensity, near the limit of detection. The diploid hybrids had five to nine sites of 5S rDNA while the tetraploid hybrid had 11 sites [86].

Additionally, a dual colour FISH showed that in all studied accessions, the satellite chromosomes carrying the 18S-25S loci did not carry the 5S loci [85]. On the other hand, the telomeric sequence was detected as pairs of dots at the ends of all the chromosomes analysed, but no intercalary sequences were seen [85].

Detection of the integration of viral sequences of banana streak badnavirus (BSV) in two metaphase spreads of Obino l’Ewai plantain (AAB) was achieved using FISH [87]. Two different BSV sequence locations were revealed in Obino l’Ewai chromosomes and a complex arrangement of BSV and Musa sequences was shown by probing stretched DNA fibres. In another study, the monkey retrotransposon was identified and localized in Musa using FISH [88]. Several copies of monkey were concentrated in the nucleolar organizer regions and colocalized with rRNA genes. Other copies of monkey appear to be dispersed throughout the genome. In addition, in order to increase the number of useful cytogenetic markers for Musa, low amounts of repetitive DNA sequences of BAC clones were used as probes for FISH on mitotic metaphase chromosomes [89]. Only one clone gave a single-locus signal on chromosomes of M. acuminate cv. Calcutta 4. The clone localized on a chromosome pair that carries a cluster of 5S rRNA genes. The remaining BAC clones gave dispersed FISH signals throughout the genome and/or failed to produce any signal. In addition, 19 BAC clones were subcloned and their ‘low-copy’ subclones were selected, to avoid the excessive hybridization of repetitive DNA sequences. Out of these, one subclone gave a specific signal in secondary constriction on one chromosome pair, and three subclones were localized into centromeric and peri-centromeric regions of all chromosomes. The nucleotide sequence analysis revealed that subclones which localized on different regions of all chromosomes contained short fragments of various repetitive DNA sequences [89].

Furthermore, a modern chromosome map technology known as high-resolution fluorescent in situ hybridization (FISH) was applied in Musa species using BAC clone positioning on pachytene chromosomes of Calcutta 4 (M. acuminate, Eumusa) and M. velutina (Rodochlamys). To make cell spread preparations appropriate for FISH, pollen mother cells were digested with pectolytic enzymes and macerated with acetic acid. BAC clones that contain markers for known resistance genes were chosen and hybridized to establish their relative positions on the
two species [90]. Centromeric retrotransposons were detected in banana chromosomes hybridized with MusA1 by FISH. Since all of the banana chromosomes are metacentric or submetacentric, signals were located around the centre of the chromosome, indicating that loci are found near or within the centromere [91]. Recently, the genomic organization and molecular diversity of two main banana DNA satellites were analysed in a set of 19 Musa accessions, including representatives of A, B and S genomes and their interspecific hybrids. The two DNA satellites showed a high level of sequence conservation within, and a high homology between Musa species. FISH with probes for the satellite DNA sequences, rRNA genes and a single-copy BAC clone 2G17 resulted in characteristic chromosome banding patterns in M. acuminata and M. balbisiana, which may aid in determining genomic constitution in interspecific hybrids. In addition, the knowledge of Musa satellite DNA was improved by increasing the number of cytogenetic markers and the number of individual chromosomes which can be identified in Musa [92].

5.2. Genomic in situ Hybridization (GISH)

Genomic in situ hybridization (GISH) is a powerful tool to differentiate alien chromosomes or chromosome segments from parental species in interspecific hybrids [93]. It has been applied to mitotic chromosomes from many plants resulting from interspecific hybridization. However, application of GISH on meiotic chromosomes is challenging, and has been reported in just a few species. In Musa, GISH was successfully applied to differentiate the chromosomes of different genomes on both mitotic and meiotic chromosomes.

The first study that used GISH in Musa was with chromosome spreads prepared from root tips. The total genomic DNA of diploid lines AA and BB was able to label the centromeric regions of all 22 chromosomes of the corresponding line. However, the two satellite chromosomes of genome B labelled strongly with genomic A DNA. GISH discriminated between A and B chromosomes in AAB and ABB cultivars. Additionally, it has immense potential for identification of chromosome origin and can be used to characterize cultivars and hybrids produced in Musa breeding [94]. In another study [95], GISH was used to determine the exact genome structure of interspecific cultivated clones AAB and ABB. As a notable exception, the clone ‘Pelipita’ (ABB) was found to have eight A and 25 B chromosomes instead of the predicted 11 A and 22 B. In addition, chromosome complement was determined by some clones that could not be classified by phenotypic characteristics and chromosome counts. Moreover, rDNA sites were located in Musa species that appeared to be frequently associated with satellites. These sites can be separated from the chromosomes providing a potential source of chromosome counting errors when conventional techniques are used. Furthermore, GISH successfully differentiated the chromosomes of the four known genomes, A, B, S and T, which correspond to the genetic constitutions of wild Eumusa species M. acuminata, M. balbisiana, M. schizocarpa and the Australimusa species, respectively [95, 96].

On the other hand, GISH has been used on meiotic chromosomes in plants. It is quite challenging, and the protocols used are complex and highly variable depending on the species. A method was developed to prepare chromosomes at meiosis metaphase I suitable for GISH in Musa [97]. The main challenge encountered was the hardness of the cell wall and the density
of the microsporocyte’s cytoplasm, which hamper the accessibility of the probes to the chromosomes and generate higher levels of background noise. It was clearly demonstrated that interspecific recombinations between *M. acuminata* and *M. balbisiana* chromosomes do occur and may be frequent in triploid hybrids. Recently, conventional cytogenetic and GISH analyses of meiotic chromosomes were used to investigate the pairing of different chromosome sets at diploid and tetraploid levels, and to reveal the chromosome constitution of hybrids derived from crosses involving allotetraploid genotype. At both ploidy levels, the analysis suggested that the newly formed allotetraploid behaves as a segmental allotetraploid. The 11 chromosomes were found as three sets in a tetrasomic pattern, three in a likely disomic pattern and the five remaining sets in an intermediate pattern. In addition, balanced and unbalanced diploid gametes were detected in progenies. The chromosome constitution was more homogenous in pollen than in ovules. The segmental inheritance pattern exhibited by the AABB allotetraploid genotype implies chromosome exchanges between *M. acuminata* and *M. balbisiana* species, and opens new horizons for reciprocal transfer of valuable alleles [97].

### 5.3. Flow cytometry

Flow cytometry (FCM) protocols have been applied for studying the natural variation in *Musa* nuclear genome size (DNA content) for taxonomic purposes and for checking ploidy among gene bank accessions and breeding materials [98-101]. The literature data suggest that, on average, the A genome of *M. acuminata* and clones with AA genome constitution is around 12% larger than the B genome of *M. balbisiana*, with small intraspecific variation in nuclear DNA found in a number of wild acuminata diploid and parthenocarpic bananas, whereas large variation seems to be exhibited among triploid varieties [102, 99].

The study of genomic composition of *Musa* accessions on a core collection based on ITS (internal transcribed spacer sequences of the nuclear ribosomal DNA) regions and SSR polymorphism, along with assessment of DNA content and ploidy by FCM, has given support to the hypothesis [103] of the occurrence of homologous recombination between A and B genomes, or between *M. acuminata* subspecies genomes, leading to discrepancies in the number of sets or portions from each parental genome [104]. It is worth mentioning that when the former published data sets were compared, clear differences in the genome size of *M. balbisiana* accessions were found, leaving open the question as to the origin of such variation. Basic research works of this type are of great interest to understanding the evolution and domestication of *Musa*, and for betterment of bananas using new approaches.

Research on the genetic stability/instability of *in vitro* somatic embryogenesis (SE) cultures by FCM for polyploid bananas [105, 106], and recently, by FCM and cytological analysis of embryogenic *M. acuminata* ssp. *malaccensis* cell suspension cultures, and of their somatic embryo-derived plantlets [107], adds support to the use of *in vitro* innovative cell biology tools for high-throughput production of clean banana planting materials, the rescue of hybrid true seeds by *in vitro* germination and their clonal propagation through SE, which assist in the acquisition of seedlings after interspecific hybridization and across ploidy to enhance the efficiency of genetic improvement in *Musa*. 
Figure 4. Typical histogram of propidium iodide (PI)-labelled nuclei at pre-DNA synthesis (G1), synthesis (S) and post-synthesis (G2) from a triploid (2n=3x=33 chromosome) regenerated M. acuminata AAA, cv. Williams plant. The x axis indicates the fluorescence signal intensity (channel number), which stoichiometrically relates to DNA content. Plant material was regenerated by somatic embryogenesis [68].

| Criterion                          | RFLP  | RAPD  | VNTR   | ISSR  | SRAP  | AFLP  | SNPs  |
|-----------------------------------|-------|-------|--------|-------|-------|-------|-------|
| Quantity of DNA required          | High  | Low   | Low/high° | Low   | Low   | Moderate | Low   |
| Quantity of information           | Low   | High  | High   | High  | High  | High   | Low   |
| Replicability                     | High  | Variable | High   | High  | High  | High   | Moderate |
| Resolution of genetic differences | High  | Moderate | High   | Moderate | High  | High   | High   |
| Abundance                         | High  | High   | Medium | High  | High  | High   | High   |
| Locus specificity                 | Yes   | No     | Yes    | No    | Yes   | No     | Yes    |
| Ease of use and development       | Difficult | Easy   | Easy   | Easy  | Easy  | Moderate° | Moderate |
| Development time                  | Long  | Short  | Moderate | Short | Short | Moderate | Moderate |
| Development costs                 | High  | Low    | Moderate | Low   | Low   | Moderate | Low    |
| Operational costs                 | High  | Low    | Low    | Low   | Low   | Moderate | Moderate / High |
| Locus specificity                 | Yes   | No     | Yes    | No    | Yes/No | No     | Yes    |
| Major application                 | Physical mapping | Gene tagging | Genetic diversity | Gene tagging | Genetic diversity | Gene tagging SNP mapping |

°VNTR requires a high quantity of DNA when analysed by RLFP, while it needs a low quantity when analysed by PCR. ²Analysis of AFLP is easy with the help of an automated genotyper, or when using low-resolution agarose gel electrophoresis, but manual polyacrylamide electrophoresis requires a certain amount of experience.

Table 1. Comparison among different molecular markers for various criteria; adapted from [22, 51, 108].
6. Protein polymorphism

Proteomics can be defined as the systematic analysis of proteome, the protein complement of the genome, which deals with information on proteins’ abundance, their variations and modifications, and their interacting partnerships and networks, in order to understand cellular processes in biological systems. Thus, proteomics is important for understanding the molecular mechanisms involved in plant and crop biodiversity, which is a driving force behind speciation, crop domestication and improvement. This is of particular interest in bananas, which are good representatives of a complex allopolyploid and an important fruit crop.

6.1. Proteome analysis

In recent years, several proteomic studies, based on combined use of two-dimensional electrophoresis (2DE) and mass spectrometric methods, have been successfully applied to investigate the effect of osmotic stresses on banana growth and development [109], cold tolerance [110], inter-and intra-cultivar protein polymorphisms [111], the fruit proteome of banana [112, 113], and the proteomic profiling of banana roots in response to *F. oxysporum* [114]. These investigations highlight the value of new sequencing technologies for integrating the biological information of a plant system usually considered a non-model crop, and open new approaches to studying biodiversity, evolution and domestication in *Musa*. In this scenario, the proteomic analysis of shoot meristem changes in the acclimation to sucrose-mediated osmotic stress of two banana varieties uncovered several genotype-specific proteins (isoforms), enzymes of the energy metabolism (e.g., phosphoglyceraldehyde kinase, phosphoglucose-mutase, UDP-glucose pyrophosphorylase) and stress adaptations (e.g., OSR40-like protein, abscisic stress ripening protein-like protein, ASR) that were associated with the dehydration-tolerant variety [109].

In contrast, comparative quantitative proteomic analysis of plantain (AAB genome) response to cold stress treatments revealed that about 23.3 % of the 3477 total proteins identified were differentially expressed. The largest parts of the expressed proteins were predicted to be involved in the oxidation reduction process (including oxylipin biosynthesis), cellular process, response to stress and primary metabolic process. Interestingly, among the cold-responsive proteins involved in the oxidation reduction process, Cu/Zn SOD (superoxide dismutase), CAT 2 (catalase isozyme 2) and LOX (lipoxygenase) were found to be differentially expressed in the cold-tolerant plantain, in contrast to the cold-susceptible banana [110]. Altogether, the previous works provided clues as to the existence of inter-variety protein polymorphism related to their *acuminata* or *balbisiana* origin, and open new approaches to examining diploid and triploid bananas’ different responses to environmental stress.

Moreover, evidence from the proteome analysis of different triploid banana varieties using 2D electrophoresis revealed the following results: i) principal component analysis (PCA) showed that the principal component PC1 (which explains 39 % of the variance information) was positively correlated with the presence of the B genome; and, ii) the hierarchical clustering revealed that the first level of clustering separates the varieties of genome BBB and ABB composition from both AAB and the two AAA varieties, the second level splits both AAB
varieties from the two AAA varieties and the ABB from the BBB varieties, and the third level divides both AAA varieties and both AAB varieties. Although proteome analysis does not always correspond to the presumed genome formulae, perhaps because following polyploidization new gene copies may undergo modifications allowing functional diversification, in general, the observations at the protein level provide good indications for a more complex genome structure and genomic rearrangement in some banana varieties [111].

7. Conclusion

The limited genetic knowledge of the banana genome and the nature of the crop as a parthenocarpic fruit and a mostly triploid, sterile plant mean that many aspects of breeding and selection that have been possible in other crops cannot be applied in the banana. Several approaches to breeding and selection have been applied in numerous plant species; however, they could not be used in banana due to the unclear genetic knowledge of its genome and the natural characteristics of the crop, including parthenocarpy, ploidy and sterility. Unconventional biotechnological strategies including DNA and protein-based marker techniques have contributed considerably in providing a vast amount of information that helps in understanding the nature of Musa genome and its genetic diversity. DNA-based markers were developed and are being used in Musa, representing powerful tools to assess the genetic diversity and clarify the individual genetic characteristics and relationships. Researchers should select the best marker for a certain task; however, the recently developed molecular markers were more informative when applied in banana. For instance, SRAPs showed better assessment of the genetic diversity and increased the clarity of genetic relationships among several Musa species, subspecies and cultivars when compared with other traditional markers, such as RAPD and AFLP. Moreover, since SRAP is based on the amplification of ORFs, this gives another advantage to this marker over others. Involving recently developed molecular markers, such as the target region amplification polymorphism (TRAP), which is based on the amplification of target EST sequences, as well as the intron targeted amplified polymorphism (ITAP), which is based on the amplification of 3’ widely distributed intron-exon splice junction sequences, could improve the assessment of genetic diversity in Musa and refine the genetic relationships among the different sections, species and mislabelled accessions. In addition, despite their relatively high cost, the high-throughput technologies based on SNPs or small-scale indels are efficient alternatives to traditional markers, because of their greater abundance, high polymorphism, ease of measurement and ability to reveal hidden polymorphisms where other methods fail. SNPs also allow easy and unambiguous identification of alleles or haplotypes. A good marker system for polyploid crops should be dosage sensitive and have the ability to distinguish heterozygous genotypes with multiple haplotypes. On the other hand, molecular cytogenetics techniques have played a great role in understanding the Musa genome construction, determining genomic constitution of the interspecific hybrids and studying the natural variation in Musa nuclear genome size, as well as checking ploidy levels among gene bank accessions and breeding materials. In addition, the knowledge on Musa satellite DNA was improved by increasing the number of cytogenetic markers and the number of individual
chromosomes which can be identified in *Musa*. The complementation of DNA based markers with protein-based ones has completed the image of unreachable variations at DNA level. Several proteomic studies, based on combined use of two-dimensional electrophoresis (2DE) and mass spectrometric methods, have been successfully applied to investigate various approaches in banana, including protein polymorphism, and biotic and abiotic tolerance. The combination of all molecular approaches surveyed and discussed in this chapter can help in the revelation of the genetic diversity in *Musa*. High-throughput technologies based on SNPs or small-scale indels are efficient alternatives for traditional markers (RFLP, RAPD or AFLP), because of their greater abundance, high polymorphism, ease of measurement and ability to reveal hidden polymorphisms where other methods fail. SNPs also allow easy and unambiguous identification of alleles or haplotypes. A good marker system for polyploid crops should be dosage sensitive, and have the ability to distinguish heterozygous genotypes with multiple haplotypes.

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References

[1] International Institute of Tropical Agriculture. Banana & Plantain. http://www.iita.org/banana-and-plantain (accessed September 7th, 2014)

[2] Ortiz R, Swennen R. From cross breeding to biotechnology-facilitated improvement of banana and plantain. Biotechnology Advances 2014; 32(1) 158-169.

[3] Feuillet C, Leach JE, Rogers J, Schnable PS, Eversole K. Crop genome sequencing: Lessons and rationales. Trends in Plant Sciences 2011; 16(2) 77-88.
[4] Liu BH. 1997. Statistical Genomics: Linkage, Mapping and QTL Analysis. CRC Press, 1997, p. 611.

[5] D’Hont A, Denoeud F, Auray JM, Baurens FC, Carreel F, Garsmeur O, Noel B, Bocs S, Droc G, Rouard M, Da Silva C, Jabbari K, Cardi C, Poulain J, Souquet M, Labadie K, Jouarda C, Lengellé J, Rodier-Goud M, Alberti A, Bernard M, Correa M, Ayyampalayam S, Mckain MR, Leebens-Mack J, Burgess D, Freeling M, Mbéguié-A-Mbéguié D, Chabannes M, Wicker T, Panaud O, Barbosa J, Hribova E, Heslop-Harrison P, Habas R, Rivallain R, Francois P, Poiron C, Kilian A, Burthia D, Jenny C, Bakry F, Brown S, Guignon V, Kema G, Dita M, Waalwijk C, Joseph S, Dievart A, Jaillon O, Leclercq J, Argout X, Lyons E, Almeida A, Jeridi M, Dolezel J, Roux N, Risterucci AM, Weissenbach J, Ruiz M, Glaszmann JC, Quétier F, Yahiaoui N, Wincker P. The banana (Musa acuminata) genome and the evolution of monocotyledonous plants. Nature 2012; 488 213-217.

[6] Davey MW, Gudimella R, Harikrishna JA, Sin LW, Khalid N, Keulemans J. A draft Musa balbisiana genome sequence for molecular genetics in polyploid, inter-and intra-specific Musa hybrids. BMC Genomics 2013; 14 683. http://www.biomedcentral.com/1471-2164/14/683 (accessed 20 October 2014).

[7] Youssef M, James A, Rivera-Madrid R, Ortiz R, Escobedo-Gracia Medrano RM. Musa genetic diversity revealed by SRAP and AFLP. Mol Biotech 2011; 47(3) 189–199.

[8] Valdez-Ojeda R, James-Kay A, Ku-Cauich J, Escobedo-Gracia Medrano RM. Genetic relationships among a collection of Musa germplasm by fluorescent-labeled SRAP. Tree Genetics and Genomes 2014; 10 (3) 465-476.

[9] Heslop-Harrison JS, Schwarzacher T. Domestication, genomics and the future for banana. Annals of Botany 2007; 100 (5) 1073-1084.

[10] Botstein D, White RL, Skolnick M, Davis RW. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 1980; 32 (3) 314-333.

[11] Bhat KV, Jarret RL. Random amplified polymorphic DNA and genetic diversity in Indian Musa germplasm. Genet Resour Crop Evol 1995; 42 (2) 107–118.

[12] Gawel NJ, Jarret RL, Whittemore AP. Restriction fragment length polymorphism (RFLP)-based phylogenetic analysis of Musa. Theor Appl Genet 1992; 84 (3-4) 286–290.

[13] Jarret R, Gawel N, Whittemore A, Sharrock S. RFLP-based phylogeny of Musa species in Papua New Guinea. Theor Appl Genet 1992; 84 (5-6) 579–584.

[14] Lanaud C, Tezena du Montcel H, Jolivot MP, Glaszman JC, Gonzalez de León D. Variation in ribosomal gene spacer length among wild and cultivated banana. Heredity 1992; 68 148-156.
[15] Jenny C, Carreel F, Bakry F. Revision of banana taxonomy: Klue Tiparot (Musa sp.) reclassified as a triploid. Fruits 1997; 52 (2) 83-91.

[16] Gawel NJ, Jarret RL. Chloroplast DNA restriction fragments length polymorphisms (RFLPs) in Musa species. Theor Appl Genet 1991; 81 (6) 783–786.

[17] Baurens FC, Noyer JL, Lanaud C, Lagoda PJJ. Sequence tagged site markers to draft the genomic structure of the banana chloroplast. Fruits 1997; 52 (4) 247-259.

[18] Hippolyte I, Bakry F, Seguin M, Gardes L, Rivallan R, Risterucci A-M, Jenny C, Perrier X, Carreel F, Argout X, Piffanelli P, Khan I, Miller R, Pappas G, Mbegue-A-Mbegue D, Matsumoto T, De Bernardinis V, Huttner E, Kilian A, Baurens F-C, D’Hont A, Cote F, Courtois B, Glaszmann JC. A saturated SSR/DArT linkage map of Musa acuminate addressing genome rearrangements among bananas. BMC Plant Biol 2010; 10: 65–65. http://www.biomedcentral.com/1471-2229/10/65 (accessed 20 October 2014).

[19] Uma S, Siva SA, Saraswathi MS, Manickavasagam M, Durai P, Selvarajan R Sathiamoorthy S. Variation and intraspecific relationships in Indian wild Musa balbisiana (BB) population as evidenced by random amplified polymorphic DNA. Genetic Resources in Crop Evolution 2006; 53 (2) 349-355.

[20] Pillay M., Ashokkumar K., James A., Prince Kirubakara SJ, Miller R, Ortiz R, Sivalingam E. Molecular markers techniques in Musa genomic research. In: Genetics, Genomics, and Breeding of Bananas, ed. Pillay M, Ude G and Kole C: Science Publishers; 2012. pp. 70-90.

[21] Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 1990; 18 (22) 6531–6535.

[22] Agarwal M, Shrivastava N, Padh H. Advances in molecular marker techniques and their applications in plant sciences. Plant Cell Reports 2008; 27 (4) 617-631.

[23] Kaemmer D. Oligonucleotide and amplification fingerprinting of wild species and cultivars of banana (Musa spp.) Bio/Technology 1992; 10 (9) 1030-1035.

[24] Howell EC, Newbury HJ, Swennen RL, Withers LA, Ford-Lloyd BV. The use of RAPD for identifying and classifying Musa germplasm. Genome 1994; 37 (2) 328–332.

[25] Bhat KV, Jarret RL. Random amplified polymorphic DNA and genetic diversity in Indian Musa germplasm. Genet Resour Crop Evol 1995; 42 (2)107–118.

[26] Jain PK, Saini MK, Pathak H, Gupta VK. Analysis of genetic variation in different banana (Musa species) variety using random amplified polymorphic DNAs (RAPDs). Afri J Biotechnol 2007; 6(17) 1987–1989.

[27] Ray T, Dutta I, Saha P, Das S, Roy SC. Genetic stability of three economically important micropropagated banana (Musa spp.) cultivars of lower Indo-Gangetic plains, as assessed by RAPD and ISSR markers. Plant Cell Tiss Org Cult 2006; 85 (1) 11–21.
[28] Bairu MW, Fennell CW, van Staden J. The effect of plant growth regulators on somaclonal variation in Cavendish banana (Musa AAA cv. ‘Zelig’). Sci Hort 2006; 108 (4) 347–351.

[29] Lakshmanan V, Venkataramareddy SR, Neelwarne B. Molecular analysis of genetic stability in long-term micropropagated shoots of banana using RAPD and ISSR markers. Elec J Biotechnol 2007; 10 (1) 106–113.

[30] Imelda M, Estiati A, Hartati NS. Induction of mutation through gamma radiation in three cultivars of banana. Ann Bogorien 2001; 7 (2) 75–82.

[31] Hautea DM, Molina GC, Balatero CH, Coronado NB, Perez EB, Alvarez MTH, Canama AO, Akuba RH, Quilloy RB, Frankie RB, Capillos AO. Analysis of induced mutants of Philippine with molecular markers. In: Banana Improvement: Cellular, Molecular Biology and Induced Mutations, ed. Jain S, Swennen R. Kluwer Academic Publishers, Dordrecht, the Netherlands; 2004. p 45–57.

[32] Faure S, Noyer JL, Horry JP, Bakry F, Lanaud C, Goazalez de Lean D. A molecular marker-based linkage map of diploid bananas (Musa acuminata). Theor Appl Genet 1993; 87(4) 517-526.

[33] Pillay M, Nwakanma DC, Tenkouano A. Identification of RAPD markers linked to A and B genome sequences in Musa L. Genome 2000; 43 (3) 763–767.

[34] Pillay M, Ogundiwin E, Tenkouano A, Dolezel J. Ploidy and genome composition of Musa germplasm at the International Institute of Tropical Agriculture (IITA). Afr J Biotechnol 2006; 5(13) 1224–1232.

[35] Crouch HK, Crouch JH, Jarret RL, Cregan PB, Ortiz R. Segregation at microsatellite loci in haploid and diploid gametes of Musa. Crop Science 1998; 38(1) 211–217.

[36] Das BK, Jena RC, Samal KC. Optimization of DNA isolation and PCR protocol for RAPD analysis of banana/plantain (Musa spp.). Int J Agric Sci 2009; 1(2) 21–25.

[37] Khatri A, Dahot MU, Khan I, Raza S, Bibi S, Yasmin S, Nizamani DGS. In vitro mutagenesis in banana and variant screening through ISSR. Pak J Bot 2011; 43(5) 2427-2431.

[38] Nadal-Medina R, Manzo-Sanchez G, Orozco-Romero J, Orozco-Santos M, Guzman-Gonzalez S. Genetic diversity of bananas and plantains (Musa spp.) determined by RAPD markers. Revista Fitotecnia Mexicana 2009; 32(1) 1–7.

[39] Tourmente S, Deragon JM, Lafleuriel J, Tutois S, Pelissier T, Cuvillier C, Espagnol MC, Picard G. Characterization of minisatellites in Arabidopsis thaliana with sequence similarity to the human minisatellite core sequence. Nucl Acid Res 1994; 22(16) 3317–3321.
[40] Kaemmer D, Fischer D, Jarret RL, Baurens FC, Grapin A, Dambier D, Noyer JL, Lainaud C, Kahl G, Lagoda PJL. Molecular breeding in the genus *Musa*: A strong case for STMS marker technology. Euphytica 1997; 96(1) 49–63.

[41] Cheung F, Town CD. A BAC end view of the *Musa* acuminata genome. BMC Plant Biol 2007; 7 29–29. http://www.biomedcentral.com/1471-2229/7/29 (accessed 20 October 2014).

[42] Jarret RL, Bhat KV, Cregan PB, Ortiz R, Vuylsteke D. Isolation of microsatellite DNA markers in *Musa*. InfoMusa 1994; 3(2) 3–4.

[43] Wang JY, Zheng LS, Huang BZ, Liu WL, Wu YT. Development, characterization, and variability analysis of microsatellites from a commercial cultivar of *Musa acuminata*. Genet Resour Crop Evol 2010; 57 (4) 553–563.

[44] Miller RNG, Passos MAN, Menenez NNP, Souza MT Jr, do Carmo Costa MM, Rennó Azevedo VCR, Amorim EP, Pappas GJ Jr, Ciampi AY. Characterization of novel microsatellite markers in *Musa acuminata* subsp. *burmannicoides*, var Calcutta 4. BMC Res Notes 2010; 3: 148. http://www.biomedcentral.com/1756-0500/3/148 (accessed 20 October 2014).

[45] Garcia SAL, Talebi R, Ferreira CF, Vroh BI, Paiva LV, Kema GHJ, Souza MT Jr. Identification and validation of est-derived molecular markers, TRAP and VNTRS, for banana research. Acta Hort 2011; 897: 69-80. http://www.actahort.org/books/897/897_6.htm (accessed 20 October 2014).

[46] Zietkiewicz E, Rafalski JA, Labuda D. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics 1994; 20(2) 176–183.

[47] Rout GR, Senapati SK, Aparajita S, Palai SK. Studies on genetic identification and genetic fidelity of cultivars banana using ISSR marker. Plant Omics Journal 2009; 2(6): 250-258.

[48] Qin XQ, Peng HX, Long X, Yao JY. Preliminary study on ISSR analysis and classification of wild *Musa* germplasm in Guangxi, China. Acta Hort 2011; 897: 259-262. http://www.actahort.org/books/897/897_29.htm (accessed 20 October 2014).

[49] Lu Y, Xu WH, Xie YX, Zhang Z, Pu JJ, Qj YX, Li HP. Isolation and characterization of nucleotide-binding site and C-terminal leucine-rich repeat-resistance gene candidates in bananas. Genet Mol Res 2011; 10(4) 3098-3108.

[50] Padmesh P, Mukunthakumar S, Vineesh PS, Hari Kumar K, Krishnan PN. Exploring wild genetic resources of *Musa acuminata* Colla distributed in the humid forests of southern Western Ghats of peninsular India using ISSR markers. Plant Cell Reports 2012; 31(9) 1591-1601.
[51] Li G, Quiros CF. Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: Its application to mapping and gene tagging in Brassica. Theor Appl Genet 200; 103(2-3) 455–461.

[52] Phothipan S, Silayoi B, Wanichkul K, Apisitwanich S. Genetic relationship among banana in AA, AAB and B groups using random amplified polymorphic DNA (RAPD) and sequence related amplified polymorphism (SRAP) techniques. Kasetsart J Nat Sci 2005; 39 703–710. http://kasetsartjournal.ku.ac.th/kuj_files/2008/A0804281451054134.pdf (accessed 20 October 2014).

[53] Xie Z-S, Liu D-B, Wei S-X, Chen Y-Y, Xie J-J, Wei J-Y. Analysis of genetic diversity of 29 banana genotypes by SRAP. Acta Botanica Boreali-Occidentalia Sinica 2012; 8 009. http://caod.oriprobe.com/articles/31027308/Analysis_of_Genetic_Diversity_of_29_Banana_Genotypes_by_SRAP.htm (accessed 20 October 2014).

[54] Till BJ, Jankowicz-Cieslak J, Sagi L, Huynh OA, Utsushi H, Swennen R, Terauchi R, Mba C. Discovery of nucleotide polymorphisms in the Musa gene pool by Ecotilling. Theor Appl Genet 2010; 121(7) 1381–1389.

[55] Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Pellem J, Kuiper J, Zabeau M. AFLP: A new technique for DNA fingerprinting. Nucleic Acids Res 1995; 23(21) 4407–4414.

[56] Bachem CWB, van der Hoeven RS, de Bruijn SM, Vreugdenhil D, Zabeau M, Visser RGF. Visualization of differential gene expression using a novel method of RNA fingerprinting based on AFLP: Analysis of gene expression during potato tuber development. The Plant Journal 1995; 9(5) 745-753.

[57] Masiga DK, Turner CMR. 2004. Amplified (restriction) fragment length polymorphism (AFLP) analysis. In: Methods in Molecular Biology, ed., Melville SE. Humana Press, USA, 2004, p173–186.

[58] Gort G, van Hintum V, van Eeuwijk F. Homoplasy corrected estimation of genetic similarity from AFLP bands, and the effect of the number of bands on the precision of estimation. Theor Appl Genet 2009; 119(3) 397–416.

[59] Crouch JH, Crouch HK, Constand H, Van Gysel A, Breyne P, Van Montagu M, Jarret RL, Ortiz R. Comparison of PCR-based molecular marker analysis of Musa breeding populations. Mol Breed 1999; 5(3) 233–244.

[60] Loh JP, Kiew R, Set O, Gan LH, Gan YY. Amplified fragment length polymorphism fingerprinting of 16 banana cultivars (Musa cvs.). Mol Phylogenet Evol 2000; 17(3) 360–366.

[61] Wong C, Kiew R, Loh JP, Gan LH, Lee SK, Ohn S, Gan YY. Genetic diversity of the wild banana Musa acuminata Colla in Malaysia as evidenced by AFLP. Ann Bot 2001; 88(6) 1017–1025.
[62] Wong C, Kiew R, Argent G, Ohn S, Lee SK, Gan YY. Assessment of the validity of the sections in *Musa* (*Musaceae*) using AFLP. Ann Bot 2002; 90(2) 231-238.

[63] U de G, Pillay M, Nwakanma A, Tenkouano A. Analysis of genetic diversity and selectional relationships in *Musa* using AFLP markers. Theor Appl Genet 2002; 104(8) 1239–1245.

[64] Wongniam S, Somana J, Swangpol S, Seelanun T, Chareonsap P, Chadchawan S, Jenjitikul T. Genetic diversity and species-specific PCR-based markers from AFLP analyses of Thai bananas. Biochemical Systematics and Ecology 2010; 38(3) 416–427.

[65] Ude G, Pillay M, Ogundiwin E, Tenkouano A. Genetic diversity in an African plantain core collection using AFLP and RAPD markers. Theor Appl Genet 2003; 107(2) 248–255.

[66] Lheureux F, Carreel F, Jenny C, Lockhart BEL, Iskra C. 2003. Identification of genetic markers linked to banana streak disease expression in inter-specific Musa hybrids. Theor Appl Genet 2003; 106(4) 594–598.

[67] James AC, Peraza-Echeverria S, Herrera-Valencia V, Martinez O. Application of the amplified length polymorphism (AFLP) and the methylation-sensitive amplification polymorphism (MSAP) techniques for the detection of DNA polymorphism and changes in DNA methylation in micropropagated bananas. In: Banana improvement: Cellular, Molecular Biology, and Induced Mutations, ed. Jain SM, Swennen R. Science Publishers, Enfield (NH) USA & Plymouth, UK, 2004, p 97-110.

[68] Vroh-Bi I, Anagbogu C, Nnadi S, Tenkouano A. Genomic characterization of natural and somaclonal variations in bananas (*Musa* spp.). Plant Mol Biol Rep 2011; 29(2) 440–448.

[69] Youssef M, Ku-Cauich R, James A, Escobedo-Gracia Medrano RM. Genetic analysis of somatic embryogenesis derived plants in banana. Assiut Journal of Agricultural Sciences 2011; 42: 287-300.http://www.researchgate.net/publication/237101174_Genetic_Analysis_of_Somatic_Embryogenesis_Derived_Plants_in_Banana/file/72e7e51b893e19bb12.pdf (accessed 17 November 2014).

[70] Buetow KH, Edmonson MN, Cassidy AB. 1999. Reliable identification of large numbers of candidate SNPs from public EST data. Nat Genet 21: 323–325. http://www.aun.edu.eg/faculty_agriculture/arabic/journal/english/w_elkhorashy.pdf (accessed 20 October 2014).

[71] Soleimani VD, Baum BR, Johnson DA. Efficient validation of single nucleotide polymorphisms in plants by allele-specific PCR, with an example from barley. Plant Mol Biol Rep 2003; 21(3) 281–288.

[72] Mammadov J, Aggarwal V, Buuyarapu R, Kumpati S. SNP Markers and their impact on plant breeding. International Journal of Plant Genomics 2012: 728398. http://dx.doi.org/10.1155/2012/728398 (accessed 20 October 2014).
[73] Wenzl P, Carling J, Kudrna D, Jaccoud D, Huttner E, Kleinhofs A, Kilian A. Diversity arrays technology (DArT) for whole-genome profiling of barley. Proc Natl Acad Sci USA 2004; 101(26) 9915-9920.

[74] Kilian A. Towards effective deployment of diversity arrays technology (DArT) in banana genomics and sequencing. Conference Proceedings January 13–17, 2007, Plant and Animal Genomes XV Conference. San Diego, USA. 2007

[75] Amorim EP, Vilarinhos AD, Cohen KO, Amorim VBO, Dos Santos-Serejo JA, Silva SO, Pestana KN, Dos Santos VJ, Paes NS, Monte DC, Dos Reis RV. Genetic diversity of carotenoid-rich bananas evaluated by Diversity Arrays Technology (DArT). Genetics and Molecular Biology 2009; 32(1) 96-103.

[76] Risterucci AM, Hippolyte I, Perrier X, Xia L, Caig V, Huttner E, Kilian A, Glaszmann JC. Development and assessment of diversity arrays technology for high-throughput DNA analyses in Musa. Theor Appl Genet 2009; 119(6) 1093-1103.

[77] Sales EK, Butardo NG, Paniagua HG, Jansen H, Dolezel J. 2001. Assessment of ploidy and genome constitution of some Musa balbisiana cultivars using DArT Markers. Philippine Journal of Crop Science 2011; 36(1) 11-18.

[78] Mbanjo EGN, Tchoumboungang F, Mouelle AS, Oben JE, Nyine M, Dochez C, Ferguson ME, Lorenzen J. Molecular marker-based genetic linkage map of a diploid banana population (Musa acuminata Colla). Euphytica 2012; 188(3) 369-386.

[79] Colbert T, Till BJ, Tompa R, Reynolds S, Steine MN, Yeung AT, McCallum CM, Comai L, Henikoff S. High-throughput screening for induced point mutations. Plant Physiol 2001; 126(2) 480–484.

[80] Till BJ, Zerr T, Bowers E, Greene EA, Comai L, Henikoff S. High-throughput discovery of rare human nucleotide polymorphisms by Ecotilling. Nucleic Acids Res 2006; 34(13) e99.

[81] Comai L, Young K, Till BJ, Reynolds SH, Greene EA, Codomo CA, Enns LC, Johnson JE, Burtner C, Odden AR, Henikoff S. Efficient discovery of DNA polymorphisms in natural populations by Ecotilling. Plant J 2004; 37 (5) 778–786.

[82] Mmeka EC, Adesoye AI, Vroh BI, Ubaoji KI. 2013. Single nucleotide polymorphism (SNP) markers discovery within Musa spp (plantain landraces, AAB genome) for use in beta carotene (Provitamin A) trait mapping. American Journal of Biology and Life Science 2013; 1 (1) 11-19.

[83] Mahendhirana M, Ramirez-Prado JH, Escobedo-Gracia Medrano RM, Canto-Canché B, Tzec-Simá M, Grijalva-Arango R, James-Kay A. Single nucleotide polymorphisms in partial sequences of the gene encoding the large sub-units of ADP-glucose pyrophosphorylase within a representative collection of 10 Musa genotypes. Electronic Journal of Biotechnology 2014; 17 (3) 137–147.

[84] Yunis JJ. 1976. High resolution of human chromosomes. Science 191(4233) 1268-1270.
[85] Doleželová M, Valárik M, Swennen R, Horry JP, Doležel J. Physical mapping of the 18S-25S and 5S ribosomal RNA genes in diploid bananas. Biol Plantarum 1998; 41(4) 497-505.

[86] Osuji JO, Crouch J, Harrison G, Heslop-Harrison JS. Molecular cytogenetics of Musa species, cultivars and hybrids: Location of 18S-5.8S-25S and 5S rDNA and telomere-like sequences. Annals of Botany 1998; 82(2) 243-248.

[87] Harper G, Osuji JO, Heslop-Harrison JS, Hull R. Integration of banana streak badnavirus into the Musa genome: Molecular and cytogenetic evidence. Virology 1999; 255(2) 207-213.

[88] Balint-Kurti PJ, Clendennen SK, Dolezelova M, Valarik M, Dolezel J, Beetham PR. Identification and chromosomal localization of the monkey retrotransposon in Musa sp. Mol Gen Genet 2000; 263(6) 908-915.

[89] Hřibová E, Doleželová M, Doležel J. Localization of BAC clones on mitotic chromosomes of Musa acuminata using fluorescence in situ hybridization. Biologia Plantarum 2008; 52 (3) 445-452.

[90] De Capdeville G, Souza Junior MT, Szinay D, Diniz L, Wijnker E, de Jong H, Swennen R, Kema GHJ, De Jong H. The potential of high-resolution BAC-FISH in banana breeding. Euphytica 2009; 166 (3) 431-443.

[91] Neumann P, Navrátilová A, Koblížková A, Kejnovský E, Hřibová E, Hobza R, Widmer A, Doležel J, Macas J. Plant centromeric retrotransposons: A structural and cytogenetic perspective. Mobile DNA 2011; 2: 4. http://www.mobilednajournal.com/content/2/1/4 (accessed 20 October 2014).

[92] Cızkova J, Hribova E, Humplıkova L, Christelova P, Suchankova P, Dolezel J. Molecular analysis and genomic organization of major DNA satellites in banana (Musa spp.). PLoS ONE 2013; 8(1) e54808. http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0054808 (accessed 20 October 2014).

[93] Schwarzacher T, Leitch AR, Bennett MD, Heslop-Harrison JS. In situ localization of parental genomes in a wide hybrid. Annals of Botany 1989; 64(3) 315–324.

[94] Osuji JO, Crouch J, Harrison G, Heslop-Harrison JS. Identification of the genomic constitution of Musa L. lines (bananas, plantains and hybrids) using molecular cytogenetics. Annals of Botany 1997; 80(6) 787–793.

[95] D’Hont A, Paget-Goy A, Escoute J, Carreel F. The interspecific genome structure of cultivated banana, Musa spp. revealed by genomic DNA in situ hybridization. Theoretical and Applied Genetics 2000; 100(2) 177-183.

[96] D’Hont A. Unraveling the genome structure of polyploids using FISH and GISH; examples of sugarcane and banana. Cytogenet Genome Res 2005; 109 (1-3) 27-33.
[97] Jeridi M, Bakry F, Escoute J, Fondi E, Carreel F, Ferchichi A, D'Hont A, Rodier-Goud M. Homoeologous chromosome pairing between the A and B genomes of *Musa* spp. revealed by genomic in situ hybridization. Annals of Botany 2011; 108(5) 975–981.

[98] Doležel J, Doleželová M, Novák F. Flow cytometric estimation of nuclear DNA amount in diploid bananas (*Musa acuminata* and *M. balbisiana*). Biologia Plantarum 1994; 36(3) 351–357.

[99] Kamaté K, Brown S, Durand P, Bureau JM, De Nay D, Trinh TH. Nuclear DNA content and base composition in 28 taxa of *Musa*. Genome 2001; 44(4) 622–627.

[100] Asif MJ, Mak C, Othman RY. Characterization of indigenous *Musa* species based on flow cytometric analysis of ploidy and nuclear DNA content. Caryologia 2001; 54(2) 161–164.

[101] Bartos J, Alkhimova O, Doleželová M, De Langhe E, Doležel J. Nuclear genome size and genomic distribution of ribosomal DNA in *Musa* and *Ensete* (Musaceae): Taxonomic implications. Cytogenet Genome Res 2005; 109(1-3) 50-57.

[102] Lysak MA, Doleželová M, Horý JP, Sweenen R, Doležel. Flow cytometry analysis of nuclear DNA content in *Musa*. Theor Appl Genet 1999; 98(8) 1344–1350.

[103] De Langhe E, Hřibová E, Carpentier S, Doležel J, Swennen R. Did backcrossing contribute to the origin of hybrid edible bananas? Annals of Botany 2010; 106(6) 849–857.

[104] de Jesus ON, Silva SdO, Amorim EP, Ferreira CF, de Campos JMS, de Gaspari GS, Figueira A. Genetic diversity and population structure of *Musa* accessions in ex situ conservation. BMC Plant Biology 2013; 13: 41. http://www.biomedcentral.com/1471-2229/13/41 (accessed 20 October 2014).

[105] Roux N, Strosse H, Toloza A, Panis B, Dolezel J. Detecting ploidy level instability of banana embryogenic suspension cultures by flow cytometry. In: Jain MS, Swennen R. (eds.) Banana Improvement: Cellular, Molecular, Biology, and Induced Mutations In Sci Publishers Inc, 2004. p251–261.

[106] Roux N, Toloza A, Radeck Z, Zapata-Arias FJ, Doležel J. Rapid detection of aneuploidy in *Musa* using flow cytometry. Plant Cell Reports 2003; 21(5) 483–490.

[107] Escobedo-Gracia Medrano RM, Maldonado-Borges J, Burgos-Tan M, Valadez-González N, Ku-Cauich JR. Using flow cytometry and cytological analyses to assess the genetic stability of somatic embryo-derived plantlets from embryogenic *Musa acuminata* Colla (AA) ssp. *malaccensis* cell suspension cultures. Plant Cell, Tissue and Organ Culture (PCTOC) 2014; 116(2) 175-185.

[108] Mueller UG, Wolfenbarger LL. AFLP genotyping and fingerprinting. TREE 1999; 14(10) 389-394.
[109] Carpentier SC, Witters E, Laukens K, Van Onckelen H, Swennen R, Panis B. Banana (*Musa* spp.) as a model to study the meristem proteome: Acclimation to osmotic stress. Proteomics 2007; 7(1) 92–105.

[110] Yang QS, Wu JH, Li CY, Wei YR, Sheng O, Hu CH, Kuang RB, Huang YH, Peng XX, McCordle JA, Chen W, Yang Y, Rose JK, Zhang S, Yi GJ. Quantitative proteomic analysis reveals that antioxidation mechanisms contribute to cold tolerance in plantain (*Musa paradisiaca* L.; ABB Group) seedlings. Mol Cell Proteomics 2012; 11(12) 853–869.

[111] Carpentier SC, Panis B, Renaut J, Samyn B, Vertommen A, Vanhove AC, Swennen R, Sergeant K. The use of 2D-electrophoresis and de novo sequencing to characterize inter-and intra-cultivar protein polymorphisms in an allopolyploid crop. Phytochemistry 2011; 72(10)1243-1250.

[112] Toledo TT, Nogueira SB, Cordenunsi BR, Gozzo FC, Pilau EJ, Lajolo FM, Oliveira do Nascimento JR. Proteomic analysis of banana fruit reveals proteins that are differentially accumulated during ripening. Postharvest Biology and Technology 2012; 70: 51-58. http://www.sciencedirect.com/science/article/pii/S0925521412000816 (accessed 20 October 2014).

[113] Esteve C, D’Amato A, Marina ML, Garcia MC, Righetti PG. In-depth proteomic analysis of banana (*Musa* spp.) fruit with combinatorial peptide ligand libraries. Electrophoresis 2013; 34(2) 207-214.

[114] Li X, Bai T, Li Y, Ruan X, Li H. 2013. Proteomic analysis of *Fusarium oxysporum* f. sp. *cubense* tropical race 4-inoculated response to Fusarium wilts in the banana root cells. Proteome Science 2013; 11: 41 http://www.proteomesci.com/content/11/1/41 (accessed 20 October 2014).
