Genetic diversity and population structure of *Musa* accessions in *ex situ* conservation

Onildo Nunes de Jesus¹,²,³*, Sebastião de Oliveira e Silva³, Edson Perito Amorim³, Claudia Fortes Ferreira³, José Marcello Salabert de Campos⁴, Gabriela de Gaspari Silva¹ and Antonio Figueira¹*

**Abstract**

**Background:** Banana cultivars are mostly derived from hybridization between wild diploid subspecies of *Musa acuminata* (A genome) and *M. balbisiana* (B genome), and they exhibit various levels of ploidy and genomic constitution. The Embrapa *ex situ* Musa collection contains over 220 accessions, of which only a few have been genetically characterized. Knowledge regarding the genetic relationships and diversity between modern cultivars and wild relatives would assist in conservation and breeding strategies. Our objectives were to determine the genomic constitution based on Internal Transcribed Spacer (ITS) regions polymorphism and the ploidy of all accessions by flow cytometry and to investigate the population structure of the collection using Simple Sequence Repeat (SSR) loci as co-dominant markers based on Structure software, not previously performed in *Musa*.

**Results:** From the 221 accessions analyzed by flow cytometry, the correct ploidy was confirmed or established for 212 (95.9%), whereas digestion of the ITS region confirmed the genomic constitution of 209 (94.6%). Neighbor-joining clustering analysis derived from SSR binary data allowed the detection of two major groups, essentially distinguished by the presence or absence of the B genome, while subgroups were formed according to the genomic composition and commercial classification. The co-dominant nature of SSR was explored to analyze the structure of the population based on a Bayesian approach, detecting 21 subpopulations. Most of the subpopulations were in agreement with the clustering analysis.

**Conclusions:** The data generated by flow cytometry, ITS and SSR supported the hypothesis about the occurrence of homeologue recombination between A and B genomes, leading to discrepancies in the number of sets or portions from each parental genome. These phenomena have been largely disregarded in the evolution of banana, as the "single-step domestication" hypothesis had long predominated. These findings will have an impact in future breeding approaches. Structure analysis enabled the efficient detection of ancestry of recently developed tetraploid hybrids by breeding programs, and for some triploids. However, for the main commercial subgroups, Structure appeared to be less efficient to detect the ancestry in diploid groups, possibly due to sampling restrictions. The possibility of inferring the membership among accessions to correct the effects of genetic structure opens possibilities for its use in marker-assisted selection by association mapping.

**Keywords:** Association mapping, Banana, Evolution, Flow cytometry, Internal transcribed spacer, Microsatellite, Simple sequence repeat, Structure
Background

Cultivated bananas and plantains (Musa spp.) originated in Southeast Asia and the Western Pacific [1,2]. From the center of origin, Musa spp. was introduced into Africa in ancient times and taken by European explorers to the Americas and other parts of the world [3,4]. Currently, bananas and plantains (hereafter jointly called bananas) are widely cultivated in tropical and subtropical regions as important staple foods and commodities in many countries [5].

The large majority of banana cultivars are derived from natural crosses between wild seeded diploid subspecies of M. acuminata Colla (A genome) and M. balbisiana Colla (B genome) [6]. Most of modern cultivars contain genome combinations with various levels of ploidy, such as diploid (AA; BB; or AB; 2n = 2x = 22); triploid (AAA; AAB; or ABB; 2n = 3x = 33); and tetraploid (AAAA; AAAB; AABB; or ABBB; 2n = 4x = 44) [6]. It is not well established how wild bananas became domesticated, but it is possible that the accumulation of sterility and acquisition of parthenocarpy with the increase of pulp mass and the absence of seeds, followed by human selection, gave rise to the modern predominantly sterile cultivars [7-10].

There are a limited number of ex situ conservation collections in the world (http://www.crop-diversity.org/banana/) and even fewer breeding programs associated with an important collection. One of these rare examples is the germplasm collection maintained at ‘Embrapa Mandioca Fruticultura’ Center, located at Cruz das Almas, Bahia, Brazil (12°39’59"S; 39°06’00"W). This ex situ collection, with over 220 individual accessions, is derived from the efforts begun in 1981 by the late Dr. Kenneth Shepherd, who used his significant personal networking and credibility with international organizations to obtain and introduce Musa spp. germplasm from various countries [11]. Despite the fact that a wide range of genetic resources is maintained, only a few accessions have been used in the breeding program, possibly because of the lack of characterization and genetic identity.

The precise determination of the ploidy and genomic composition of the accessions are of great interest to define hybridization programs, as the combination of these two genomes (A and B) defines the agronomical attributes (for e.g., yield; resistance to biotic factors) as well as the fruit flavor and quality of the resulting hybrid plants [12-14]. In addition, estimation of genetic diversity and genetic relationships among the various wild and cultivated accessions will help to develop novel approaches for breeding and assist long-term conservation strategies.

To determine ploidy in Musa spp., chromosome counting [15], estimation of the stomata size and density, or measurement of the pollen grain sizes have been employed [16], whereas for the characterization of the genomic composition (genome A and/or B), a set of 15 standard morphological descriptors have been traditionally used [6]. However, these conventional methods are imprecise, suffering from large environmental effects, and they are tedious and time-consuming, and not applicable on a large scale. Flow cytometry is a quick method that is able to detect small variations in DNA content and efficient for determining ploidy level in Musa spp. [17-19]. To determine the genomic composition of the Musa genus, PCR-RFLP markers based on the rDNA region developed by Nwakanma et al. [20] appeared to be effective [21], but the results are limited in terms of the ability to estimate the genetic diversity. On the other hand, simple sequence repeat (SSR) loci with genome-specific alleles [22,23] offer the possibility to identify genomic composition and to estimate the genetic diversity and relationships among accessions from an ex situ conservation collection.

Despite the multiallelic and highly informative nature of microsatellite (SSR) loci, the allelic information in Musa had usually been converted into binary data due to the difficulty in establishing allelic relations between heterozygous genotypes with distinct levels of ploidy [9,21,22,24-29] and polysomic inheritance [29]. The exploration of the co-dominant nature of SSR loci using Bayesian models implemented using the software Structure [30-32] might enable new perspectives of establishing allelic relationships between various accessions to infer about ancestry between cultivars and wild accessions and M. acuminata subspecies. The determination of the genetic structure in ex situ collections is important to determine the genetic relationships [11,33] and to establish core collections [34]. Further, the use of Structure would enable the estimation of a membership matrix among the accessions, adopted in association mapping models [35] to correct the genetic structuring that leads to false associations (false positives). Association mapping is an approach particularly well suited for Musa spp., because non-related individuals can be sampled in a population, such as an ex situ germplasm collection or collections of elite varieties [36-38], without the requirement to develop segregating populations, limited in Musa by sterility, incompatibility [39], low viability of the hybrids due to chromosomal aberrations, and segregation of unviable gene alleles [40,41].

Therefore, the objectives of this study were (i) to characterize the accessions of the ex situ conservation collection in Brazil regarding ploidy and the genomic constitution by flow cytometry and PCR-RFLP; and (ii) to establish the genetic relationships by exploring the co-dominant nature of the SSR loci using the Bayesian model implemented on Structure.
Methods

Plant material
A total of 224 accessions of the Musa genus were analyzed, including wild and cultivated materials with apparent diverse ploidy and genomic constitution (Table 1; Additional file 1: Table S1). The only passport information available was the origin of the accessions, with a presumed genomic constitution. Classification of the banana accessions as members of subgroups (such as ‘Pome’; ‘Silk’; and ‘Cavendish’) had previously been performed by breeders. Other information, such as the subspecies or subgroup, was obtained from the Musa Germplasm Information System (http://www.cropdiversity.org/banana/) [42].

Flow cytometry analyses
To determine the ploidy, approximately 20 to 30 mg of fresh young healthy leaf tissue from each sample, in addition to the same amount of internal standard Pisum sativum [43], were finely chopped with a blade in a Petri dish containing appropriate buffer [44] to lyse the cells and release the nuclei into the suspension. The nuclei suspension was then filtered through a 50 μm screen and stained with 25 μL of 1 mg mL^(-1) propidium iodide, followed by the addition of 5 μL of RNase solution (100 μg mL^(-1)). Each accession was represented by samples from three individual with one leaf each. For each sample, at least 10,000 nuclei were analyzed using a FACS Calibur flow cytometer (Becton Dickinson & Co.; San Jose, CA, USA), and histograms with the nuclei counts and fluorescence values were obtained using the software CellQuest (Becton Dickinson). Statistics for DNA content were estimated using WinMDI 2.8 (http://fac.scripps.edu/software.html). The DNA content was expressed in pg (2C), estimated based on the P. sativum standard as 2C = 9.09 pg.

Amplification of the internal transcribed spacers (ITS) for PCR-RFLP
The ITS1-5.8S-ITS2 regions of the nuclear ribosomal gene were amplified using the primers ITS1 and ITS4 [45] for the PCR-RFLP method [20]. The amplification reaction (with a final volume of 25 μL and 25 ng genomic DNA) and cycling conditions were identical as proposed by [20], except for primer concentration (0.2 μM of each primer). Five μL of each reaction were used to confirm the amplification by gel electrophoresis. The remaining 20 μL were then digested with 2 U Rsal (Fermentas), after adding 2.5 μL 1X Tango buffer, for 3 h at 37°C and visualized by 2% agarose gel electrophoresis in 0.5X TBE (90 mM Tris; 90 mM boric acid; 2.5 mM EDTA, pH 8.3) ran for 2 h at 4 V cm^-1.

To discriminate mixtures of genomes at various dosages, the profiles of fragments and band intensities were initially established by sequential mixtures of DNA samples from the M. acuminata (AA; ‘Calcutta 4’) and the M. balbisiana (BB; ‘Butuhan’) genomes to obtain various artificial combinations of genomes. In a first assay, equimolar amounts of DNA from AA and BB were combined in the following molar proportions: 1AA:2BB; 1AA:1BB; 2AA:1BB; and 3AA:1BB to simulate ABB, AB, AAB, and AAAB, respectively. For the second assay, the ratios 2AA:1BB; 1AA:1BB; 1AA:2BB; and 1AA:3BB were prepared to simulate AAB, AB, ABB and AABB genotypes, respectively. Accessions 20 (ABB); 53 (AAB); 84 (AAAB); and 142 (AA) with known genomic constitutions (Additional file 1: Table S1) were used as positive controls for both assays (Figure 1).

Analyses of SSR loci
A total of 21 SSR loci were tested (Additional file 1: Table S2), including two loci from the ‘Ma’ series [46]; three from the ‘AGMI’ series [47]; four ‘Mb’ locus derived from M. balbisiana [48]; eight derived from the M. acuminata commercial cultivar ‘Ouro’(AA) (MaO) [23]; and four new loci, being two from ‘Ouro’ (MaOCEN) and another two from M. acuminata ‘Calcutta 4’ (MaC-CEN). The amplification reactions contained 25 ng of DNA; 1.5 mM MgCl_2; 100 μM of each dNTPs; 0.2 μM of each primer and 1.2 U Taq polymerase in 1x PCR buffer (Fermentas) in a final volume of 10 μL. The amplifications were conducted using a touchdown cycle [23]. The loci were analyzed in an automatic DNA analyzer, and the amplification reactions were conducted for each locus separately, each with a forward primer containing one of the three additional tail sequences [49] equivalent to a fluorescent primer that was at a concentration of 0.02 μM. An aliquot of 1 μL of each amplification reaction for each one of the three fluorescence of each individual was mixed with 12 μL of Hi-Di formamide (Applied Biosystems) and 0.5 μL of the ROX-500 size standard (35–500 bp) (Applied Biosystems) at an original concentration of 8 nM. This mixture was then denatured at 94°C for 5 min and kept on ice before injection. The samples were loaded into an ABI PRISM 310 Genetic Analyzer, and the results were analyzed using a GeneScan and Genotyper (Applied Biosystems).

Statistical analysis of the SSR data
For all accessions (2x; 3x; and 4x), the polymorphic information content (PIC) was estimated for each SSR locus as \( PIC = 2f_i (1 - f_i) \), where \( i \) is the information of the \( i^{th} \) marker; \( f_i \) is the frequency of the amplified allele (presence of a band) and \( (1 - f_i) \) is the frequency of null alleles [50]. PIC was presented as the mean over the various loci. The Marker Index (MI) was estimated as \( MI = PIC \times EMR \), where \( EMR \) is the effective multiplex relation given by the product between the total number
Table 1  *Musa* accessions from the *ex situ* collection of ‘Embrapa Mandioca Fruticultura’ Center (Cruz das Almas, Brazil) with information from passport data, and characterization of genomic constitution by flow cytometry, PCR-RFLP of internal transcribed spacers (ITS) regions, and Simple Sequence Region (SSR) loci

| No | Acession      | Genome | 2C pg | Ploidy | Molecular markers | ITS fragments (bp) | Genotype | SSR  |
|----|---------------|--------|-------|--------|-------------------|-------------------|----------|------|
| 1  | *Musa basjoo* | ES     | 1.23  | 2x     | ++                | +                 | ++       | -    |
| 2  | Pirai         | BB     | 1.22  | 2x     | ++                | +                 | ++       | BB   |
| 3  | Butuhan       | BB     | 1.23  | 2x     | ++                | +                 | ++       | BB   |
| 4  | BB Panama     | BB     | 1.30  | 2x     | ++                | +                 | ++       | BB   |
| 5  | Balbisiana França | BB | 1.27  | 2x     | ++                | +                 | ++       | BB   |
| 6  | Musa Balbisiana | BB | 1.25  | 2x     | ++                | +                 | ++       | BB   |
| 7  | TIP           | ABB    | 1.98  | 3x     | ++                | +                 | +        | AAB  |
| 8  | Saba Honduras | ABB    | 1.93  | 3x     | ++                | +                 | ++       | ABB  |
| 9  | Saba          | ABB    | 1.95  | 3x     | ++                | +                 | ++       | ABB  |
| 10 | Prata Zulu    | ABB    | M     | ?      | ++                | +                 | +        | ABB  |
| 11 | Poteau Nain   | ABB    | 1.89  | 3x     | ++                | +                 | ++       | ABB  |
| 12 | Pelipita      | ABB    | 1.91  | 3x     | ++                | +                 | ++       | ABB  |
| 13 | Namwa Khorn   | ABB    | 1.94  | 3x     | ++                | +                 | ++       | ABB  |
| 14 | Namwa Daeng   | ABB    | 1.90  | 3x     | ++                | +                 | ++       | ABB  |
| 15 | Muisa Tia     | ABB    | 1.93  | 3x     | ++                | +                 | ++       | ABB  |
| 16 | Monthan       | ABB    | 1.94  | 3x     | ++                | +                 | ++       | ABB  |
| 17 | Ice Cream     | ABB    | 1.93  | 3x     | ++                | +                 | ++       | ABB  |
| 18 | Ice Cream     | ABB    | 1.93  | 3x     | ++                | +                 | ++       | ABB  |
| 19 | Gia Hui       | ABB    | 1.90  | 3x     | ++                | +                 | ++       | ABB  |
| 20 | Figo Cinza    | ABB    | 1.86  | 3x     | ++                | +                 | ++       | ABB  |
| 21 | Espermo       | ABB    | M     | ?      | ++                | +                 | ++       | ABB  |
| 22 | Champa Madras | ABB    | 1.98  | 3x     | ++                | +                 | ++       | ABB  |
| 23 | Cachaco       | ABB    | 1.93  | 3x     | ++                | +                 | ++       | ABB  |
| 24 | Cacambou Naine| ABB    | 1.93  | 3x     | ++                | +                 | ++       | ABB  |
| 25 | Bendetta      | ABB    | 1.93  | 3x     | ++                | +                 | ++       | ABB  |
| 26 | Abuperak      | ABB    | 1.94  | 3x     | ++                | +                 | ++       | ABB  |
| 27 | IAC           | AB(H)  | 1.26  | 2x     | ++                | +                 | +        | AB   |
| 28 | Yangambi nº 2 | AAB    | 1.93  | 3x     | +                 | +                 | AAA      | AAB  |
| 29 | Warik         | AAB    | 1.94  | 3x     | +                 | +                 | +        | AAB  |
| 30 | Walha         | AAB    | 1.95  | 3x     | +                 | +                 | +        | AAB  |
| 31 | Ustrali       | AAB    | 1.93  | 3x     | +                 | +                 | +        | AAB  |
| 32 | Umpako        | AAB    | 1.89  | 3x     | +                 | +                 | +        | AAB  |
| 33 | Thap Maero    | AAB    | 1.94  | 3x     | +                 | +                 | +        | AAB  |
| 34 | Trois Vert    | AAB    | 1.90  | 3x     | +                 | +                 | +        | AAB  |
| 35 | Tomnam        | AAB    | 1.94  | 3x     | +                 | +                 | +        | AAB  |
| 36 | Tipo Velhaca  | AAB    | 1.92  | 3x     | +                 | +                 | +        | AAB  |
| 37 | Tip Kham      | AAB    | 1.87  | 3x     | +                 | +                 | +        | AAB  |
| 38 | Thong Ruong   | AAB    | 1.96  | 3x     | +                 | +                 | +        | AAB  |
| 39 | Terrinha      | AAB    | 1.94  | 3x     | +                 | +                 | +        | AAB  |
| 40 | Terra S/ Nome | AAB    | 1.94  | 3x     | +                 | +                 | +        | AAB  |
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| No. | Accession   | Type | Cytotype | ITS 1 | ITS 2 | ITS 3 | SSR 1 | SSR 2 | SSR 3 | SSR 4 | SSR 5 | SSR 6 | SSR 7 |
|-----|-------------|------|----------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 41  | Tai         | ABB  | 1.99     | 3x    | ++    | ++    | ++    | ABB   | ABB   |
| 42  | Sempre Verde| AAB  | 1.94     | 3x    | ++    | +     | +     | AAB   | AAB   |
| 43  | Saney       | AAB  | 1.94     | 3x    | ++    | +     | +     | AAB   | AAB   |
| 44  | Samurá B    | AAB  | 1.89     | 3x    | ++    | +     | +     | AAB   | AAB   |
| 45  | Red Yade    | AAB  | 1.95     | 3x    | ++    | +     | +     | AAB   | AAB   |
| 46  | Pulut       | AAB  | 1.94     | 3x    | ++    | +     | +     | AAB   | AAB   |
| 47  | Pratão      | AAB  | 1.95     | 3x    | ++    | +     | +     | AAB   | AAB   |
| 48  | Prata Sta. Maria | AAB  | 1.94   | 3x    | ++    | +     | +     | AAB   | AAB   |
| 49  | Prata P. Aparada | AAB  | 1.95   | 3x    | ++    | +     | +     | AAB   | AAB   |
| 50  | Prata Maceió | AAB  | 1.93     | 3x    | ++    | +     | +     | AAB   | AAB   |
| 51  | Prata Comum | AAB  | 1.90     | 3x    | ++    | +     | +     | AAB   | AAB   |
| 52  | Prata IAC   | AAB  | 1.99     | 3x    | ++    | +     | +     | AAB   | AAB   |
| 53  | Prata Anã   | AAB  | 1.94     | 3x    | ++    | +     | +     | AAB   | AAB   |
| 54  | Prata Branca| AAB  | 2.29     | 4x    | ++    | +     | +     | AAB   | AAB   |
| 55  | Poovan      | AAB  | 1.94     | 3x    | ++    | +     | +     | AAB   | AAB   |
| 56  | Plantain N. 2| AAB  | ?       | ?     | ++    | +     | +     | ?     | AAB   |
| 57  | Pinha       | AAB  | 1.94     | 3x    | ++    | +     | +     | AAB   | AAB   |
| 58  | Padath      | AAB  | 1.95     | 3x    | ++    | +     | +     | AAB   | AAB   |
| 59  | Pacovan     | AAB  | 1.93     | 3x    | ++    | +     | +     | AAB   | AAB   |
| 60  | N. 113      | AAB  | 1.89     | 3x    | ++    | +     | +     | AAB   | AAB   |
| 61  | Mysore      | AAB  | 1.95     | 3x    | ++    | +     | +     | AAB   | AAB   |
| 62  | Muracho     | AAB  | 1.95     | 3x    | ++    | +     | +     | AAB   | AAB   |
| 63  | Mongolo     | AAB  | 1.98     | 3x    | ++    | +     | +     | AAB   | AAB   |
| 64  | Moenang     | AAB  | 1.99     | 3x    | ++    | +     | +     | AAB   | AAB   |
| 65  | Maçã Caule Roxo| AAB  | 1.90   | 3x    | ++    | +     | +     | AAB   | AAB   |
| 66  | Kune        | AAB  | 1.92     | 3x    | ++    | +     | +     | AAB   | AAB   |
| 67  | Kingala N.1 | AAB  | 1.95     | 3x    | ++    | +     | +     | AAB   | AAB   |
| 68  | Kepok Bung  | AAB  | 1.95     | 3x    | ++    | +     | +     | ABB/AAB | ABB/AAB |
| 69  | Kelat       | AAB  | 1.87     | 3x    | ++    | +     | +     | AAB   | AAB   |
| 70  | Java IAC    | AAB  | 1.99     | 3x    | ++    | +     | +     | AAB   | AAB   |
| 71  | Garoto      | AAB  | 1.93     | 3x    | ++    | +     | +     | AAB   | AAB   |
| 72  | Figue Rose Naine | AAB  | 1.93     | 3x    | ++    | +     | +     | AAB   | AAB   |
| 73  | Eslesno     | AAB  | 1.95     | 3x    | ++    | +     | +     | AAB   | AAB   |
| 74  | Curare Enano| AAB  | 1.99     | 3x    | ++    | +     | +     | AAB   | AAB   |
| 75  | Comprida    | AAB  | 1.98     | 3x    | ++    | +     | +     | AAB   | AAB   |
| 76  | Chifre De Vaca| AAB  | 1.92     | 3x    | ++    | +     | +     | AAB   | AAB   |
| 77  | Adimoo      | AAB  | 1.93     | 3x    | ++    | +     | +     | AAB   | AAB   |
| 78  | AAB S/Nome  | AAB  | 1.95     | 3x    | ++    | +     | +     | AAB   | AAB   |
| 79  | BRS Tropical| AAAB | 2.50     | 4x    | ++    | +     | +     | AAAB  | AAAB  |
| 80  | Preciosa    | AAAB | 1.94     | 3x    | ++    | +     | +     | AAB   | AAAB  |
| 81  | Porp        | AAAB | 2.43     | 4x    | ++    | +     | +     | AAAB  | AAAB  |
| 82  | Platina     | AAAB | 2.56     | 4x    | ++    | +     | +     | AAAB  | AAAB  |
| No. | Accession | Code | Cytot. | Chrom. | Genomic Constit. | Parentage | Variety |
|-----|-----------|------|--------|--------|------------------|-----------|---------|
| 83  | Pacova Ken | AAAB | 2.45   | 4x     | ++               | +         | AAAB    |
| 84  | BRS Platina | AAAB | 2.45   | 4x     | ++               | +         | AAAB    |
| 85  | Ouro Da Mata | AAAB | 2.46   | 4x     | ++               | +         | AAAB    |
| 86  | Ngern      | AAAB | 2.55   | 4x     | ++               | +         | AAAB    |
| 87  | Langka     | AAAB | 2.48   | 4x     | ++               | +         | AAAB    |
| 88  | Garantida  | AAAB | 2.48   | 4x     | ++               | +         | AAAB    |
| 89  | FHIA-21    | AAAB | 2.49   | 4x     | ++               | +         | AAAB    |
| 90  | FHIA-18    | AAAB | 2.48   | 4x     | ++               | +         | AAAB    |
| 91  | FHIA-02    | AAAB/AAAA | 2.40 | 4x | ++ | + | AAAB | AAAB |
| 92  | FHIA-01    | AAAB | 2.49   | 4x     | ++               | +         | AAAB    |
| 93  | IC - 2     | AAAA | 2.49   | 4x     | ++               |           | AAAA    |
| 94  | Calypso    | AAAA | 2.43   | 4x     | ++               |           | AAAA    |
| 95  | Buccaneer  | AAAA | 2.45   | 4x     | ++               |           | AAAA    |
| 96  | Ambrosia   | AAAA | 2.47   | 4x     | ++               |           | AAAA    |
| 97  | Yangambi Km 5 | AAA | 1.92 | 3x | ++ | | AAA | AAA |
| 98  | Wasolay    | AAAA | 1.92   | 3x     | ++               |           | AAAA    |
| 99  | Walebo     | AAAA | 1.98   | 3x     | ++               |           | AAAA    |
| 100 | Valery     | AAAA | 1.98   | 3x     | ++               |           | AAAA    |
| 101 | Umbuk      | AAAA | 1.95   | 3x     | ++               |           | AAAA    |
| 102 | Tugoomomboo | AAA | ? | ? | ++ | ++ | AAAB | AAA |
| 103 | Caipira    | AAAA | 1.98   | 3x     | ++               |           | AAAA    |
| 104 | Towoollee  | AAAA | 1.90   | 3x     | ++               |           | AAAA    |
| 105 | Torp       | AAAA | 1.90   | 3x     | ++               |           | AAAA    |
| 106 | Sári       | AAAA | 1.93   | 3x     | ++               |           | AAAA    |
| 107 | Sapon      | AAAA | 1.93   | 3x     | ++               |           | AAAA    |
| 108 | São Tomé   | AAAA | 1.92   | 3x     | ++               |           | AAAA    |
| 109 | Roomburn   | AAAA | 1.92   | 3x     | ++               |           | AAAA    |
| 110 | Poyo       | AAAA | 1.91   | 3x     | ++               |           | AAAA    |
| 111 | Pirua      | AAAA | 1.91   | 3x     | ++               |           | AAAA    |
| 112 | Pagatow    | AAAA | 1.91   | 3x     | ++               |           | AAAA    |
| 113 | Ouro Mel   | AAAA | 1.92   | 3x     | ++               |           | AAAA    |
| 114 | Orotawa    | AAAA | 1.93   | 3x     | ++               |           | AAAA    |
| 115 | Nancão     | AAAA | 1.94   | 3x     | ++               |           | AAAA    |
| 116 | Nam        | AAAA | 1.94   | 3x     | ++               |           | AAAA    |
| 117 | Muga       | AAAA | M | ? | ++ | A? | AAA | AAA |
| 118 | Morong     | AAAA | 1.92   | 3x     | ++               |           | AAAA    |
| 119 | Markatooa  | AAAA | 1.91   | 3x     | ++               |           | AAAA    |
| 120 | Maída      | AAAA | 1.94   | 3x     | ++               |           | AAAA    |
| 121 | Leite      | AAAA | 1.93   | 3x     | ++               |           | AAAA    |
| 122 | Lacatan    | AAAA | 1.90   | 3x     | ++               |           | AAAA    |
| 123 | Azedinha   | AAB  | 2.36   | 4x     | ++               | +         | AAAB    |
| 124 | Imperial   | AAAA | 1.93   | 3x     | ++               |           | AAAA    |
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| Accession  | Name            | AAA   | AA   | AA   |
|------------|-----------------|-------|------|------|
| 125        | Highgate        | AAA   | 1.91 | ++   | AAA  |
| 126        | Gros Michel     | AAA   | 1.94 | ++   | AAA  |
| 127        | Grande Naine    | AAA   | 1.92 | ++   | AAA  |
| 128        | Dois Cachos     | AAA   | 1.91 | ++   | AAA  |
| 129        | Dodogá          | AAA   | 1.94 | ++   | AAA  |
| 130        | Cocos           | AAA   | 1.95 | ++   | AAA  |
| 131        | Caru Verde      | AAA   | 1.96 | ++   | AAA  |
| 132        | Caru Roxa       | AAA   | 1.95 | ++   | AAA  |
| 133        | Canela          | AAA   | 1.94 | ++   | AAA  |
| 134        | Bakar           | AAA   | 1.91 | ++   | AAA  |
| 135        | Bagul           | AAA   | 1.93 | ++   | AAA  |
| 136        | Amritsagar      | AAA   | 1.93 | ++   | AAA  |
| 137        | Ambei           | AAA   | 1.94 | ++   | AAA  |
| 138        | AAA Desconhecida| AAA   | 1.93 | ++   | AAA/AAB |
| 139        | Zebrinha        | AA(W) | 1.23 | 2x   | AA  |
| 140        | Selangor        | AA(W) | 1.25 | 2x   | AA  |
| 141        | Perak           | AA(W) | 1.26 | 2x   | AA  |
| 142        | Pa Songkla      | AA(W) | 1.23 | 2x   | AA  |
| 143        | Pa Rayong       | AA(W) | 1.26 | 2x   | AA  |
| 144        | Pa Phatthalung  | AA(W) | 1.23 | 2x   | AA  |
| 145        | Pa Musore 3     | AA(W) | 1.25 | 2x   | AA  |
| 146        | Pa Musore 2     | AA(W) | 1.27 | 2x   | AA  |
| 147        | Pa Abissinea    | AA(W) | 1.28 | 2x   | AA  |
| 148        | N.118           | AA(W) | 1.29 | 2x   | AA  |
| 149        | Monyet          | AA(W) | 1.23 | 2x   | AA  |
| 150        | Modok Gier      | AA(W) | 1.25 | 2x   | AA  |
| 151        | Microcarpa      | AA(W) | 1.26 | 2x   | AA  |
| 152        | Malaccensis     | AA(W) | 1.23 | 2x   | AA  |
| 153        | Kraasn Saichon  | AA(W) | 1.22 | 2x   | AA  |
| 154        | Khae            | AA(W) | 1.28 | 2x   | AA  |
| 155        | Jambi           | AA(W) | 1.27 | 2x   | AA  |
| 156        | Cici            | AA(W) | 1.24 | 2x   | AA  |
| 157        | Calcutta 4      | AA(W) | 1.23 | 2x   | AA  |
| 158        | Burmannica      | AA(W) | 1.23 | 2x   | AA  |
| 159        | Buintenzorg     | AA(W) | 1.27 | 2x   | AA  |
| 160        | Birmanie        | AA(W) | 1.28 | 2x   | AA  |
| 161        | M 61            | AA(H) | 1.23 | 2x   | AA  |
| 162        | M 53            | AA(H) | 1.27 | 2x   | AA  |
| 163        | M 48            | AA(H) | 1.28 | 2x   | AA  |
| 164        | F3P4            | AA(H) | 1.23 | 2x   | AA  |
| 165        | F2P2            | AA(H) | 1.25 | 2x   | AA  |
| 166        | Tuugia          | AA(C) | 1.28 | 2x   | AA  |
Table 1 Musa accessions from the *ex situ* collection of 'Embrapa Mandioca Fruticultura' Center (Cruz das Almas, Brazil) with information from passport data, and characterization of genomic constitution by flow cytometry, PCR-RFLP of internal transcribed spacers (ITS) regions, and Simple Sequence Region (SSR) loci (Continued)

| Accession  | Name     | diploid | ploidy | ploidy | Pollen* | Inflorescence* | Fruit* | Seed* |
|------------|-----------|---------|--------|--------|---------|---------------|--------|-------|
| 167        | Tongat    | AA(C)   | 1.23   | 2x     | ++      |               |        |       |
| 168        | Giral     | AAB     | 1.90   | 3x     | ++      | +             | +      | AAB   |
| 169        | Tjau Lagada | AA(C) | 1.23   | 2x     | ++      |               |        |       |
| 170        | Thong Dok Mak | AA(C) | 1.24   | 2x     | ++      |               |        |       |
| 171        | TA        | AA(C)   | 1.24   | 2x     | ++      |               |        |       |
| 172        | Sowmuk    | AA(C)   | 1.26   | 2x     | ++      |               |        |       |
| 173        | SA        | AA(C)   | 1.23   | 2x     | ++      |               |        |       |
| 174        | S/N. 2    | AA(C)   | 1.25   | 2x     | ++      |               |        |       |
| 175        | Raja Uter | AA(C)   | 1.25   | 2x     | ++      |               |        |       |
| 176        | Pipit     | AA(C)   | 1.28   | 2x     | ++      |               |        |       |
| 177        | Ouro      | AA(C)   | 1.23   | 2x     | ++      |               |        |       |
| 178        | Niyarma Yik | AA(C) | 1.28   | 2x     | ++      |               |        |       |
| 179        | NBF 9     | AA(C)   | 1.27   | 2x     | ++      |               |        |       |
| 180        | NBA 14    | AA(C)   | 1.23   | 2x     | ++      |               |        |       |
| 181        | Mangana   | AA(C)   | 1.23   | 2x     | ++      |               |        |       |
| 182        | Mambee Thu | AA(C) | 1.27   | 2x     | ++      |               |        |       |
| 183        | Malbut    | AA(C)   | M      | ?      | ++      |               |        |       |
| 184        | Lidi      | AA(C)   | 1.27   | 2x     | ++      |               |        |       |
| 185        | Khi Maeo  | AA(C)   | 1.27   | 2x     | ++      |               |        |       |
| 186        | Khai Nai On | AA(C) | 1.27   | 2x     | ++      |               |        |       |
| 187        | Khai      | AA(C)   | 1.23   | 2x     | ++      |               |        |       |
| 188        | Jari Buaya | AA(C) | 1.25   | 2x     | ++      |               |        |       |
| 189        | Jaran     | AA(C)   | 1.26   | 2x     | ++      |               |        |       |
| 190        | Fako Fako | AA(C)   | 1.28   | 2x     | ++      |               |        |       |
| 191        | Berlin    | AA(C)   | 1.23   | 2x     | ++      |               |        |       |
| 192        | Babi Yadefana | AA(C) | 1.26   | 2x     | ++      |               |        |       |
| 193        | Prata Manteiga | AAB | 1.92   | 3x     | ++      |               |        |       |
| 194        | Borneo    | AA (W)  | 1.25   | 2x     | ++      |               |        |       |
| 195        | Madu      | AA      | 1.28   | 2x     | ++      | ++            | +      | AB?   |
| 196        | Prata Maçã | AAAB   | 2.46   | 4x     | ++      | +             | +      | AAB   |
| 197        | Verde     | AAB     | 1.90   | 3x     | ++      | +             | +      | AAB   |
| 198        | Prata Anã 2 | AAB | 1.90   | 3x     | ++      | +             | +      | AAB   |
| 199        | Prata Anã 3 | AAB | 1.91   | 3x     | ++      | +             | +      | AAB   |
| 200        | Pacovan Ken-? | AAAB | 2.46   | 4x     | ++      | +             | +      | AAB   |
| 201        | Pitogo    | ABB     | 1.24   | 2x     | ++      | ++            | +      | AB?   |
| 202        | Pacha Nadan | AB | 1.97   | 3x     | ++      | +             | +      | AAB   |
| 203        | Njok Kon  | AAB     | 1.94   | 3x     | ++      | ++            | +      | ABB   |
| 204        | Marmelo   | YNI     | 1.25   | 2x     | ++      | ++            | +      | AB?   |
| 205        | Lareina BT100 | YNI | 1.30   | 2x     | ++      |               |        | AAA   |
| 206        | Pisang Ceylan | AAB | ?      | ?      | ++      | +             | +      | AAB   |
| 207        | Pisang Nangka | AA | 1.28   | 2x     | ++      |               |        |       |
| 208        | Willians  | AAA     | 1.92   | 3x     | ++      |               |        |       |
Table 1  *Musa* accessions from the *ex situ* collection of ‘Embrapa Mandioca Fruticultura’ Center (Cruz das Almas, Brazil) with information from passport data, and characterization of genomic constitution by flow cytometry, PCR-RFLP of internal transcribed spacers (ITS) regions, and Simple Sequence Region (SSR) loci (Continued)

| Accession   | Origin   | Ploidy | Percent | cv (%) | Assay I | Assay II |
|-------------|----------|--------|---------|--------|---------|---------|
| PV42-114    | AAAB     | 2.28   | ++      | AAAB   | AAAB    |
| PV03-76     | AAAB     | 2.29   | ++      | AAAB   | AAAB    |
| Khai Prae   | AA       | 1.23   | ++      | AA     | AA      |
| Pitu        | AA       | 1.23   | ++      | AA     | AA      |
| Paka IV     | AA       | 1.26   | ++      | AA     | AA      |
| Ido 110     | AA       | 1.28   | ++      | AA     | AA      |
| P.Kermain   | NI       | 1.23   | ++      | AA     | AA      |
| P.Serum     | AA       | 1.24   | ++      | AA     | AA      |
| Pisang Mas  | AA       | 1.25   | ++      | AA     | AA      |
| Uw Ati      | AA?      | ?      | ++      | AA?    | AA      |
| Diplóide Bélgica | AA | 1.24 | ++ | ++ | BB | BB |
| BB França   | BB       | 1.27   | ++      | ++     | BB      | BB      |
| BB IAC      | BB       | 1.28   | ++      | ++     | BB      | BB      |
| Musa laterita | Musa | 1.28 | ++ | - | - | - |
| Tambi       | AAA      | 1.92   | 3x      | ++     | AAA     | AAA     |
| Musa royal  | Musa     | 1.27   | 2x      | ++     | -       | -       |

**Assay I:** AA (1AA:0BB); BB (0AA:1BB); ABB (1AA:2BB); AB (1AA:1BB); AAB (2AA:1BB); AAAB (3AA:1BB); ABB, AAB, AAAB, AA. **Assay II:** AA (1AA:0BB); BB (0AA:1BB); AAB (2AA:1BB); AB (1AA:1BB); ABB (1AA:2BB); AAAB (1AA:3BB); ABB; AAB; AAAB; AA. M: 100 bp ladder.

**Figure 1** Restriction profiles of the amplified ITS regions (negative picture). Assays to verify competition between doses of the A and/or B genomes for amplification and digestion of a rDNA region in *Musa*. **Assay I:** AA (1AA:0BB); BB (0AA:1BB); ABB (1AA:2BB); AB (1AA:1BB); AAB (2AA:1BB); AAAB (3AA:1BB); ABB, AAB, AAAB, AA. **Assay II:** AA (1AA:0BB); BB (0AA:1BB); AAB (2AA:1BB); AB (1AA:1BB); ABB (1AA:2BB); AAAB (1AA:3BB); ABB; AAB; AAAB; AA. M: 100 bp ladder.

++: fragment with strong signal; +: fragment with weak signal.
Information based on the *Musa* MGIS database [42].
NI: No information.
M: Mixoploidy; C: cultivated; W: wild; H: hybrid.
?: undefined name or ploidy, or unresolved genomic constitution.
Genome composition based on groups formed based on clustering analysis derived from SSR data (Figure 3).
of fragments (Na) and the fraction of polymorphic bands (β = number of polymorphic bands/total number of bands) [51]. To compare diploids, the PIC and mean heterozygosity (Ho) were estimated using PowerMarker v3.25 [52].

Two approaches were adopted to investigate the genetic structure and diversity among the accessions. In the first case, polymorphisms were treated as binary data (presence or absence). The binary data were then used to obtain a dissimilarity matrix using the Jaccard index employing the software Genes [53]. The matrix was used to run a cluster analysis based on Neighbor-joining [54] using Mega 4.0 [55]. To determine the genetic structure among accessions, a second approach based on the co-dominant nature of the marker was adopted using the Bayesian method implemented using the software Structure 2.3.2, assuming that some fraction of the genome of each individual came from k populations, characterized by their allelic frequencies [31,56]. The input file was prepared accordingly to multiple ploidies [32] with adaptations. As the tetraploid accessions revealed a similar pattern of alleles as triploids, with the majority of the loci displaying from 1 to 3 alleles, all accessions were standardized as triploid. For diploid accessions with more than two alleles and triploids with more than three alleles, the locus with excess alleles was removed from the analysis and considered missing. For the triploid and tetraploid accessions revealing only two alleles, it was necessary to consider one allele as duplicated. Two alternative matrices were generated: one considering the smallest allele in terms of base pairs as duplicated, and the other, based on the largest allele, as duplicated in the matrix. In this way, a triploid with the allelic profile A1A2A2 (A1 < A2) was considered either as A1A1A2 or A1A2A2, creating two files for analysis (Analysis I and Analysis II, respectively). After determining the number of populations (k), the memberships (matrices q) of Analysis I and Analysis II according to Structure were compared by Pearson correlation as proposed by Jing et al. [57]. Thus, a high correlation value between matrices would suggest a similar genetic structure among the approaches.

The origin of the modern banana cultivars involved intra- and interspecific hybridizations, and the mixture model and allelic frequency correlated was adopted. A burn-in of 150,000, followed by 70,000 Monte Carlo Markov Chain, was used for each k, varying from 2 to 30, with ten runs for each k. The choice of the likely number of populations was performed based on the highest log value of the likelihood (LnP(K)) and using the method developed by Evanno et al. [58].

Results
Ploidy determination by flow cytometry
Leaf samples from each accession were analyzed by flow cytometry to determine ploidy, and the 2C values were estimated in pg (Table 1). The 77 diploid accessions (AA; BB, and Rhodochlamys) presented an average of 2C = 2x = 1.26 pg, ranging from 1.22 to 1.30 pg. The 115 triploids (AAA; AAB; or ABB) displayed an average of 2C = 3x = 1.93 pg, varying from 1.86 to 1.99 pg, whereas the 23 tetraploid accessions (AAAA or AAAB) had a mean of 2C = 4x = 2.45 pg, ranging from 2.28 to 2.56 pg (Table 1). The overall average M. acuminata genome (A) and M. balbisiana (B) was estimated to be 2C = 1.25 pg. The overall coefficient of variation between samples was 3.31%, ranging from 1.23 to 4.56%.

From the 224 accessions evaluated, 221 were from section Musa and three were from section Rhodochlamy. From the Musa section (Table 1; Additional file 1: Table S1), three accessions (204, 205 and 215) had their ploidy defined for the first time, while for another five (54, 80, 123, 201 and 202), the ploidy level was not in agreement with the passport information. For four accessions (56, 102, 206 and 218), it was not possible to determine the ploidy by flow cytometry, whereas five accessions (10, 11, 21, 117 and 183) exhibited mixoploidy (Table 1).

Curiously, accessions 201 (‘Pitogo’) and 204 (‘Marmelo’), classified as diploid by flow cytometry, presented a typical ABB profile by ITS PCR-RFLP (compare lanes 7 and 8, top panel Figure 2). Both accessions were grouped as ABB in the clustering and Structure analyses (Figure 3 and 4 below).

Characterization of the genomic constitution based on ITS-PCR-RFLP
To evaluate whether the method proposed by Nwakanma et al. [20] would enable the discrimination of genomic constitution and ploidy, preliminary assays were carried out using mixtures of DNA samples from the M. acuminata (‘Calcutta 4’) and M. balbisiana (‘Butuhan’) genomes to obtain various artificial combinations of genomes, mimicking the natural ones. In the first assay, an increase in genome dose revealed more intense B-specific bands (350 and 180 bp) for BB, followed by ABB, AB, AAB and AAAB (Figure 1; Assay I). A clear distinction between genome composition was possible for BB, ABB and AB, but not between AAB and AAAB. Similarly, no clear difference between the reference genomes ’Prata Anã‘ (53; AAB) and ’BRS Platina‘ (84; AAAB) was detected (Figure 1). In the second assay, the increasing dose of the B genome did not allow the discrimination between ABB and ABBB (Figure 1; Assay II), but both differed from AAB and AB in the band intensity pattern. Thus, this simulation demonstrated the possibility of
genome constitution discrimination for accessions when the ploidy level had been previously determined.

Amplification of the ITS regions produced a fragment of ~ 700 bp from all 224 accessions and disclosed the expected fragments that characterized the presence of genome A and/or B after digestion with RsaI (Figure 2). From the 224 accessions evaluated, three accessions without previous information (204, 205 and 215) had their genomic constitution defined, while 13 (5.8%) disagreed with the information available about genomic constitution defined based on previous published or characterized by morphological descriptors, including accessions 7, 10, 11, 28, 68, 72, 79, 102, 195, 201, 202, 203, and 219 (Table 1). But from these 13 accessions, only four (28, 79, 102 and 195) appeared to truly demonstrate inconsistencies for the genomic constitutions established by morphology (see below), genomic constitution for accessions 28, 79, 102 and 195 were confirmed as AAB, AAAB, AAB, and AA, respectively.

For the _Musa_ ornamental diploid species represented by _M. basjoo_ (accession 1; Table 1) and the hybrid ‘Royal’ (224), derived from a cross between two species of the section _Rhodochlamys_ (_M. ornata x M. velutina_) [59], a slightly larger fragment than the 350 bp from _M. balbisiana_ and the 530 bp from the _M. acuminata_ fragment were observed. For _M. laterita_ (222; section _Rhodochlamys_), only the typical _M. acuminata_ 530 bp fragment was detected (Figure 2; Table 1).

**SSR and genetic diversity analyses**

Of the 21 loci tested, only five (MaOCEN09, Mb1-69, Mb1-134; Mb1-139; and AGM124-25) failed to amplify consistently, while sixteen SSR loci successfully amplified 182 alleles from the 224 accessions, with an average of 11.5 alleles per locus and a range from 7 to 15 alleles (Additional file 1: Table S2). The discriminatory power of each locus was evaluated by estimating the Polymorphic Information Content (PIC) and the Marker Index (MI). To estimate the PIC, the microsatellite data were converted into a binary format (presence or absence of bands), and therefore, the maximum PIC

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**Figure 2** Restriction profile of the amplified ITS regions from _Musa_ accessions with distinct genomic composition. Amplification products of the ITS1-5.8S-ITS2 region after digestion with RsaI. Accessions 1: ‘Butuhan’; 2: BB‘Panama’; 3: ‘Figue Rose Naine’; 4: ‘Tugoomombo’; 5: ‘Madu’; 6: ‘PachaNadan’; 7: ‘Njok Kon’; 8: ‘Marmelo’; 9: ‘Lareina BT100’; 10: ‘PisangCeylan’; 11: ‘PV42-114’; 12: ‘PV03-76’; 13: ‘Diploide Belgica’; 14: _Musa laterita_; 15: ‘Musa Royal’ (_M. ornata x M. velutina_); 16: ‘Prata Ponta Aparada’; 17: ‘Chifre Vaca’; 18: ‘Pulut’; 19: ‘Pratão’; 20: ‘Pacovan Ken’; 21: ‘Garantida’; 22: ‘Kelat’; 23: ‘Java IAC’; 24: ‘BRS Tropical’. Genomic composition determined by morphology is between parentheses. NI no information on genome composition; (Musa): accession from Rhodochlamys; M: 100 bp ladder marker. Arrows point to fragments of 530 bp specific for A genome (A1); 350 and 180 bp specific for the B genome (B1 and B2).
MaOCEN03 to 3.24 for file 1: Table S2). The MI [51,60] ranged from 1.57 for discriminatory power for the analyzed loci (Additional could be 0.5. The average PIC over 16 loci was 0.20, ranging from 0.16 to 0.30 per locus, indicating a large discriminatory power for the analyzed loci (Additional file 1: Table S2). The MI [51,60] ranged from 1.57 for MaOCEN03 to 3.24 for MaC-CEN04, with an average of 2.28. Considering the mean value of 2.28 as a reference, seven loci (Ma1-17; AGMI 93/94; MaOCEN01; MaOCEN10; MaOCEN14; MaOCEN19; and MaC-CEN04) revealed more diversity in the banana (Additional file 1: Table S2).

Overall, regardless of ploidy, there was a predominance of accessions with two alleles (35.2 to 55.8%), followed by those with one (14.1% to 60.7%); three (3.5 to 32.8%); and only a small fraction with four alleles (0.3 to 15.6%) (Table 2). BB and ABB were the groups with the largest proportion of accessions displaying a single allele (60.7% and 41.9%, respectively), followed by wild (41.3%) and cultivated AA diploids (39.7%). A small fraction of diploid accessions revealed three alleles in cultivated AA (4.2%), BB (4.1%) and wild AA (3.5%).

Accessions with three alleles predominated in triploids (ranging from 18.3% for AAB to 24.3% for AAA) and tetraploids (28.2% for AAAB and 32.8% forAAAA). Few accessions revealed four alleles, mostly were tetraploid hybrids AAAA, with 15.6% of accessions, and AAAB with 3.0% (Table 2).

The relationship among the 20 most frequent alleles in the cultivated AA and BB accessions was investigated in relation to the other genomic and ploidy groups. In general, the most frequent alleles in cultivated AA tended to increase in frequency according to the dose of the A genome (M. acuminata) in the higher ploidy genetic groups (Figure 5A). Similarly, the most frequent alleles in BB decreased proportionally with the reduction in the dose of the B genome (M. balbisiana) in the accessions (Figure 5B).

Cultivated diploids displayed higher mean heterozygosity (62.4%) than the wild diploids (overall average 56.4%). The lowest mean heterozygosity (37.4%) was detected among the M. balbisiana accessions (Additional file 1:
Figure S1), while *M. acuminata* ssp. microcarpa and *M. acuminata* ssp. banksii revealed the largest mean heterozygosity (74% and 71.9%, respectively). The lowest PIC values were detected for the BB accessions and *M. acuminata* ssp. banksii with 34.2% and 36.6%, respectively.

**Clustering analyses of the collection**

Clustering analysis based on Neighbor-joining essentially allowed the detection of two major clusters (Figure 3). The first cluster contained accessions with at least one copy of the B genome, while the second one contained those exclusively with the A genome (Figure 3), with the exception of the AAB accessions 38, 46, and 69, allocated together with genome A accessions (Table 1). Similar grouping was obtained by Structure analysis (Figure 4). Within these two main clusters, sub-clusters were formed with accessions according to genome composition and ploidy level. Within the major A or AB clusters, the main clusters usually corroborated the classification of subgroups, such as ‘Pome’ and derived hybrids; ‘Plantain’; ‘Silk’; ‘Pisang awak’; ‘Bluggoe’; ‘Cavendish’; and ‘Gros Michel’ (Figure 3). Accessions without previous classification were allocated into the main subgroups, allowing novel categorization, while two sub-clusters (denominated ‘unknown’ in Figure 3) require further investigations to define proper subgroup classification. Some accessions did not differ for their SSR profiles, possibly representing duplicated accessions (Figure 3), including accessions 45 and 63 from the ‘Plantain’ subgroup; 15 and 19 from ‘Pisang awak’ (ABB); 11 and 16, and 20, 21, and 24 from ‘Bluggoe’ (ABB).

**Population structure analysis**

The co-dominant nature of the SSR markers was exploited to analyze the structure of the populations using a Bayesian approach. The number of subpopulations (k) tested ranged between 2 and 30 (Figure 6A).
From the 21 groups formed by Structure (Figure 4), five contained only diploid accessions (group I, II, VII, XI, and XIII); six contained triploids or tetraploids (III, IV, VI, XV, XX, and XXI); and the other ten contained mixtures of diploids and triploids with the following (2x:3x:4x) proportion for each group: V (2:2); VIII (6:1); IX (17:7); XII (1:12); XIV (4:3); XVI (1:10); XVII (9:13); XVIII (9:3); and XIX (1:3), and X (1:2: 14:3x: 4:4x).

The membership value (q) for the 21 subpopulations (224 accessions) varied from 0.24 to 0.60 for 41 accessions; 0.61 to 0.80 for 58 accessions; 0.81 to 0.90 for 33 accessions; and greater than 0.90 for 92 accessions (Figure 7A). The largest frequencies of accessions with higher membership (0.90 < q ≤ 0.98) were from the genomic groups ABB; BB and AAA with 87.5%; 62.5%; and 60.5%, respectively (Figure 7E; D; G). On the other hand, the lowest values of membership (q varying from 0.24 to 0.50) were observed for the wild AA diploids [AA(W)], the cultivated diploids [AA(C)], and AAAB, at 30.4%, 23.2%, and 22.2% of accessions, respectively (Figure 7B; C; I). Accessions from the main banana cultivated subgroups (AAA, AAB, ABB) in general exhibited higher membership (0.90 < q) were also encountered, such as 43, 71, 68, 77, and 138 in group XII (‘Saba’ subgroup); accessions 28, 33, 55, 61, 65, and 67 in group XVI (‘Silk’/‘My-sore’ subgroups); 101, 136, and 208 in group X (‘Cavendish’/‘Gros Michel’ subgroups); accessions 30, 52, 59, 193, and 206 in group XX (‘Pome’ subgroup); and 107, 113, 114, and 116 in group XXI. Other triploid accessions with admixture were distributed in groups V; VI; VII; IX; XII; XIV; XV; XVII; and XIX (Figure 4).

Essentially, the triploid/tetraploid groups generated by Structure were identical to the clusters revealed by clustering analysis for the major banana subgroups, such as ‘Pisang awak’ (group III; Figure 3 and 4); ‘Plantain’ (group IV); ‘Cavendish’ and ‘Gros Michel’ (group X); ‘Bluggoe’ (group XVII); ‘Pome’ (group XX), and groups XV, XII and XXI with non-categorized accessions (Figure 3 and 4).

Regarding the diploid accessions analyzed by Structure, all eight *M. balbisiana* accessions were placed in subpopulation XVII, together with 12 ABB accessions (80%) (Figure 4). The *M. acuminata* subspecies (Additional file 1: Table S1) were distributed into various clusters: ssp. *malaccensis* with two accessions at group I; one at VII; three at VIII; and one at XIX; ssp. *errans* with one accession at group XVIII; ssp. *banksi* with 5 accessions at group IX; ssp. *burnhamica/burnamnicoides* with two accessions at XI; and one at XVII; ssp. *siamea* with one accession at VII; two at XI; and one at XVIII; ssp. *zebrina* with one accession at XI; and two at XVIII; and ssp. *microcarpa* with one accession at XI; and two at XVII (Figure 4).

To estimate the approximate number of subpopulations, the maximum estimated value of the logarithm of likelihood (LnP(K)) was used. However, for the evaluated accessions, the value for LnP(K) did not reach a clear plateau, continuing to increase together with the variences between the tested k (Figure 6A). Under these circumstances, the number of subpopulations (k) was projected to be between 16 and 23 (Figure 6A). For k = 20, 21 or 22, there was no large variation for the main groups formed (Figure 6; panels C1, C2 and C3). The method that calculates the second order of likelihood change (Δk) is more sensitive than the previous one to detect the number of subpopulations under these circumstances [58]. Adopting this approach, Δk peaked at k = 21 (Figure 6B).

The two alternative matrices tested (Analysis I and II) presented little differences for genotype allocation and membership values (q). The Pearson correlations (r) between the two distinct alternative approaches was high and significant (p ≤ 0.01) for most groups (r = 0.65 to 0.99), indicating a good adjustment between the co-ancestries that the alternative matrices generated (not shown), except for group VI, which did not show any correspondence between the two analyses (Figure 4). Therefore, only results from Analysis I (see Methods) was used for the purpose of discussion.
Figure 6 Left panel: Selection of the most likely number of subpopulations (k) for the evaluated accessions. A. Mean values of $\text{LnPI}k$ for 10 independent runs for each k. B. Plot of $\Delta k$ values for each k based on the second order change of the likelihood function. Right panel C. Graph for ancestralities for $k = 20$ (C1), $k = 21$ (C2), and $k = 22$ (C3). Group colors are function of colors observed for $k = 21$.

Figure 7 Percent of accessions within intervals of membership ($q$) for all accessions: A) general; B) wild AA [AA(W)]; C) cultivated AA [AA(C)]; D) BB; E) ABB; F) AAB; G) AAA; H) AAAA; and I) AAAB.
Diploid accessions were highly heterogeneous (mixture), and their ancestry remained restricted to other group of diploids, except for accessions 161, 162, 183 and 195, which exhibited ancestry with group XXI of AAA triploids, and BB ‘IAC’ (221) with ancestry to group III of the subgroup ‘Pisang awak’ (ABB) (Figure 4).

Discussion
Characterization of ploidy and genomic constitution
Flow cytometry was used to define the genome size (2C content) and the ploidy level of 224 accessions. From the 221 section Musa accessions, only five (2.3%) presented conflicting results with the passport data. Similar discrepancy between estimation of ploidy by morphological characterization and flow cytometry had been reported [61,62]. Previously, it was believed that nuclear DNA content would be a good predictor of genomic constitution [63], as the BB genome was thought to be on average 12% smaller than the AA genome [64]. However, in our study the estimated size of genome A or B did not differ among the various ploidy and genomic groups, and therefore, estimating C values by flow cytometry alone could not distinguish the genomic constitution. The predicting value of genomic constitution might be affected by minute differences in the size of individual A and B genomes; variation in the number of sets of chromosomes from distinct genomes in triploids or tetraploids, including the occurrence of aneuploids [65]; the involvement of other Musa genomes, such as the presence of S or T genomes (from M. schizocarpa or M. textilis, respectively) in some cultivars [65]; or the lack of additiveness of genome size, caused by recombination, resulting in different proportions of genomes A or B [66,67].

Determination of genomic constitution by molecular markers has long been sought, with attempts to use RAPD [68] or SSR [23,28,47,69,70], but with limited precision to determine the genome dosage. When we evaluated the ITS PCR-RFLP approach using standard cultivars, it was possible to identify all expected digested fragments, except the smallest one (50 bp) reported by Nwakanma et al. [20], which was not predicted by in silico digestion (not shown). Simulating the various A and B genome constitution and dosages indicated the ability to distinguish most genome combinations (BB, AAB, ABB and AB); however AAB could not be distinguished from AAAB, and ABB could not be distinguished from AABB, possibly because of amplification competition. For successful adoption of this approach, knowledge about ploidy is essential [20]. When the ITS PCR-RFLP approach was applied to the whole collection, the genomic constitution of most of the accessions was congruent with the morphologic classification available, as previously reported [21]. Our data indicated that determination of ploidy and genomic constitution using morphologic descriptors can still be considered reliable and useful in most cases, with few exceptions.

Noteworthy, our study revealed that a few accessions presented unexpected behavior, such as ‘Yangambi no.2’ (28) and ‘BRS Tropical’ (79), recognized as AAB and AAAB, respectively, but they exhibited typical AAA and AAAA digestion profiles. These changes in the restriction profiles for ‘Yangambi no.2’ and ‘BRS Tropical’ (a tetraploid hybrid from ‘Yangambi no.2’) might have derived from a variant of the B genome rDNA-locus. Other unusual alleles were identified. For example, ‘Tugoomombo’ (102), considered as AAA, exhibited an ABB PCR-RFLP profile, but it was classified as AAB by clustering analysis, suggesting the occurrence of the B genome allele for the ITS regions in one of the A genomes. The diploid AA ‘Madu’ (195) was indicated to be AB, with a slight change in the restriction fragment size for the B genome. This alteration in size was derived from a change in the RsaI restriction site, later confirmed by sequencing (not shown). This accession also exhibited ancestriality from group VI of AAB and AAAB and XVIII of AAA/AA/AABB (Figure 4). Such results can be related to the occurrence of recombination between the A and B genomes [5,66,67].

Incomplete concerted evolution of ITS sequences observed in Musa hybrids, with the predominance of the original parental alleles, might derive from the absence of sexual reproduction [71]. But the observation of unexpected genotypes, demonstrated by sequence analyses of ITS and ETS regions of rDNA, have pointed to the occurrence of recombination between A and B or between M. acuminata subspecies genomes [5,20,71]. Homeologue pairing and recombination between A and B chromosomes have been actually observed in meiosis of triploid hybrid accessions (AAB and ABB) and an allotetraploid (AABB), and appeared to occur at some frequency [66,70].

Therefore, despite fact that small differences in genome size between M. acuminata and M. balbisiana are recognized, the occurrence of chromosome recombination and multivalent pairing during meiosis, leading to unbalance genome segregation, could generate a continuum in genome sizes among accessions, overlapping differences and impairing the ability to distinguish genomic constitution, as corroborated by our results and others [61,62]. Similarly, our results from PCR RFLP of ITS sequences pointed to the occurrence of recombinants, with the lack of B alleles in two hybrid accessions (AAB and AAAB), or the B genome allele in one of the A genomes for a ABB and AA. Exceptions from the commonly observed incomplete concerted evolution might be associated with the occurrence of sexual reproduction, with meiosis offering the possibility for
homeologue chromosome pairing generating recombinant chromosomes.

**Genetic diversity and clustering analysis**

Sixteen SSR loci were used, revealing 182 alleles, with an average of 11.5, while Christelová et al. [29] detected an average of 15.4 and 14 alleles for 70 diploid and 38 triploid accessions, respectively. Within each ploidy level, the BB genome group presented a higher proportion of accessions with only one allele (homozygosis) as previously reported [7], suggesting a lower genetic variability [72] or the occurrence of a large number of null alleles among the accessions evaluated. Conversely, in cultivated AA accessions, structural heterozygosity [9,73] might justify larger average heterozygosity (62.4%), as well as limited fertility [7,9,73,74], in comparison to the wild diploids (mean 56.4%) (Additional file 1: Figure S1). Previous studies reported heterozygosity of 61% for cultivated AA and 53% for wild diploid accessions based on SSR markers [26], and 61% for cultivated AA and 53% for wild AA using RFLP markers [7].

In our study, it was verified a high proportion (more than 75%) of accessions producing one and two alleles among triploids. Banana triploid cultivars supposedly originated from crosses between non-reduced 2n gamete (restitution of the first or the second division) and reduced n gamete. The formation of non-reduced gametes tends to be higher when two different genomes are involved, such as in the case of AB or AA hybrids between subspecies of *M. acuminata*, as in the cultivated diploids [8,9]. In the case of triploids, they most likely resulted from crosses between heterozygote diploid individuals, such as the cultivated diploids with non-reduced gametes (2n) and another individual (n) carrying a similar allele to one found in the other parent. This hypothesis is supported by the finding that the most frequent alleles found in cultivated AA diploids were observed in increasing frequency in triploid and tetraploid accessions, containing increasing dosages of the *M. acuminata* genome (Figure 5A). The association with cultivated diploids is justified by the presence in cultivated triploids and tetraploids of domestication traits, such as parthenocarpy, sterility and pulp yield [9]. Further, Ortiz [75] investigated the occurrence of non-reduced gametetes and observed that all genotypes that produced 2n gametes also produced fruits by parthenocarpy. Many cultivated triploids presented the same mitochondrial and chloroplast patterns as the cultivated diploids [2]. The *M. acuminata* spp. banksii and *M. a. spp. errans* subspecies, characterized as cultivated diploids, are involved in the development of almost all the cultivated diploids and triploids and parthenocarpic cultivars [2,9,10].

Despite the fact that there was a trend of the participation of AA(C) in some accessions, only 34% (ABB); 39% (AAB); 57% (AAA); 42% (AAAB); and 70% (AAAA) of the accessions contained such alleles. This fact reinforces the previous observation from PCR-RFLP, that the origin of cultivated bananas might have involved recombination events (inter- and intraspecific) and backcrosses between species as well as human intervention. Therefore, a cultivar cannot carry the whole allelic complement from a specific genome A or B [66]. On the other hand, 40% of the alleles present in the eight BB accessions were not detected on ABB, most likely because there is a larger diversity of BB in the formation of ABB. Hippolyte et al. [76] also verified a larger diversity in the B genome of interspecific hybrids, such as ABB, than in BB, suggesting an under-representation of the *M. balbisinana* diversity or the extinction of the parental donor of the B genome in these hybrids. Our study also detected these differences (Additional file 1: Figure S2), but when compared to BB, ABB showed to be more uniform (q > 0.91 for 62.5% and 87.5% of accessions) in the Structure analysis (Figure 7 and 4).

The analysis performed by converting SSR genotyping into binary data and using it to estimate dissimilarities among genotypes revealed a broad genetic variability among *Musa* accessions (Additional file 1: Table S2). SSR loci enabled the separation of accessions into two major clusters (one with at least one copy of the B genome, and the second with those exclusively with the A genome) and according to genomic constitution. Further subdivision, in general, corroborated the classification into banana subgroups ('Pome', 'Plantain','Cavendish','Gros Michel','Bluggoe 'Silk,' and 'Pisang awak'). The most diverse accessions were AA diploids and the less diverse were subgroups of commercial interest, such as 'Pome','Plantain','Cavendish','Gros Michel,' and 'Bluggoe', corroborating previous studies [21,22,28,29,70,77-79]. Banana subgroups are characterized by genotypes that share similar agronomic and fruit quality traits [22], which are believed to originate from a common ancestor, meaning, one single meiotic event and the total lack of a sexual stage in the evolution of these subgroups [78], which justifies the small genetic differences. However, large morphological differences are observed in the field maintained by asexual propagation [78-80]. Epigenetic regulation might help to elucidate phenotypic differences within subgroups not correlated with genetic differences [66,76].

In addition to the contribution regarding the identification of duplicated accessions, definition of the ploidy level and genomic constitution of the accessions, the cluster analysis based on SSR also enabled us to infer to which subgroup the natural triploid accessions belong, according to their allocation in the phenogram. This is a key aspect because it enabled us to separate accessions with similar agronomic attributes. This information can be used by
breeding programs to develop hybrids, which requires certain agronomic or qualitative requisites of the subgroups. However, two clusters (identified as 'unknown'; Figure 3) need to be further investigated for proper categorization.

Population structure and genetic relationships of accessions

To our knowledge, this is the first work to explore the co-dominant nature of the SSR markers in Musa accessions with distinct ploidy levels using the Bayesian model from Structure. Establishing the relationships and evolution of the genomes of modern cultivars, landraces and their wild relatives is of great importance to determine the effect of human intervention on the process of domestication and to understand the geographic dimension of the diversity and the domestication process of wild species [11]. Many species have undergone a long and complex period of domestication and breeding with limited gene flow, it is expected that there is a complex population structure [81,82].

Here, we suggested the separation of 224 accessions into 21 subpopulations (groups) based on the method proposed by Evanno et al. [58]. Such elevated number of groups was expected considering that accessions with proposed by Evanno et al. [58]. Such elevated number of groupings with distinct ploidy levels using the Bayesian model from Structure. Establishing the relationships and evolution of the genomes of modern cultivars, landraces and their wild relatives is of great importance to determine the effect of human intervention on the process of domestication and to understand the geographic dimension of the diversity and the domestication process of wild species [11]. Many species have undergone a long and complex period of domestication and breeding with limited gene flow, it is expected that there is a complex population structure [81,82].

However, accessions from subgroups 'Plantain' (group V), 'Cavendish' and 'Gros Michel (X), and 'Pome' (XX) were highly homogeneous, with a few exceptions.

The subgroup 'Pome' (AAB; group XX; Figure 4) contained the most cultivated accessions in Brazil, and the Embrapa’s breeding program has focused on the development of tetraploids derived from crosses between a partially fertile cultivated female parent (AAB), producing non-reduced gametes (2n), with a male diploid pollen-donor (AA), with novel desirable characters, such as disease resistance. Here, all these 'Pome' tetraploid hybrids from Embrapa demonstrated ancestry to the parental diploids 'M53' (Group IV) or 'Calcutta 4' (Group XI). Similar to what was observed for 'Pome' tetraploid hybrids, all the improved AAAA hybrids from 'Gros Michel' (94, 95, and 96) presented ancestry to diploid groups VII or II. In the 'Pome' subgroup (XX), from five triploids inferred as mixture, only 59 and 193 displayed a clear ancestry to groups XVI and II, respectively. Curiously, ‘FHIA-02’ (91) is reported to be an AAAA hybrid, from a cross between ‘Williams’ and the diploid ‘SH3393’ with characteristics of the ‘Cavendish’ subgroup [83], but here it presented only 22% of the genome as ‘Cavendish’, suggesting to be ‘Pome’ (Table 1; Figure 3 and 4). Other FHIA hybrids, whose diploid parents were probably not represented in this study displayed ancestry in groups X ('Cavendish'/‘Gros Michel’), XVI ('Silk'/‘Mysore’) and XIX (Figure 4).

‘Cavendish’ and ‘Gros Michel’ were separated into two close subgroups in the cluster analysis (Figure 3); however, according to Structure (Figure 4), representative accessions from these subgroups appeared in the same group, most likely because they share common alleles [2,8]. Similar results were also observed using RFLP [8], microsatellite [22], and DArT markers [84], while sharing the same cytotype for organellar genomes as shown based on PCR-RFLP [85]. Hippolyte et al. [76] proposed that accessions from subgroup ‘Cavendish’ and ‘Gros Michel’ are derived from a common 2n gamete donor, and most likely two different, but genetically close, n donors. Raboin et al. [8] proposed the accessions ‘Sa’ and ‘Khainai On’ as the probable n gamete donor for ‘Gros Michel’ subgroups. In our study, two diploids with identical denominations (173 and 186) were allocated to group IX, but only accession 136 (‘Amritsagar’) from group X (‘Cavendish’/‘Gros Michel’) presented ancestry (q ~ 18%) to group IX, which gives support to the proposed diploid origins of subgroup ‘Cavendish’ and ‘Gros Michel’. In addition, the diploid ‘Lareina BT100’ (205) was placed in group X and it could be a potential 2n gamete donor for ‘Cavendish’ and ‘Gros Michel’. Therefore, diploids from group IX and ‘Lareina BT100’ appeared as potentially related parentals of the ‘Cavendish’ and ‘Gros Michel’, which could be used in crossing programs or chromosome manipulations (doubling) to obtain/re-synthesize ‘Gros Michel’/‘Cavendish’ hybrids.
Noteworthy, some AAB and AAA triploid accessions demonstrated ancestry to other groups, containing other accessions with similar genomic constitution. It is known that some hybrids showed various degree of residual fertility and it is possible that their evolution involved episodes of sexual reproduction, as suggested by the backcross hypothesis [66].

Our results indicated that Structure was efficient in the detection of ancestry of recently developed tetraploid hybrids by breeding programs in Brazil (‘Pome’) and Jamaica (‘Gros Michel’) with a defined genealogy, and for some triploid cultivars. However, this approach appeared to be less efficient to detect the ancestry of most of the primeval triploid accessions, which make up the main commercial subgroups (‘Pisang awak’; ‘Gros Michel’; ‘Cavendish’; ‘Pine’; ‘Plantain’). This absence of detection of ancestry might be explained by a series of hypotheses.

One possibility is that potential parental diploids for the main commercial subgroups were under-represented in the collection, such as demonstrated by the absence of ancestry in diploids groups for some recent tetraploid hybrids developed by FHIA evaluated in this study (Figure 4). Secondly, the long and uncertain evolutionary period that these triploid cultivars went through since they originated might have resulted in changes/mutations in loci, which could result in complete elimination or modification of the alleles in one of the parents. The ability to detect ancestry for recently developed tetraploid hybrids is important evidence to support this hypothesis. The process of allopolyploidyization can lead to activation of retrotransposons; elimination and rearrangements of parental chromosomes [86,87], DNA sequence losses, apparently from the largest parental genome [89] and from highly repetitive sequence regions [89]. Such events might have occurred in M. acuminata, with a larger genome [62,63] and more repetitive sequences than M. balbisiana [90]. Thirdly, the limited number of loci used can also be a reason for the lack of precision in identifying the ancestry of commercial accessions, as a large number of loci would increase the chances of finding equivalent alleles in a group of conserved polymorphic loci among the cultivated triploids and the ancestral diploids. For example, other researchers did not find differences between accessions of the ‘Cavendish’ subgroup [22], but differences between the accessions of this subgroup have been identified here and by Christelová et al. [29], most likely because of the larger number of alleles identified per locus.

The relationship between diploids and AAB could have been affected by the potential occurrence of recombinations between homeologue chromosomes with distinct structural organization, contributing to large genetic changes in allopolyploids [88]. Recombinations between the A and B genomes can occur, and it can be frequent in triploid hybrids, while it might lead to unbalanced genome transmission with respect to the parental species [66,67], justifying variations in AAB genomes, morphological expression of A and B characters, and no addictiveness, as hybrids may carry different recombinant A and B chromosomes (e.g. A and B) [66]. Therefore, all these processes, occurring in isolation or combined, especially in M. acuminata subspecies can obstruct the inference of ancestry for most of the triploid accessions.

Concerning diploids, the groups formed by clustering analysis presented distinct behavior as to the one observed for the triploid and tetraploid accessions. In the Structure approach, the groups were defined based on the likelihood probability using allelic frequencies that characterize each population [30], making this method more reliable to evaluate the group of individuals. In our study, a limited number of accessions of the distinct subspecies were analyzed (seven accessions of ssp. malaccensis at groups I, VII, VIII, XIX; one ssp. errans at XVIII; five ssp. banksii at group IX; three ssp. burmannica/burmannicoides at XI, XVIII; four ssp. siamea at VII, XI, XVIII; two ssp. microcarpa at XI, XVIII; and three ssp. zebrina at XI, XVIII), which limit inferences about the relationships among these distinct subspecies. Further, some of these AA diploids can intercross, and the classification in subspecies was merely based on spatial and temporal isolation, and some of the accessions might have an inter-subspecific origin [2].

Despite the limited number of accessions for each subspecies, inferences from previous studies were supported. For instance, the grouping of five ssp. banksii (group IX) accessions with cultivated diploids have been reported [2,84] with a clear distinction from other subspecies [84]. Musa acuminata ssp. banksii originated in Papua New Guinea and the Northern Indonesian islands, geographically isolated from the other subspecies, and it is a preferential autogamous [2]. Accession of this subspecies, presented low average heterozygosity (55.8%) and PIC value (36.6%). These homozygous loci for banksii and the cultivated diploids were also reported by Grapin et al. [73]. When compared with the other subspecies, banksii presented high membership values (Figure 4).

In general, there was a diversified behavior of diploids with accessions of the same subspecies in different groups and/or with different subspecies, as verified for groups XI and XVIII (Figure 4). These two groups contained a few accessions of ssp. burmannica/burmannicoides; ssp. siamea; ssp. microcarpa and ssp. zebrina, corroborating the grouping obtained based on DARt [84], and the closer relationships between ssp. errans and ssp. microcarpa [73]. However, these subspecies demonstrated distinct cyotypes based on PCR-RFLP [85]. Assembling the distinct
subspecies into the same cluster has been reported [2,9,84]. This behavior could be associated with the broad variability that exists within M. acuminata [91] or the presence of many rare alleles in the subspecies [73] that may obscure genetic relationships. Further, differences in markers and methods of analysis, together with distinct accession names [76], and the identification of some accessions as being from a determined subspecies is still questionable [2] makes direct comparison between studies difficult.

Conclusions
The ex situ collection at ‘Embrapa Mandioca Fruticultura’ Center represents an important source of Musa spp. genetic resources. The accessions are characterized according to their agronomic traits, and they have been screened for disease resistance to Black- and Yellow-Sigatoka, Fusarium wilt and Moko, and now their ploidy, genomic constitution and genetic diversity have been established. This study represents an initial effort to define genetic relationships within Musa using Bayesian statistics implemented in Structure, while exploring the co-dominant nature of microsatellites, not previously performed in Musa.

DNA content was believed to be a good predictor of genomic constitution in Musa, but our results confirmed that these small differences are potentially overlapped by the occurrence of homeologue recombination, discrepancies in the number of sets or portions from each parental genome, including aneuploidy. Similarly, detection of unexpected ITS rDNA alleles corroborated the hypothesis about the occurrence of recombination between the A and B genomes or between M. acuminata subspecies genomes. The occurrence of these phenomena has been largely disregarded in the evolution of banana cultivars, as the “single-step domestication” hypothesis had long predominated, and these findings will have an impact in future breeding approaches.

Structure analysis enabled the efficient detection of ancestry of recently developed tetraploid hybrids by breeding programs, and for some triploid cultivars. However, for the main commercial subgroups, Structure appeared to be less efficient to detect the ancestry in diploid groups, possibly either due to diploid under-representation in the collection; limited number of analyzed loci evaluated; or allelic changes during evolution of the subgroups, especially the allopolyploids.

Establishing ancestry and genetic relationships by Structure allowed the identification of diploids from group IX and ‘Lareina BT100’ as potentially related to parentals of the sterile ‘Cavendish’ and ‘Gros Michel’ accessions, which could be used in crossing programs or chromosome manipulations (doubling) to obtain/re-synthesize ‘Gros Michel’/‘Cavendish’ hybrids. The possibility of inferring the membership of the accessions using Bayesian analysis opens possibilities for its use in marker-assisted selection by association mapping by incorporating the effects of the structure (matrix of the membership; q matrix) in the population to control false positives (type I error) [35,92].

With the completion of the Musa genome sequencing [93], together with the development of next-generation sequencing technology, increasing the precision of genomic information will enable an improved definition of the relationships among cultivated bananas and its diploids parental. The evaluation of a larger number of diploid accessions from the various subspecies would allow a better definition of the relationships among diploids and among triploid cultivars, therefore, to use this approach to assist and develop new strategies in breeding programs.

Additional file

Additional file 1: Table S1. Musa accessions from the ex situ collection of ‘Embrapa Mandioca Fruticultura’ Center (Cruz das Almas, Brazil) with original provenance and information on ploidy and genomic composition derived from morphological characterization or information from origin (passport data). Table S2. Loci used for the characterization of the ex situ Musa collection from ‘Embrapa Mandioca Fruticultura’ Center, containing a tail for fluorescent labeling, with number of observed alleles (Na), Polymorphic Information Content (PIC), Markers Index (MI). Underlined regions refer to tail used to label products with fluorescence FAM, HEX, or NED. Figure S1. Mean observed heterozygosity (H), and Polymorphic Information Content (PIC) for all microsatellite loci.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
ONJ, SOS and AF conceived the study, which was the Doctoral project of ONJ. SOS, EPA and CFF maintained and provided material from the ex situ Musa collection, and participated in the interpretation of the data. ONJ and GGS conducted the molecular analyses. JMCs conducted the flow cytometry analyses. ONJ and AF discussed the results and wrote the manuscript with the help of CFF. All authors read and approved the final manuscript.

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Author details
1Centro de Energia Nuclear na Agricultura, Universidade de São Paulo, Av. Centenário, 303, CP 96, Piracicaba, SP 13400-970, Brazil. 2Escola Superior de Agricultura ‘Luiz de Queiroz’, Universidade de São Paulo, Av. Pádua Dias, 11, Piracicaba, SP 13418-900, Brazil. 3EMBRAPA Mandioca Fruticultura, R. Embrapa s/n, Cruz das Almas, BA 44380-000, Brazil. 4Instituto de Ciências Biológicas, Universidade Federal de Juiz de Fora, Campus Martelos, Juiz de Fora, MG 36016-900, Brazil.

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