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Foundation characteristics of edible *Musa* triploids revealed from allelic distribution of SSR markers

I. Hippolyte[1,*], C. Jenny[2], L. Gardes[3], F. Bakry[1], R. Rivallan[1], V. Pomies[1], P. Cubry[1], K. Tomekpe[1,2,3,4], A. M. Risterucci[1], N. Roux[5], M. Rouard[5], E. Arnaud[6], M. Kolesnikova-Allen[7] and X. Perrier[1]

[1]*CIRAD, UMR AGAP, F-34980 Montferrier sur Lez, France,* [2]*CIRAD, UMR AGAP, F-97170 Petit Bourg, Guadeloupe, France,* [3]*CIRAD, UMR CMAEE, F-34398 Montpellier, France,* [4]*CARBAP, BP 832 Douala, Cameroon,* [5]*Bioversity International, Parc Scientifique Agropolis II, 34397 Montpellier Cedex 5, France,* [6]*Bioversity International, Via dei Tre Denari 472/a, 00057 Maccarese (Fiumicino), Rome and* [7]*IITA. PMB 5320, Ibadan, Oyo State, Nigeria*
* For correspondence. E-mail isabelle.hippolyte@cirad.fr

• *Background and Aims* The production of triploid banana and plantain (*Musa* spp.) cultivars with improved characteristics (e.g. greater disease resistance or higher yield), while still preserving the main features of current popular cultivars (e.g. taste and cooking quality), remains a major challenge for *Musa* breeders. In this regard, breeders require a sound knowledge of the lineage of the current sterile triploid cultivars, to select diploid parents that are able to transmit desirable traits, together with a breeding strategy ensuring final triploidization and sterility. Highly polymorphic single sequence repeats (SSRs) are valuable markers for investigating phylogenetic relationships.
• *Methods* Here, the allelic distribution of each of 22 SSR loci across 561 *Musa* accessions is analysed.
• *Key Results and Conclusions* We determine the closest diploid progenitors of the triploid 'Cavendish' and 'Gros Michel' subgroups, valuable information for breeding programmes. Nevertheless, in establishing the likely monoclonal origin of the main edible triploid banana subgroups (i.e. 'Cavendish', 'Plantain' and 'Mutika-Lujugira'), we postulated that the huge phenotypic diversity observed within these subgroups did not result from gamete recombination, but rather from epigenetic regulations. This emphasizes the need to investigate the regulatory mechanisms of genome expression on a unique model in the plant kingdom. We also propose experimental standards to compare additional and independent genotyping data for reference.

Key words: 'Cavendish', lineage, *Musa acuminata*, *Musa balbisiana*, 'Mutika-Lujugira', 'Plantain', phylogeny, polyploidy, SSR, triploid.

INTRODUCTION

Banana (*Musa* spp.) is the number one tropical fruit in production, exceeding 100 million metric tonnes worldwide in 2009, with the 'Cavendish' variety comprising over 50 % of this (Loeillet *et al.*, 2011). Banana provides a staple food for more than 400 million people (Loeillet, 2008). The genus *Musa* is divided into four sections: *Callimusa* and *Australimusa* have a chromosome number of $2n = 20$, while *Eumusa* and *Rhodochlamys* have a chromosome number of $2n = 22$ (Bakry *et al.*, 2009; Christelová *et al.*, 2011).

Most edible cultivars derived from two species of the around 30 in sect. *Eumusa*, namely *Musa acuminata* and *Musa balbisiana*, contributing the A and B genome, respectively. The naturally occurring genotypes are classified in six groups (AA, AAA, AB, AAB, ABB and ABBB) on the basis of their ploidy level and on the basis of a taxonomic scoring method encompassing 15 morphological characters (Simmonds and Shepherd, 1955). The S genome from *Musa schizocarpa* has also been shown in some edible cultivars (Carreel *et al.*, 1994). The T genome from species in sect. *Australimusa* might also be part of rare cultivated accessions.

Wild diploid accessions are seeded whereas edible bananas are seedless, parthenocarpic and vegetatively propagated. Most of these cultivars are triploid, even if some edible AAs are cultivated in Asia, the origin of *Musa* species. These triploid cultivars were clustered in subgroups such as 'Plantain' (AAB), 'Cavendish' (AAA) or 'Mutika-Lujugira' (AAA), based on typical morphological traits (IPGRI-INIBAP(Bioversity)/CIRAD, 1996).

Work on *Musa* has to deal with the complexity of the different ploidy levels of the genotypes, the mixture of intra- and inter-specific hybrids, the sterility which prevents allele shuffling and the vegetative multiplication which fixes some selected genetic events.

Diversity has been analysed over the past 70 years using agro-morphological traits, and these have been standardized in the *Musa* descriptors reference list (IPGRI-INIBAP(Bioversity)/CIRAD, 1996). Data on agro-morphological characterizations of accessions in *Musa* collections are published in the *Musa* Germplasm Information System (IPGRI-INIBAP(Bioversity), 2003). Based on these characteristics, around 1200 cultivars are currently distinguished. Wild diploids are also used for other purposes, such as a source of fibre and feed (Lescot, 2008).

More recently, studies based on polyphenols, isozymes, molecular markers on nuclear and cytoplasmic DNAs, AFLP (amplified fragment length polymorphism), RAPD (random amplification of polymorphic DNA), RFLP (restriction fragment length polymorphism), STMS (sequence tagged microsatellite sites), IRAP (inter-retrotransposon amplified polymorphism), DArT (diversity arrays technology), rRNA, SRAP (sequence-related amplified polymorphism) or retroelement markers and molecular cytogenetics (Gawel *et al.*, 1992; Lanaud *et al.*, 1992; Horry *et al.*, 1997; Carreel *et al.*, 2002; Ude *et al.*, 2002; Wong *et al.*, 2002; D'hont, 2005; Raboin *et al.*, 2005; Heslop-Harrison and Schwarzacher, 2007; Risterucci *et al.*, 2009; Hribova *et al.*, 2011; Youssef *et al.*, 2011) have sustained, and sometimes refined, the agromorphological classification.

In the last decade, single sequence repeat (SSR) markers have been tested to analyse *Musa* diversity for their properties of genetic co-dominance, high reproducibility, high overall mutation rate and high polymorphism (Weber and Wong, 1993; Ellegren, 2002; Vigouroux *et al.*, 2002). Despite the economic importance of *Musa*, the development of these markers remains limited; until recently, fewer than 100 SSR markers were available (Kaemmer *et al.*, 1997; Crouch *et al.*, 1998; Lagoda *et al.*, 1998; Buhariwalla *et al.*, 2005). Only some of these have been used in diversity analysis, and the analyses have been conducted on a limited number of banana samples (Grapin *et al.*, 1998; Creste *et al.*, 2003, 2004; Ning *et al.*, 2007). The present study represents the first attempt to obtain an overview of *Musa* diversity, with more than 500 genotyped accessions. The large amount of data were analysed for different purposes such as understanding the domestication process (Perrier *et al.*, 2009, 2011). Beside these synthetic analyses, which aggregate SSR marker results in a single overall similarity between accessions, here we investigated the resolving power of each marker at the species, subspecies, subgroup and accession level. We analysed the information provided by 22 SSR markers at the interspecific level between the A and B genomes. We compared allelic patterns within and between the main triploid subgroups. Lineages between diploid and triploid accessions were also investigated.

MATERIAL AND METHODS

Plant material

In total, 561 *Musa* accessions were genotyped. The initial germplasm sample consisted of 547 accessions of cultivated and wild bananas classified in section *Eumusa*, which are currently conserved within three significant field collections. Of these accessions, 236 were obtained from CIRAD Neufchateau (Guadeloupe), 192 from IITA (Ibadan, Nigeria) and 119 from CARBAP (Cameroon) (Hippolyte *et al.*, 2011). Each genotype was documented with the genome constitution and subgroup classification according to the current agro-morphological classification (IPGRI-INIBAP(Bioversity), 2003) as well as the ploidy level identified by flow cytometry (Dolezel *et al.*, 1997). In the sample, no duplicated accession (ITC code) was found within a collection or between collections.

The germplasm sample included 186 *M. acuminata*, 12 *M. balbisiana*, 16 interspecific diploid accessions (AB, AS, AT), 287 triploid bananas (AAA, AAB, ABB, BBB, AAS, AAT) and 30 tetraploid accessions (AAAA, AAAB, AABB, ABBT) (Hippolyte *et al.*, 2011). In the genus *Musa*, other wild species from section Eumusa (two *Musa basjoo*, one *Musa schizocarpa)*, from section Callimusa (one *Musa beccarii*, one *Musa coccinea*), from section Rhodoclamys (one *Musa laterita*) and from section Australimusa (one *Musa jackeyi*) were also included.

Ten diploid and triploid accessions from the Comoros islands were added to the initial sampling (ID 550–558 and 563; Hippolyte *et al.*, 2011), as well as three *M. balbisiana* originating from China (ID 560–562) and one *Ensete superbum* (ID 559). They were genotyped independently of the other experiments.

To check the reliability of the genotyping, four 'Cavendish' (germplasm ID: 179, 183, 306, 508), five 'Mutika-Lujugira' (germplasm ID: 109, 115, 116, 487, 508) and 12 'Plantain' (germplasm ID: 91, 96, 113, 195, 312, 356, 359, 419, 428, 429, 430, 491) accessions were genotyped twice with 16 SSR markers (Ma3_90, mMaCir01, mMaCir03, mMaCir07, mMaCir08, mMaCir13, mMaCir152, mMaCir164, mMaCir195, mMaCir196, mMaCir214, mMaCir264, mMaCir27, mMaCir307, mMaCir39, mMaCir40). These experiments were conducted independently of the main analysis.

DNA isolation

Three grams of frozen leaves were ground in liquid nitrogen using a mortar and pestle. Leaf DNA was extracted using the modified Matab method (Risterucci *et al.*, 2000). DNA was re-suspended in PCR-grade water after isopropanol evaporation. DNA of samples from the CIRAD and CARBAP were extracted at CIRAD, while DNA from IITA was extracted there.

SSR markers

Twenty-two SSR primer pairs (Table 1) were selected to analyse the accessions. Twelve SSRs were identified from *M. acuminata* 'Gobusik' (Lagoda *et al.*, 1998), while the ten others were newly defined from *M. balbisiana* 'Pisang Klutuk Wulung' (Hippolyte *et al.*, 2010). The 22 SSRs were distributed across ten of the 11 linkage groups (Hippolyte *et al.*, 2010) (Table 1).

For all SSR loci, the forward primer was designed with a 5′-end M13 extension (5′-CACGACGTTGTAAAACGAC-3′). This extension enabled the generation of fluorescent amplicons after fluorescent dye hybridization.

Ten nanograms of *Musa* DNA was PCR amplified in a 384-well Eppendorf mastercycler with 10 μL final volume of buffer [10 mM Tris-HCl (pH 8), 100 mM KCl, 0·05 % (w/v) gelatin and 2·0 mM MgCl$_2$] containing 0·08 μM of the M13-labelled primer, 0·1 μM of the other primer, 160 μM dNTP, 1 U *Taq* DNA polymerase (Life Technologies, Foster City, CA, USA) and 0·06 μM M13 primer-fluorescent dye IRD700 or IRD800 (Eurofins MWG Operon, Ebersberg, Germany).

TABLE 1. *Characteristics of the 22 SSR markers used for genotyping*

Origin	SSR	EMBL[1]	No. of indels[2]	Min.−max. size (bp)	Al < 1 %[4]	Main allele frequency[5]	Total alleles	LG[6]	Motif
AA Gobusik	Ma1-32	(Crouch *et al.* 1998)	3*x*; 1 bp	208−251	7	0·53	20	4	$(GA)_{17}AA(GA)_8AA(GA)_2$
	Ma3-90	(Crouch *et al.* 1998)	–	123−157	4	0·54	18	3	$(CT)_{11}$
	mMaCIR01	X87262[3]	1*x*; 16 bp	219−295	5	0·39	22	2	$(GA)_{20}$
	mMaCIR03	X87263[3]	3*x*; 1 bp	91−119	4	0·71	14	1	$(GA)_{10}$
	mMaCIR07	X87258[3]	–	127−165	3	0·58	18	1	$(GA)_{13}$
	mMaCIR08	X87264[3]	–	233−279	3	0·85	12	1	$(TC)_6N_{24}(TC)_7$
	mMaCIR13	X90745[3]	–	251−279	0	0·82	12	3	$(GA)_{16}N_{76}(GA)_8$
	mMaCIR24	Z85972[3]	–	218−278	9	0·84	19	5	$(TC)_7$
	mMaCIR27	Z85962[3]	–	212−240	4	067	12	5	$(GA)_9$
	mMaCIR39	Z85970[3]	–	310−350	7	0·77	20	2	$(CA)_5GATA(GA)_5$
	mMaCIR40	Z85977[3]	1 bp	149−187	8	0·62	17	8	$(GA)_{13}$
	mMaCIR45	Z85968[3]	4*x*; 1 bp	253−275	1	0·84	9	10	$(TA)_4CA(CTCGA)_4$
BB Pisang Klutuk Wulung	MMaCIR150	AM950440	1 bp	238−251	0	0·84	05	6	$(CA)_{10}$
	MMaCIR152	AM950442	1 bp	139−175	1	0·41	12	4	$(CTT)_{18}$
	MMaCIR164	AM950454	1*x*; 52 bp + 1*x* 1 bp + 1*x*; 38 bp	236−390	7	0·37	17	4	$(AC)_{14}$
	mMaCIR195	AM950461	–	239−295	11	0·55	21	5	$(GA)_{17}$
	mMaCIR196	AM950462	2*x*; 1 bp	147−173	3	0·70	12	7	$(TA)_4(TC)_{17}(TC)_3$
	mMaCIR214	AM950480	1*x*; 1 bp	96−116	2	0·69	6	–	$(AC)_7$
	mMaCIR231	AM950497	1*x*; 1 bp	219−267	5	0·53	19	–	$(TC)_{10}$
	mMaCIR260	AM950515	–	175−211	6	0·69	10	9	$(TG)_8$
	mMaCIR264	AM950519	3*x*; 1 bp	215−273	7	0·44	18	4	$(CT)_{17}$
	mMaCIR307	AM950533	–	141−153	0	0·82	6	–	$(CA)_6$

[1] EMBL, registration number on EMBL database or publication reference.
[2] Number of alleles deviating from stepwise model (number *x*); size of the observed indels (bp).
[3] Lagoda *et al.* (1998).
[4] Rare alleles with a frequency lower than 1 %.
[5] Highest frequency of an allele observed at this locus.
[6] Linkage groups (Hippolyte *et al.*, 2010).

The amplification was performed on a 384-well plate under touchdown PCR conditions: after an initial denaturation at 94 °C for 60 s, touchdown cycles were performed at a rate of −1 °C per cycle. These initial cycles were followed by 35 cycles at 94 °C for 30 s (lowest T_m −1 °C) for 1 min and 72 °C for 2 min, and a final elongation stage at 72 °C for 5 min.

Gel standards

A classical ladder covering a range of 71–367 bp was added to each gel. We refined the calibration of the allele sizes using accessions within the sample. These accessions belonged to the three triploid subgroups largely represented in the study: AAA 'Cavendish', AAB 'Plantain' and AAA 'Mutika-Lujugira'. We defined these accessions as CPM ('Cavendish', 'Plantain', 'Mutika-Lujugira') standards in the text. These numerous accessions were distributed on the different migration gels of the analysis. They were also added later for genotyping the additional accessions (ID 550–560, ID 562 and 563) (Hippolyte et al., 2011).

Data analysis

The gel pictures were analysed using AFLP-Quantar Pro software (Keygene, Wageningen, the Netherlands), which can record more than two alleles for one individual. Each genotype was evaluated according to the presence (1) and absence (0) of an allele. Two independent readings were performed for each individual.

We observed differences in band intensity, which disturbed the recording of the data, especially in the case of extremely weak bands compared with other band(s) at the same locus. We chose to allocate a presence score to any clearly detectable band. When the intensity of a band at a locus was too weak to assume its presence, the marker was scored as missing data. This decision reinforced the robustness of our data, but consequently increased the number of missing data.

Eleven per cent (1361/12 342) of the data were missing (no amplification, unreadable pattern and null alleles). Accessions displaying more than six missing loci (45) were removed from the analyses (diversity tree, lineage determination and comparison within and between triploid subgroups) and are indicated in the 'accessions' sheet, column missing data (Hippolyte et al., 2011). This brought the proportion of missing data to 8 % (907/11 352 loci).

In addition, accessions displaying doubtful passport data were also excluded from the analyses (Hippolyte et al., 2011).

All genotyping data from the 22 SSR are available on the Generation Challenge Program registry (Hippolyte et al., 2011, 'data_list' sheet). Allele sizes (bp) are provided for each locus and each accession. The independent genotyping data of some new accessions using CPM standards ('Cavendish', 'Mutika-Lujugira' and 'Plantain') are also presented on the GCP registry website (Sample ID B5 is Ensente superbum, B6–B8 are M. balbisiana accessions and samples B9–B18 are diploid and triploid Comorian samples).

Diversity tree construction

A diversity tree (see Fig. 2 below) was constructed using the Darwin software (Perrier and Jacquemoud-Collet, 2006). To deal with the mixture of several levels of ploidy, a specific measure of dissimilarity was defined as the probability of parentage between two accessions, regardless of ploidy level. Based on this dissimilarity, a first tree was build on the subset of AA diploids, using the neighbour-joining (NJ) algorithm (Saitou and Nei, 1987). A tree built on the AA diploids and our target triploids was constructed using a modified version of the NJ tree proposed by the Darwin software. These triploid accessions belong to the subgroups reported in this paper: AAA 'Cavendish', 'Gros Michel', 'Mutika-Lujugira', 'Ibota' and 'Red' subgroups and AAB 'Plantain', 'Pome', 'Nendra Padathi' and 'Nadan' subgroups. This modified version exhibits a solution in the NJ sense, but such that the a priori known topology of a subset is forced. In this case, the topology observed on the AA diploids was used as constraint in order to insert the triploid subgroups in the structure of their parental diploids.

Parental lineage determination

In addition to indices based on allelic frequencies observed in the defined groups, a specific method was developed to detect direct affiliations between triploids and their diploid parents. For a target triploid tested against a pair of potential diploid parents, a marker was regarded as positive when two alleles were found in the first putative parent, regarded as the $2n$ gamete donor, and the third one was in the second parent. Each triploid was successively taken as a target and a kinship score was calculated for each pair of diploids as the proportion of positive markers. High scores indicated that the two diploids were potential parents or, more exactly, were closely related to these parents.

RESULTS

SSR characteristics

Twelve markers displayed dinucleotide motifs. Nine markers exhibited imperfect dinucleotide motifs and one an imperfect trinucleotide motif (Tables 1 and 2).

The number of alleles per marker ranged from five to 22, with a mean of 14. Rare alleles, those present on less than 1 % of the sample (Kimura, 1983), ranged from zero to 11 per marker. The frequency of the most frequent allele for each locus ranged from 0·37 to 0·85 (Table 1).

From the 22 SSR markers, 12 generated the expected pattern, with allele sizes following strictly stepwise the repeated motif (2 bp) (Table 2). Ten other SSR markers displayed both stepwise alleles and alleles with a shift of 1 bp from stepwise alleles, possibly an indel in the flanking or repeated regions. These differences of 1 bp were efficient and in several cases had a clear evolutionary interpretation. For example, the alleles mMaCir03 of 121 and 127 bp were specific to M. balbisiana (these alleles have also been recorded independently with the capillary systems genotyper, C. Billot, CIRAD, 2011, pers. comm.), while the alleles of 120, 122, 126 and 128 bp were found in accessions

TABLE 2. *Allele sizes of each of the 22 SSR markers*

SSR	Alleles (bp)																						
Ma 1–32	227	229	**232**	233	235	237	239	**242**	245	247	249	251	**252**	254	258	260	262	264	266	270			
Ma 3–90	142	144	**146**	148	150	152	154	156	158	160	162	164	166	168	170	172	174	176					
mMaCIR01	238	240	248	250	252	254	256	258	260	262	264	266	268	270	274	276	290	292	298	300	304	310	314
mMaCir03	110	114	116	**119**	120	**121**	122	124	126	**127**	128	130	134	138									
mMaCir07	146	152	154	156	154	156	154	158	160	162	164	166	168	170	172	174	176	178	180	184			
mMaCir08	255	257	259	261	263	265	267	269	271	275	279	285											
mMaCir13	274	276	278	282	284	286	290	292	294	296	298												
mMaCir150	257	259	261	263	267																		
mMaCIR152	158	161	164	**165**	167	170	173	176	179	182	185	191	194										
mMaCIR164	255	293	**298**	313	315	323	329	339	347	401	403	405	407	409	413								
mMaCIR195	258	262	268	270	276	284	286	288	290	292	294	296	298	300	302	304	308	310	312	314			
mMaCIR196	166	168	170	172	174	176	**177**	**179**	180	182	184	192											
mMaCIR214	115	119	123	125	**128**	135																	
mMaCIR231	238	242	244	246	248	**249**	250	252	254	256	260	262	264	270	274	276	278	282	286				
mMaCIR24	235	237	239	241	243	247	249	251	253	255	257	261	267	269	271	273	281	285	289	297			
mMaCIR260	194	200	204	206	208	210	212	214	216	218	220	222	226	230									
mMaCIR264	234	236	238	240	242	244	246	248	250	**251**	252	254	256	**257**	258	260	262	**263**	264	266	268	270	274
mMaCIR27	231	235	237	239	241	243	245	247	249	251	253	259											
mMaCIR307	160	162	164	166	168	172																	
mMaCIR39	329	331	333	335	337	339	341	343	345	347	349	351	353	355	357	359	361	363	365	369			
mMaCIR40	168	172	174	176	**177**	178	180	182	184	186	190	192	196	200	202	204	206						
mMaCIR45	272	**274**	277	279	281	283	**284**	289	**294**														

Bold type indicates alleles differing from the expected stepwise model.
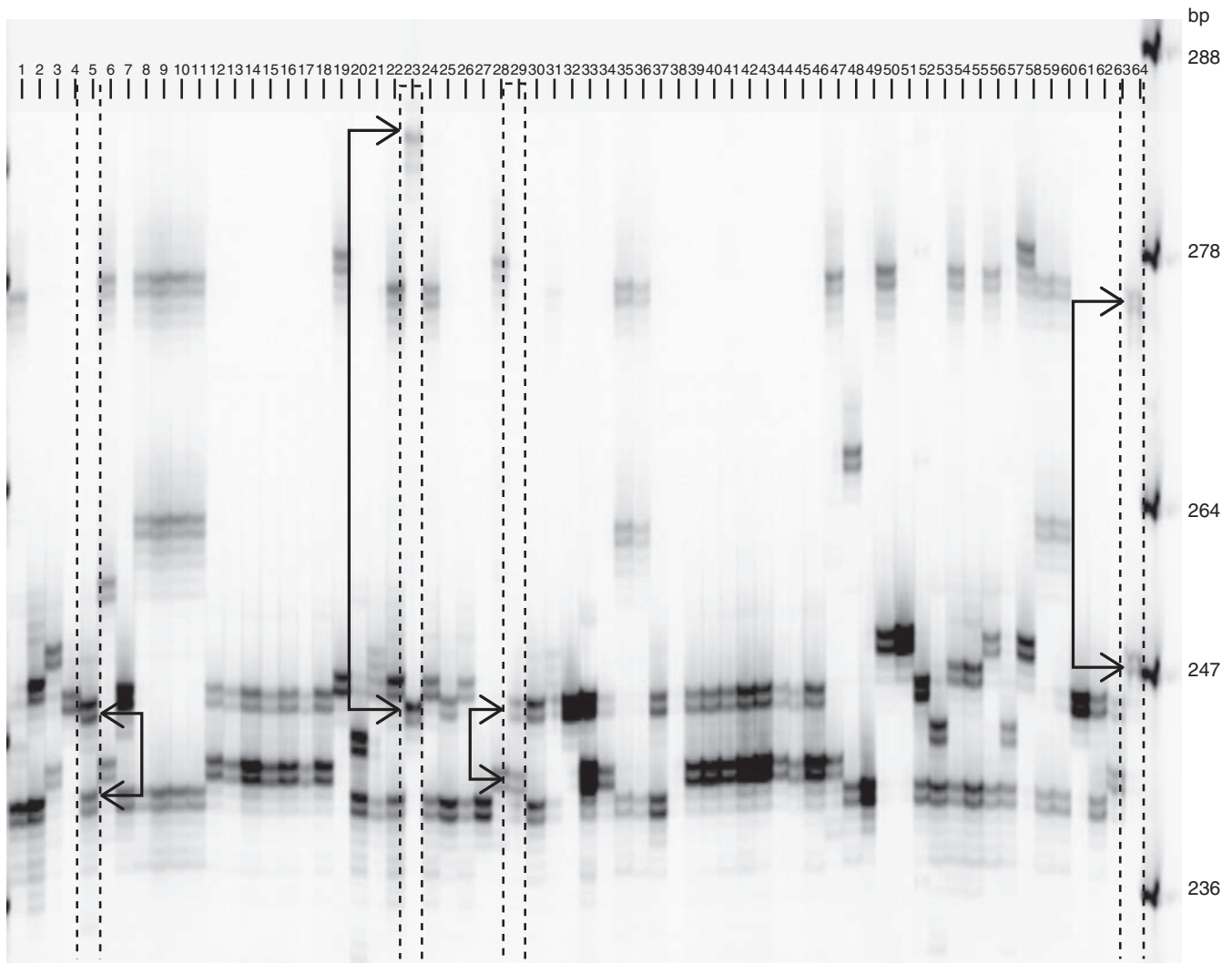
FIG. 1. Migration profile of amplicons generated by mMaCIR231 on different accessions. Lane 1–64 are samples: 1, T1; 2, S. F. (265) (AA); 3, Uwati (AA); 4, Maduranga (ABB); 6, Ouro Mel (AAA); 7, Blue Torres Strait (ABB); 19, No 110 (AA); 20, P. Gigi Buaya (AA); 21, Klue Roi Wee (AAB); 25, Kinkala (ABB); 26, Pitu (AA); 27, P. Tongat (AA); 28, Guyod (11–33) (AA); 30, Ney Poovan (AB); 31, Oura da Mata (AAAB); 32, Singapuri (BB); 37, Kamaramasenge (AB); 47, Gwanhour (AA); 48, Selangor (AA); 49, Hy (302) (AA); 50, Lai THA002 (AAA); 51, Malaccensis Holotype (AA); 52, Sabra (ABB); 53, Morong Princesa (AA); 55, Galeo (AA); 56, Highgate (AAA); 57, Saing Todloh (AA); 58, P. Mulik (AA); 61, Los Banos (BB); 62, T1. 'Cavendish' accessions (AAA) (C standard): 22, Robusta 133; 24, P. Masak Hijau; 54, Valery. 'Mutika-Lujira' accessions (AAA) (M standard): 8, Indemera y' Ymbihire; 9, Kibungo; 10, Makara; 11, Kitawira; 35, Imbogo; 36, Igisahira Gisanzwe; 38, Igihuni; 59, Igitsiri; 60, Bakurura. 'Plantain' accessions (AAB) (P standard): 12, Dominico Rojo (641); 13, Nazika; 14, Motouka 1; 15, Apem Onniaba; 16, Zue Ekon; 17, Harton Maqueno (628); 18, Purple Plantain; 33, Banane Serpent; 34, Currare; 39, O. Ntanga G. M; 40, Mbi Egome; 41, Gabon 4; 42, Diby 2 off-type; 43, Atali Kiogo; 44, Red Plantain Hembra; 45, Moto Ebanga; 46, Msisa; 63, Agbagba. Bold numbers and dotted lines highlight profiles exhibiting unexpected band intensities; alleles are indicated with bold arrows: 5, P. madu (AA); 23, Maia Oa (AA); 29, Laknau (AV-66) (AAB); 64, Red (AAA); L, ladder 98–364 bp.

containing *acuminata* genomes. Among these ten markers, two also displayed larger indels (54 bp for mMaCIR164 and 16 bp for mMaCIR01).

The migration profile of amplicons generated by mMaCIR231 on different accessions showed several unexpected features (Fig. 1). For example, the same band intensity expected for a heterozygous diploid was often not verified, as illustrated by the diploid accessions *M. acuminata* 'Zebrina maia oa' (Fig. 1, lane 23), for which the intensity of the 286-bp allele was weaker than for the 248-bp allele, and by the accession *M. acuminata* 'Pisang madu' (Fig. 1,

lane 5), for which the intensity of the 242-bp allele was weaker than for the 248-bp allele. On the other hand, the triploids AAB 'Laknau' (Fig. 1, lane 29) and AAA 'Red' (Fig. 1, lane 64) displayed two bands of the same intensity, preventing us from determining which of the alleles is double dose or simple dose. Because the intensity of an allele cannot be read in number of doses, the allelic distribution within the triploids displaying two bands remained partly undetermined. This inability to estimate the number of doses of each allele at a locus also hampered estimation of null alleles within the dataset.
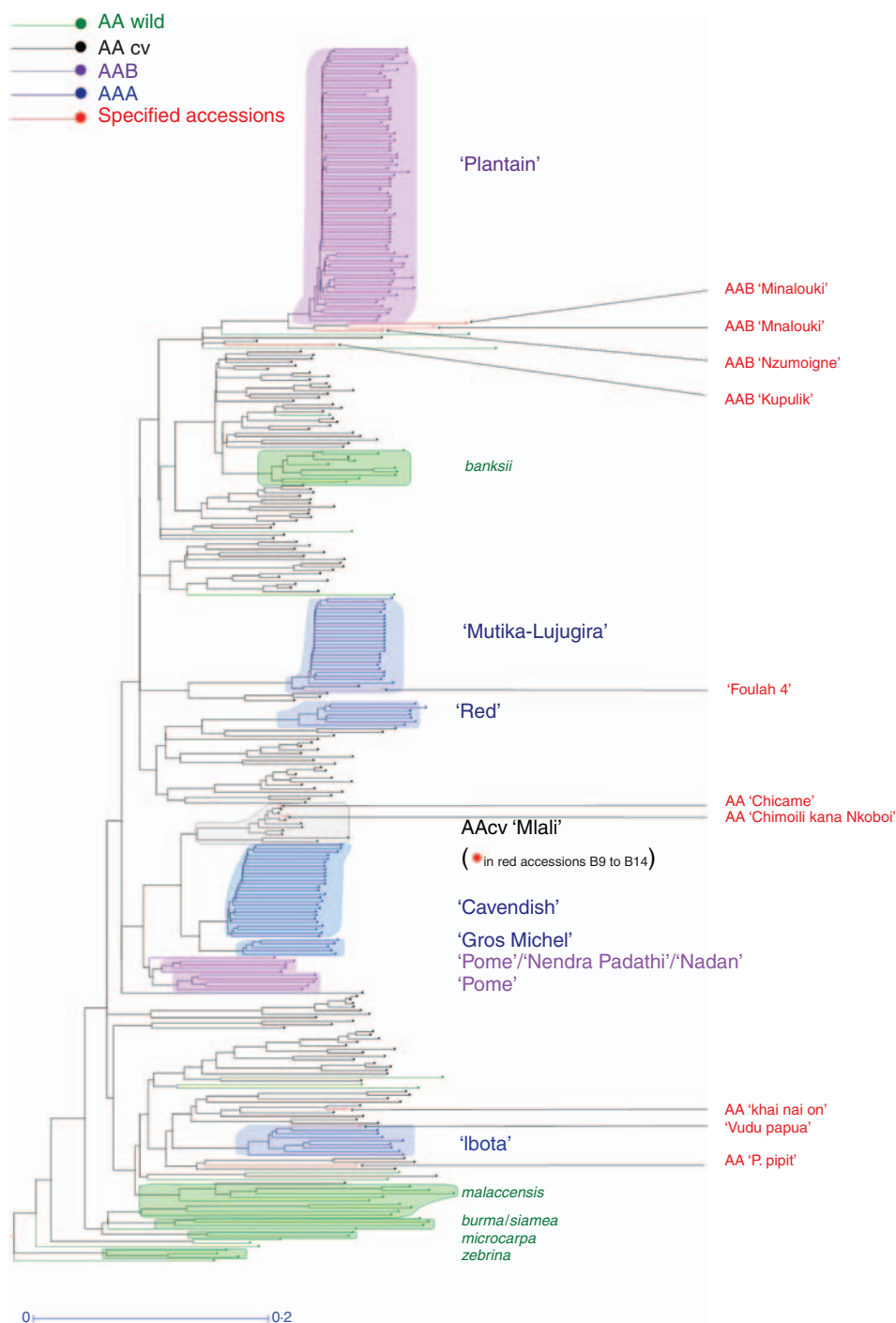
FIG. 2. NJ diversity tree constructed with accessions from AAA subgroups ('Cavendish', 'Gros Michel', 'Ibota', 'Mutika-Lujugira' and 'Red') and from AAB subgroups ('Nadan', 'Nendra padathy', 'Pome' and 'Plantain') under constraint of 172 diploid *M. acuminata* accessions. AA wild, *M. acuminata* wild-type accessions; AAcv, *M. acuminata* cultivars. Accessions in red in the diploid Mlaly group are from the Comoros islands and have been genotyped independently of the others. Accessions names on the right in red are accessions discussed in the text.

Gel standards

Because the classical standard ladder used in the experiments, covering broadly 100–400 bp of the genotyped SSRs, generated very large gaps between successive amplicons and hampered precise reading, we calibrated the allele sizes by directly using accessions from the sample itself. Three triploid subgroups, AAA 'Cavendish', AAB 'Plantain' and AAA 'Mutika-Lujugira', were over-represented in the study. Within these triploid subgroups the allelic polymorphism between the accessions was very low, with a main pattern

present on more than 99 % of the accessions at every locus (see below). The accessions belonging to these subgroups, spread among the different gels, could thus be used for allele size calibration. The CPM standards covered a broad part of the *Musa* diversity because the CPM subgroups originate from different *M. acuminata* subspecies and include the *M. balbisiana* genome. Using these references, a scoring precision of 1 bp appeared to be of phylogenetic significance (see above).

These CPM accessions are publicly available upon request from the International Transit Center of Bioversity International hosted by the Katholieke Universiteit (Leuven, Belgium).

Analysis of main edible triploid subgroups

Investigation of the common alleles at each locus between the AA diploids and the accessions from the triploid 'Cavendish', 'Gros Michel', 'Mutika Lujugira', 'Red', 'Ibota', 'Pome' and 'Plantain' subgroups provided contrasting results.

The results of putative lineages of 'Cavendish', 'Gros Michel' and 'Pome' subgroups are shown in Table 3. For the 'Mutika Lujugira', 'Red', 'Ibota' and 'Plantain' subgroups, such likely ancestors were not found.

The *M. balbisiana*-specific alleles and their presence in 'B'-containing accessions are shown in Table 4. From a total of 329 alleles defined (Table 2), four alleles recorded on B-containing genomes were absent from all AA and AAA genotypes (mMaCIR01, 298 bp; mMaCIR01, 310 bp; mMaCIR03, 121 bp; mMaCIR03, 127 bp). Six other alleles were highly infrequent (<2 %) in these AA or AAA accessions (Table 4).

For the triploid subgroups, we identified loci with triallelic combinations, which were specific to a triploid subgroup (Table 5).

Polymorphism within triploid subgroups

The analysis was restricted to the triploid subgroups represented by more than 20 exploitable accessions: AAA 'Cavendish' (27), AAA 'Mutika-Lujugira' (25) and AAB 'Plantain' (78) (Hippolyte *et al.*, 2011). From the 22 SSRs analysed, the number of loci with an identical allelic pattern for all the accessions ranged from 14 in 'Plantain' to 20 in 'Cavendish' and 'Mutika-Lujugira' (Table 6). The variations around this main pattern were spread between different accessions and were not concentrated in just a few accessions. From more than 2800 allelic profiles within the three subgroups, we recorded 19 deviations from the main pattern, which could be divided into two classes: loss of main alleles (11) or emergence of new alleles in addition or as substitution to the main alleles (eight).

DISCUSSION

Diversity in the genus *Musa* has previously been analysed based on levels of dissimilarity calculated between accessions in these 22 SSR loci (Perrier *et al.*, 2009). The resulting phylogenetic structure of *Musa* species was congruent with previous results using molecular markers (Grapin *et al.*, 1998; Creste

et al., 2004; Risterucci *et al.*, 2009). Furthermore, the large degree of polymorphism of these markers enabled us to refine the typology, particularly for genetically close subgroups, and to elucidate the relationships between wild diploids and cultivated diploids, and also between edible diploids and the main triploid subgroups (Perrier *et al.*, 2009). In this paper, we investigated the resolving power of each marker at the species, subspecies and subgroup and accession levels in order to refine the lineage.

SSR characteristics

The observed allelic ranges were derived from a typical SSR stepwise mutation model, with some additional events (often indel), as observed in other species (Colson and Goldstein, 1999). Variations in band intensities have been observed previously for *Musa* (Creste *et al.*, 2004) (Fig. 1). They probably resulted from preferential amplification of shorter alleles (Wattier *et al.*, 1998) as seen in the diploid *M. acuminata* 'Zebrina maia oa', i which the intensity of the 248-bp allele was higher than that of the 286-bp allele (Fig. 1). These intensity variations could also result from mutations in annealing sequences (Ishibashi *et al.*, 1996; Colson and Goldstein, 1999) leading to a null allele (Dakin and Avise, 2004; Chapuis and Estoup, 2007) or competitive amplification, if annealing still occurs.

Paying particular attention to these scoring difficulties, we showed that the use of the CPM standards allowed the recording of reliable independent data. For example, when the additional Comorian accessions were added to the initial diversity tree, they co-localized with the 'Mlali' diploid subgroup originating from these islands, and the triploid 'Minalouki' also co-localized with the 'Mnalouki' accessions of the initial sampling (Fig. 2).

Therefore, using the protocol defined here and the publicly available genotyping data of this analysis (Hippolyte *et al.*, 2011), there is an opportunity to compare local collections with this broad *Musa* sample as reference.

Lineage between triploid subgroups and AA diploids

The analysis confirmed that the 'Mlali' subgroup was the closest 2*n* gamete donor for the 'Cavendish' and 'Gros Michel' subgroups, as previously proposed (Raboin *et al.*, 2005). We identified *M. acuminata* 'Chimoili Kana Nkoboï', a Comorian diploid, as the best 2*n* gamete donor to 'Cavendish' and 'Gros Michel' accessions (Table 3). This accession was a better candidate than *M. acuminata* 'Akondro mainty', originating from Madagascar, which has been suggested previously (Raboin *et al.*, 2005), although the accessions within the 'Mlali' subgroup are roughly genetically homogeneous. We also defined *M. acuminata* 'Pisang pipit' as the putative *n* gamete donor for the 'Cavendish' accessions and *M. acuminata* 'Khai nai on' as *n* gamete donor for the 'Gros Michel' subgroup (Table 3). This analysis also demonstrated that the 'Mlali' subgroup is probably the *M. acuminata* 2*n* gamete donor of the AAB 'Pome' subgroup, *M. acuminata* 'Samba kundre' or *M. acuminata* 'Chicame' being the best candidates (Table 3). These relationships

TABLE 3. *Putative diploid parents (2*n *and* n *gamete donors) of 'Cavendish', 'Gros Michel' and 'Pome' triploid subgroups*

	Ma1_32	Ma3_90	mMa CIR01	mMa CIR03	mMa CIR07	mMa CIR08	mMa CIR13	mMa Cir150	mMa CIR152	mMa CIR164	mMa CIR195	mMa CIR196	mMa CIR214	mMa CIR231	mMa CIR24	mMa CIR260	mMa CIR264	mMa CIR27	mMa CIR307	mMa CIR39	mMa CIR40	mMa CIR45
'Cavendish' AAA	**235**	**150**	**254**	**122**	**158**	**261**	**286**	**257**	**164**	**401**	**298**	**168**	**119**	**242**	**237**	**212**	**250**	**235**	**162**	*331*	**176**	**284**
	245	*162*	**258**	**124**	**170**	**265**		**261**		**407**		**180**	**123**	*250*	**247**			**258**	**243**	**164**	**335**	**178**	**289**
		168	**264**											**276**	**253**			**245**				**180**
'Chimoili Kana Nkoboï' AA 2n donor	245	150	254	122	158	261	286	261	164	401	298	180	119	242	247	*210*	258	243	162	335	178	284
		168	258	124	170	265		257					123	276	253	212		245	164		180	289
P. pipit AA n donor	235	*x*	250	120	170	261	286	257	167	407	*258*	168	119	242	*x*	208	250	235	160	*341*	176	284
	254		**264**	124	172	271	257		164				128	270		212	264	243	162	*349*	178	289
'Gros Michel' AAA	**235**	**150**	**254**	**122**	**158**	**261**	**286**	**257**	**164**	**401**	258	176	**119**	**242**	*252*	**212**	258	243	**162**	*329*	**176**	**284**
		168	**258**	**124**	**170**	**265**		**261**			**298**	**180**	**123**		**253**		266		**164**	**335**	**178**	**289**
			270											*276*								**180**
'Chimoili Kana Nkoboï' AA 2n donor	245	150	254	122	158	261	286	257	164	401	298	180	119	242	247	*210*	258	243	162	335	178	284
		168	258	124	170	265		261					123	276	253	212		245	164		180	289
'Khai nai on' AA n donor	235	162	250	120	170	261	286	257	*161*	*403*	258	168	115	250	*x*	*210*	252	*235*	162	*x*	176	284
	254	168	270	124		269			*165*			176	119				266	*241*			178	289
'Pome' AAB	**239**	**150**	**254**	**122**	**158**	**261**	**286**	**257**	**164**	**298**	**298**	177	**119**	**242**	**247**	**210**	242	**245**	**164**	**335**	**178**	274
	245	**168**	**258**	**124**	**166**	**265**		**261**	**182**	**401**			**123**	*252*	**253**		**258**	**243**	**168**	**357**	**180**	**284**
			290		**170**								**125**	**276**								**289**
'Samba Nkundre'/ Chicame' AA 2n donor	245	150	254	122	158	261	286	257	164	401	298	*180*	119	242	247	210	258	243	164	335	178	284
		168	258	124	170	265		261					123	276	253			245			180	289
'Lal velchi' BB n donor	239	*152*	**290**	*121*	**166**	257	282	**257**	173	**298**	296	177	**123**	249	*241*	212	**242**	*235*	**168**	**357**	**180**	274
			310			**261**	*290*	**261**	**182**	313		182			*243*							

For each locus, allele sizes are given in base pairs. The allele pattern given for each triploid subgroup is the most common pattern observed within these subgroups at the considered locus.

X, missing data; italic indicates allelic discrepancy between the major pattern observed within the subgroup and the putative ancestor pattern; bold indicates allelic identity between the major pattern within the subgroup and the putative ancestor pattern.

TABLE 4. *Number of 'B-specific alleles' encountered in accessions of the sampling in each group of the Simmonds and Shepherd's classification (1955) (AA, AAA, AAB, AAB 'Plantain's, AB, ABB, BB).*

Locus	'B allele' (bp)	AA, 186	AAA, 100	AAB 'Plantain', 78	AAB others, 63	AB, 9	ABB, 7	BB, 12
mMaCIR01	298	–	–	56	20	2	12	1
mMaCIR03	127	–	–	76	34	2	20	7
mMaCIR01	310	–	–	–	–	–	21	8
mMaCIR03	121	–	–	–	10	5	24	10
Ma1_32	239	–	3	–	21	7	30	10
mMaCIR39	355	1	–	–	8	1	6	6
mMaCIR214	125	2	1	73	30	7	25	9
mMaCIR260	225	3	–	–	11	2	10	2
mMaCIR164	298	3	–	–	20	5	17	3
mMaCIR39	357	4	–	76	43	6	24	7
mMaCIR264	242	4	2	75	51	7	36	12

TABLE 5. *SSR markers displaying specific allelic combinations for accessions belonging to the same triploid subgroup*

Subgroup	SSR marker	Allele 1	Allele 2	Allele 3	Out of subgroup*
'Plantain', AAB	mMaCIR01	258	266	298	Nzumoigne, Kupulik indet
	mMaCIR264	242	251	258	M009
'Mutika-Lujugira', AAA	mMaCIR242	231	260	276	Foulah 4
	mMaCIR307	160	164	172	Foulah 4
	Ma 3_90	156	166	170	Foulah 4
'Cavendish', AAA	mMaCIR01	254	258	264	
	mMaCIR231	242	250	276	Pisang bakar, Hom Thong Mokho (Ambon)
	mMaCIR 24	237	247	253	
'Gros Michel', AAA	mMaCIR01	254	258	270	
'Pome', AAB	mMaCIR01	254	258	290	Lady finger
	mMaCIR45	274	284	289	Lady finger Rajapuri india
'Red', AAA	mMaCIR264	250	264	266	Mata kun
	mMaCIR 39	329	331	339	
	mMaCIR40	176	180	196	Leite (Rio)
'Ibota', AAA	mMaCIR03	110	120	124	Vudu papua
	mMaCIR07	152	162	170	Vudu papua beccarii
	mMaCIR27	235	241	245	Vudu papua

* Accessions displaying the same SSR triallelic allelic pattern of the subgroup, but not belonging to the subgroup.

between triploid subgroups and the 'Mlali' accessions are also clear from the diversity tree (Fig. 2)

For the 'Mutika-Lujugira', 'Red', 'Ibota' and 'Plantain' subgroups, we did not find any convincing lineage with the diploid *M. acuminata* accessions sampled. Nevertheless, analysis based on allele frequencies clearly showed that the 'Plantain' subgroup has a dominant *banksii* subspecies origin, while the 'Mutika-Lujugira' accessions have a binary *banksii-zebrina* subspecies origin. While the 'Ibota' subgroup displays a main *malaccensis* subspecies origin, the putative origin of the 'Red' subgroup is much less clear (results not shown). This information has been useful for refining the geographical origin (Perrier *et al.*, 2009, 2011), but is not detailed enough to assist with breeding strategy.

Specificity of balbisiana *alleles*

Four alleles from two loci were fully discriminating between A- and B-genome-containing accessions (Table 4). For those displaying highly imbalanced occurrence between A and B accessions (Table 4), the introgression of B alleles into the A genome is very unlikely and could only be cautiously hypothesized for 'Calcutta 4' (mMaCIR39_355), a wild diploid from *M. acuminata* subspecies *burmanicoides*, which is sympatric with *M. balbisiana* in Burma and north Thailand. For all other cases, the most likely explanation is that alleles of the same length (bp) arose from different alleles with convergent evolution (i.e. homoplasy), which is known to be frequent in microsatellite evolution (Estoup *et al.*, 2002).

The distribution of these B-specific alleles was not homogeneous between wild BB accessions and interspecific cultivars (AB, AAB, ABB). For example, the 298-bp allele from mMaCIR01 was only found in *M. balbisiana* 'ITC 0626' while it was widely recorded within interspecific cultivars, most of which were of 'Plantain' genotypes (Table 4). Nevertheless, only 50 % of the loci of *M. balbisiana* 'ITC 0626' displayed alleles also recorded in AAB 'Plantain', excluding it as the potential *M. balbisiana* parent of this subgroup. Conversely, the 239-bp allele from MA1–32 was present on nearly all BBs (Table 4) and most AB and ABB cultivars, but it was absent from 'Plantain' and 'Iholena' AAB subgroups. For this locus, none of the alleles of 'Plantain' or 'Iholena' was found in any BB accession. We only found

TABLE 6. *Accessions from the 'Plantain', 'Cavendish' and 'Mutika-Lujugira' subgroups deviating from common patterns of their subgroup at 22 SSR loci*

	'Plantain' (78)			'Cavendish' (27)			Mutika/Lujugira (25)		
	Main profile	Other profiles		Main profile	Other profiles		Main profile	Other profiles	
	bp	bp	accessions	bp	bp	accessions	bp	bp	accessions
Ma 1_32	245−251			235−245			260		
Ma3_90	152−162−166	152−166	Bobby Tanap	150−162−168			156−166−170		
mMaCir01	258−266−298			254−258−264			256−266		
mMaCIR03	122−127	122	Okoyo Ukom	122−124	122	Chinese Cavendish	120−122		
mMaCIR07	158−160−172	158−160−174	Atali Kiogo	158−170			152−154−160		
mMaCIR08	261			261−265			261−263−267	261−267	Ingumba y' inyamunyo-Ingoromoka-Indemera y' imbihire
mMaCIR13	286	274−286−298 274−286 286−298	Haton Tigre mbouroukou kelong mekintu	286			286−298		
mMaCIR24	247			237−247−253			241−247		
mMaCIR27	235−243			235−243−245	235−241	901	235−243		
mMaCIR39	335−357	335	Bungaoisan	331−335			335−345		
mMaCIR40	182			176−178−180			180		
mMaCIR45	284−289			284−289			284−289		
mMaCIR150	257			257−261			257−263		
mMaCIR152	194	185 182−194	Mbi Egome 1 Okyo ukom	164			164		
mMaCIR164	313−407	313	Nselouka Obino L'ewai Njock Kon	401−407			409		
mMaCIR195	290−294			298			298		
mMaCIR196	177−180			168−180			180−184		
mMaCIR214	125−128	119−123−125	Ndingo Liko	119−123			123		
mMaCIR231	244−249			242−250−276			242−260−276		
mMaCIR260	210			212			210	206−210	Bolo Bigouyo
mMaCIR264	242−251−58			250−258			256−266		
mMaCIR307	164−168			162−164			160−164−172		

this for the AAB 'Pome' subgroup, a potential close donor of the *balbisiana* genome, which is *M. balbisiana* 'Lal velchi' (Table 3).

The *balbisiana* diversity provided by the interspecific cultivars seemed larger than the BB diploid diversity present in our BB sample, given that several alleles from the cultivars were not present in diploid *M. balbisiana* sampled at numerous loci, suggesting an under-representation of the whole *balbisiana* diversity in collections or extinction of the BB parents of the current hybrids. In fact, available BB genotypes are sparse and their origin is poorly documented (IPGRI-INIBAP(Bioversity), 2003). This gap could be related to the level of diversity in BB accessions, which is lower than in AA accessions (De Langhe and De Maret, 1999; Swangpol *et al.*, 2007), with no subspecies clustering (Sotto and Rabara, 2000).

Nevertheless, *M. balbisiana* originated from a broad area ranging from India (Simmonds, 1962; Uma *et al.*, 2006) to the south of China (Wang *et al.*, 2007) and possibly the Philippines (Sotto and Rabara, 2000). Several studies have demonstrated the existence of a local diversity of these *M. balbisiana* (Uma *et al.*, 2006; Ning *et al.*, 2007; Swangpol *et al.*, 2007; Wang *et al.*, 2007). Unfortunately, differences in analytical methods, as well as the lack of accessions in common and the use of vernacular names prevented cross-analysis.

Analysis of a broad sample, enriched by additional collecting efforts, would be of significant importance to better characterize *M. balbisiana* genomes, particularly for lineage studies of the interspecific cultivars and consequently for breeding programmes. This would also provide information on the possible extinction of BB ancestors of the current hybrids.

From the present set of 22 SSR markers, several highlighted the divergence between *acuminata* and *balbisiana* species, which would have occurred between 4 Mya (Lescot *et al.*, 2008) and 28 Mya (Christelová *et al.*, 2011). The absence of discriminating alleles between *M. acuminata* subspecies meant that no specific allele has been generated since their divergence, which is assumed to have begun with the maximal geographical isolation of the south-east Asian islands, during the last interglacial period. SSR markers trace recent evolution events as their mutation rate is quite high, roughly between 10^{-3} and 10^{-6}, depending on species and location on the genome (Vigouroux *et al.*, 2002). Despite the absence of specific alleles discriminating between *M. acuminata* diploids, allele frequencies distinguished AA wild subspecies (*banksii*, *zebrina*, *malaccensis* and *burmanica*) (Perrier *et al.*, 2009) and enabled the determination of the wild origin of AA cultivars.

Discrimination between triploid subgroups

The triploid accessions clustered in subgroups based on agromorphological characters (IPGRI-INIBAP(Bioversity)/CIRAD, 1996; Pollefeys *et al.*, 2004), despite broad phenotypic diversity within these subgroups, as illustrated for 'Plantain' (Ortiz *et al.*, 1998; Lescot *et al.*, 2008) and 'Cavendish' (Simmonds, 1954). The genetic status of 'Plantain' (i.e. intra-subgroup homogeneity versus intra-subgroup heterogeneity) has been investigated using molecular markers. There was no clear outcome from these studies, as two studies using AFLP, SSR, MSAP or DArT markers (Noyer *et al.*, 2005; Risterucci *et al.*, 2009) found genetic homogeneity within this subgroup, whereas two other studies based on RAPD and AFLP markers (Crouch *et al.*, 2000; Ude *et al.*, 2003) found 'Plantain' subgroup genetic heterogeneity.

Our results suggested predominantly genetic homogeneity within six triploid subgroups (Table 5). The specific triallelic combinations, at fully heterozygous loci, were accurate both for the discrimination of a subgroup from the other subgroups and for the allocation (or rejection) of accessions to (from) a subgroup (Table 5). As an example of subgroup discrimination, the locus mMaCIR01 generated specific triallelic combinations for each of four subgroups: 'Plantain' (AAB), 'Pome' (AAB), 'Cavendish' (AAA) and 'Gros Michel' (AAA), although the last two are genetically very close (Table 3; Raboin *et al.*, 2005). Regarding the triallelic combinations, the relationships between AAB Indian dessert bananas, classified within 'Pome', 'Nadan' or 'Nendra padathi', need to be clarified. Although they shared common triallelic combinations, Fig. 2 suggests clustering into two subgroups. These examples indicate that the triallelic combinations should conveniently help in defining or refining subgroup clustering, the NJ tree providing a graphical tool for investigating putative clustering.

Concerning allocation to a subgroup, the 'Nzumoigne' accession, classified as a 'Plantain' based on morphological characteristics, differs from the mMaCIR01 triallelic pattern of 'Plantain' and did not cluster with this subgroup (Fig. 2). In fact, it has only been found in the Comoros Islands other Indian Ocean islands, and probably has a different history (domestication period, human migration, etc.) than African 'Plantain'. Most of the morphological traits of the 'Kupulik' accession fit the characteristics of the AAB 'Plantain' subgroup, but some others, such as rounded fruit apex, prevented its classification to this subgroup (C. Jenny, CIRAD, 2009, pers. comm.). This accession displayed some of the triallelic combinations characterizing 'Plantain', but the discrepancies at some loci prevented its genetic classification in the 'Plantain' subgroup (Fig. 2). The accession 'Foulah 4', classified as ABB, shared all specific allelic combinations of the AAA 'Mutika-Lujugira' subgroup. This probably resulted from a mislabelling, and according based on the diversity tree (Fig. 2) and its allelic pattern (Hippolyte *et al.*, 2011, data sheet), differing only by two missing alleles, the sample analysed should be classified into the 'Mutika-Lujugira' subgroup of East Africa. Similarly, the AA accession 'Vudu Papua, ITC0590' should be included in the AAA 'Ibota' subgroup based on triallelic combinations and its location in Fig. 2. Checking all loci, 'Vudu papua' most closely matches 'Ibota' subgroup profiles and differs for one allele only. Therefore, the 'Nzumoigne', 'Kupulik', 'Foulah' and 'Vudu papua' cases reveal that the stringency of these triallelic combinations allows refinement of subgroup classification and that these allelic combinations might be used as easy keys for assigning accessions to subgroups.

Polymorphism within triploid subgroups

The genetic homogeneity within subgroups showed some exceptions. From more than 2800 allelic profiles obtained in this study with accessions belonging to the 'Cavendish', 'Mutika-Lujugira' and 'Plantain' subgroups, 19 deviations from the main allelic pattern were established: 11 were due to missing alleles (null alleles), and eight arose from the occurrence of an extra allele (Table 6). The occurrence and transmission of mutations in the flanking region were as frequent as the mutations occurring in repeated regions in the triploid samples.

Independently, by genotyping 21 accessions of the initial sample with 16 SSR markers, confirmed differences in band intensities at some loci, corroborating the previous hypothesis of allelic preferential amplification. This duplicated genotyping also confirmed deviations from the main profiles of these loci, such as the presence of the 274-bp extra allele of mMacir13 or the absence of the 407-bp allele from mMaCIR164 on some 'Plantain' accessions (Table 6).

According to the process of banana triploidization, resulting from the association of a non-reduced 2n gamete (gamete with sporophytic chromosome number) and an n gamete (Ortiz, 1997), two main hypotheses might explain this reduced intra-subgroup diversity: (1) all accessions in a subgroup were derived from the same initial clone and evolved by somatic mutations fixed through vegetative propagation; and (2) the genotypes of these subgroups arose from sexual events from the same parents or from genetically related parents. Most authors have suggested a mix of these two hypotheses, with a diversification of 'Plantain' by somatic mutations from a few introduced cultivars (Simmonds, 1966; De Langhe *et al.*, 1994–1995; Crouch *et al.*, 2000; Ude *et al.*, 2003).

The widely different frequencies between main and extra alleles or null alleles within the 'Cavendish', 'Mutika-Lujugira' and 'Plantain' subgroups (Table 6) favour the first hypothesis. Based on RFLP markers (Raboin *et al.*, 2005) and SSR markers (this study), the two AAA subgroups 'Cavendish' and 'Gros Michel' were found to derive from a common 2n gamete donor and probably two different, but genetically close, n donors. For these two much closer subgroups, nine SSR loci were different for at least one allele, while we never found more than one deviating loci per accession within the analysed triploid subgroups. It is therefore likely that each triploid subgroup arose from a unique clone, as hypothesized by Noyer *et al.* (2005), and that sparse somatic mutations have been 'inherited' (transmitted through clonal propagation), leading to new SSR alleles (or null allele from mutations in annealing sequences). The transmission of somatic mutations is possible through vegetative propagation, leading to non-chimeric or to chimeric plant structure (Marcotrigiano, 1997; Klekowski, 2003) and a mosaic state (i.e. initial cells associated with mutated cells) (Gill *et al.*, 1995; Santelices, 1999; Pineda-Krch and Lehtilä, 2004).

Nevertheless, the low number and in most cases the absence of genetic differences between the accessions of a subgroup cannot explain the huge phenotypic diversity observed within these subgroups (Ortiz *et al.*, 1998; Daniells *et al.*, 2001; IPGRI-INIBAP(Bioversity), 2003). Therefore other possibilities, such as epigenetic regulation, need to be explored.

Human migration brought the 'Cavendish', 'Mutika-Lujugira' and 'Plantain' subgroups from Asian centres of origin to Africa in the case of 'Plantain' and 'Mutika-Lujugira' (De Langhe *et al.*, 1994–1995), and more recently worldwide in the case of 'Cavendish'. Several genetically close accessions were found in the centres of origin, but they could not be allocated to the three subgroups. They probably resulted from crosses of close diploid parents or the same parents (full-sibling). This is illustrated for the 'Gros Michel' and 'Cavendish' subgroups (half-sibling) or for a single accession such as 'Kupulik' or 'Nzumoigne' compared with the 'Plantain' subgroup. Human migrations introduced a drastic bottleneck in the diffusion of these triploids, with only some suckers of the same clonal origin being exported and then spread. This human influence therefore shaped the triploid diversity landscape, favouring an over-representation of sparse genotypes, which evolved phenotypically.

CONCLUSIONS

Using a broad sample, this study contributed to improving our understanding of *Musa* species diversity. The accuracy of the results depended greatly on experimental control, which limited the impact of preferential amplification, and thus misinterpretation. Moreover, the CPM standards enabled accurate scoring. Studying additional accessions, with these experimental procedures (including CPM standards), allowed us to add these independent data to previous diversity analysis.

By using the co-dominance of SSR markers for parentage analysis, we showed that the high polymorphism of the SSR markers was able to identify specific loci efficient for discrimination and assignment. At the interspecific level, some alleles discriminated genotypes containing the B genome from strictly *M. acuminata* genotypes. These specific alleles showed also that the *balbisiana* species diversity displayed through interspecific cultivars was broader than that of the available *M. balbisiana* diploids. Therefore, new exploration for and collection of *balbisiana* species is recommended, especially if *M. balbisiana* provides agronomic traits to important AAB cultivars, such as 'Plantain'. We did not find any specific allele of *M. acuminata* subspecies, probably due to the more recent divergence within *acuminata* species (diploids and triploids).

The analysis of allelic distributions supported the monoclonal origin of the major triploid subgroups, 'Cavendish', 'Mutika-Lujugira' and 'Plantain', despite wide geographical distribution and huge phenotypic diversity. The current CIRAD's breeding strategy to develop triploid cultivars consists of crossing a diploid accession with an auto-tetraploid accession ($2n = 4x$), obtained through chromosome doubling using colchicine treatment (Bakry and Horry, 1994; Bakry *et al.*, 2001, 2009). With this approach, using the identified putative parents, it should be possible to generate genotypes very close to those of 'Cavendish' or 'Gros Michel'. For the other triploid subgroups, the lack of close ancestors of *M. acuminata*, as shown for the 'Mutika-Lujugira' subgroup, or the lack of both *M. acuminata* and *M. balbisiana* putative parents, as for the 'Plantain' subgroup, hampers this kind of process and other strategies have to be developed.

The ongoing full genome sequencing of the double haploid of *M. acuminata* 'Pahang' will provide data useful for the comparison between cultivars and probably also structural comparisons. Our results showed that the origin of the huge and valuable phenotypic diversity within the different triploid subgroups, which is essential for breeding programmes, will have to be investigated within epigenetic mechanisms in addition to genetic mechanisms and inherited somatic mutations. The genetically homogeneous cultivars within these triploid subgroups are well characterized phenotypically and differentiated from each other. They represent unique models to investigate and compare the influence of more than 1000 years of epigenetic regulation through mitosis on the same genomes, without any interference with meiosis. Furthermore, evolution and diversification processes mixing sexuality and clonality within *Musa* should be compared with current studies on other vegetatively propagated crops, such as grape, potato and fruit trees.

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