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RESEARCH ARTICLE

Collection of new diversity of wild and cultivated bananas (*Musa* spp.) in the Autonomous Region of Bougainville, Papua New Guinea

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Abstract Bananas (*Musa* spp.), including dessert and cooking types, are of major importance in the tropics. Due to extremely high levels of sterility, the diversity of cultivated bananas is fixed over long periods of time to the existing genotypes. This pattern puts banana-based agrosystems at risk. Therefore, assessing the extent of wild and cultivated banana diversity, conserving it and making it available for further use is a priority. We report here the collection of new wild and cultivated banana germplasm in the Autonomous Region of Bougainville, Papua New Guinea. In total, 61 accessions were collected and their names and uses were recorded when possible. Classification was also provided based on the observations made in the field. Three wild specimens were collected. Among the 58 cultivated accessions, we noted that eight were used as ornamental plants, seven were edible varieties of the Fe'i type and two were natural tetraploids from the *Musa* section. The ploidy was then checked by flow cytometry and the accessions were genotyped with a set of 19 SSR markers. The genotyping results were merged to the dataset from Christelová et al. (Biodivers Conserv 26:801–824, 2017). This joint analysis helped refine

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or confirm the classification of the collected accessions. It also allowed to identify 10 private alleles and 35 genotypes or Genotype Groups that were not present in the wider dataset. Finally, it shed light on the diversification processes at work in the region, such as the capture of mutations by farmers and the likely occurrence of geneflow within the cultivated genepool.

Keywords Banana · Collecting mission · Crop diversification · Genetic diversity · *Musa* · Microsatellites

Introduction

Bananas (*Musa* spp.), including dessert and cooking types such as Plantain, are of major importance in the tropics for both subsistence and food security (FAO 2014). Originating from the South-East Asia/West Oceania region, this crop has a complex domestication history (De Langhe et al. 2009; Perrier et al. 2011). The name "banana" corresponds to different species of the *Musa* genus, and to their hybrids. The genus *Musa* is divided into two sections corresponding to distinct phylogenetic clades, *Musa* (2n = 22) and *Callimusa* (2n = 20 or 18) (Häkkinen 2013; Janssens et al. 2016). The edible bananas from the section *Musa* are composed of either genome A (*Musa acuminata* Colla), or A in combination with B (*M. balbisiana* Colla) or S (*M. schizocarpa* N.W. Simmonds). Edible bananas from the *Musa* section are diploid, triploid or more rarely tetraploid, the most popular cultivars being triploid from Groups such as Cavendish (AAA) and Plantain (AAB). Edible bananas from the section *Callimusa*, called Fe'i bananas, are associated with T genome (*M. textilis* Née), but have been far less studied and their origin remains obscure. Recent results indicate that the Fe'i bananas arose from complex domestication schemes and comprise accessions with different ploidy (Christelová et al. 2017; www.crop-diversity.org/mgis/). As edible bananas bear seedless fruits, they are propagated vegetatively. This mode of propagation and extremely low level of fertility make cultivated bananas particularly vulnerable to diseases and abiotic stress and the occurrence of new, potentially better adapted genotypes is limited to rare mutation events. Therefore, the diversity of cultivated bananas was fixed over long periods of time to the existing genotypes. Even if the diverse genetic make-up of banana varieties grown worldwide exhibit a wide range of traits (Heslop-Harrison and Schwarzacher 2007), this pattern slows down cultivated bananas' evolution and puts banana-based agrosystems at risk by hampering rapid adaptation of the crop to new threats.

Breeding programs in banana focus primarily on creating improved triploid or tetraploid varieties (Ortiz 2013; Tomekpe et al. 2004). In addition to multiple ploidy levels, the sterility associated with the production of seedless fruits is a challenge for breeders. However, the use of fully or partially fertile, parthenocarpic, edible diploids eases the process of creating progenies in a largely sterile crop (Tomekpe et al. 2004; Tenkouano et al. 2011). In this context, assessing the extent of wild and cultivated banana diversity, conserving it and making it available for further use is a priority.

Papua New Guinea (PNG), including neighbouring islands, is a recognized centre of diversity and potentially a domestication centre for banana (Christelová et al. 2017; Lebot 1999; Sardos et al. 2016b). Four banana collecting missions[1] were organized to mainland PNG and the Bismarck Archipelago in 1988–1989 which revealed high levels of diversity in the country. In total, 264 wild and cultivated accessions were collected, of which 86% appeared to be unique genotypes (Arnaud and Horry 1997). These accessions were sent to both the National Banana Germplasm Collection at Laloki, Port Moresby and to the Bioversity International *Musa* Germplasm Transit Centre (ITC)[2] in Belgium for conservation purposes. Currently, more than 25 years after the PNG missions, 230 of the accessions collected in PNG are still conserved in vitro in the ITC. Over the years, the PNG accessions have been valuable resources for breeders and researchers and have significantly improved our knowledge of banana (e.g. Ploetz et al. 1999; Geering et al. 2005; Raboin et al. 2005; Ball et al. 2006;

[1] These missions were organized by IBPGR and QDPI (current Queensland DAF) in co-operation with the PNG Department of Agriculture and Livestock (current NARI) and supported by INIBAP (current Bioversity International).

[2] Since 1994 and the signature of an agreement between Bioversity International and FAO, all the germplasm conserved in the ITC, including the PNG material, is available to all on the understanding that it remains in the public domain.

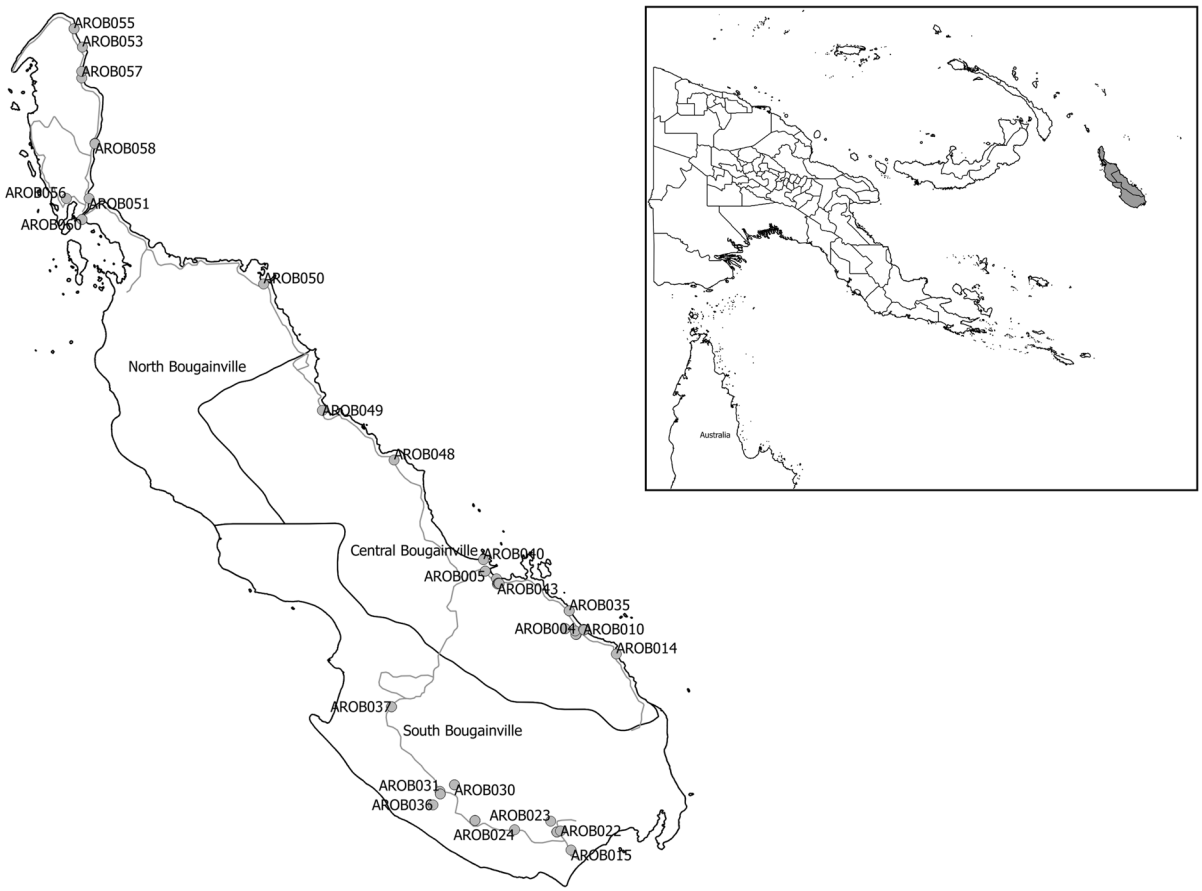

Fig. 1 Map of the Autonomous Region of Bougainville and of the collection sites

Volkaert 2011; Till et al. 2010; Hřibová et al. 2011; Teo et al. 2011; Christelová et al. 2011, 2017; Valdez-Ojeda et al. 2014; Janssens et al. 2016; Sardos et al. 2016a, b).

Interestingly, out of the 177 cultivated accessions collected in PNG that are still conserved in the ITC, 100 are cultivated diploids with AA genome composition. These accessions are mainly used by local populations as cooking varieties and are therefore of great interest for the improvement of cooking triploids such as Plantains. Due to a civil conflict (1988–1998), the region which later became the Autonomous Region of Bougainville (AROB) was not visited by the expeditions in the 1980′s (Fig. 1).

Located in Near Oceania, the AROB is composed of two main islands, Bougainville and Buka, and of smaller surrounding islands. While it is an autonomous region of Papua New Guinea, it belongs geographically and ecologically to the Solomon Islands Archipelago. Agricultural systems in Bougainville and Buka are typical of the West Oceania region, where people practise a rain fed and shifting agriculture with fallow periods that can reach up to 15 years. The most important staple crop all over these islands is sweet potato (*Ipomoea batatas* (L.) Lam.) followed by banana, coconut (*Cocos nucifera* L.) and a range of root and tuber crops (Bourke et al. 2002).

Organized by NARI and Bioversity International, a banana collecting mission in the AROB took place from 19 October to 31 October 2016, and explored the islands of Bougainville, Buka and Sohano. This report presents the genetic diversity collected during the prospections, which targeted new wild and cultivated germplasm for conservation purposes. When possible, the prospections were coupled with the systematic ploidy measurement and SSR genotyping of the collected accessions. These two activities took place at the *Musa* Genotyping Centre (MGC) held in the

Institute of Experimental Botany (Olomouc, Czech Republic). The SSR profiles of the collected accessions were added to the results obtained in the study published by Christelová et al. (2017) in order to better characterize the genetic diversity of the new accessions collected in this region. We discuss here the diversity of the genotypes collected in Bougainville and show that doubling plant prospection with "real-time" ploidy measurements and SSR genotyping adds substantial value to collecting missions.

Materials and methods

Collecting trip and documentation

The collecting trip was set up from 19 October to 31 October 2016, just before the rainy season. The team was composed of JS, JP, SJ, GSS and GR. Due to potential safety uncertainty, a local guide, Mr Zohn Bosco Miriona from Bougainville Experience Tours (BET), was contracted. Officers from Department of Primary Industries (DPI) offices in Buka, Kieta and Buin were also contacted to support the team locally.

The aim of this expedition was to seek new germplasm to enrich the national banana collection of NARI-Laloki and ultimately the ITC, in order to ensure the conservation and use of a wider diversity of *Musa*. The range of diversity encountered was documented (Sachter-Smith et al. 2017), but suckers and leaf samples were collected only when cultivated varieties were unknown to the team based on their morphology. Once suckers were collected, an accession code was provided and each accession was documented with local names, their meaning, origins, uses and GPS coordinates (Fig. 1). Potential classifications were also given based on the morphology of the plants. All collections, except from abandoned food gardens, were made with the authorization of the field's owner, or a relative. The Fe'i types being quite uncommon, all Fe'i bananas encountered were collected along with a few samples of wild *Callimusa* previously described by Argent (1976) (*M. bukensis* Argent and *M. maclayi* F. Muell. subsp. *maclayi* var. *erecta* (N. W. Simmonds) Argent).

Sample processing

Once identified as potentially absent from the NARI collection, the varieties and wild specimens were given unique ID codes and became accessions. Suckers were collected for further ex-situ conservation and fresh leaf tissues, preferentially cigar leaves, were collected from all the accessions and conserved in an electric cooler that could be plugged to the car and in any guesthouse equipped with power. Two types of back-up samples were kept for DNA: one in DNAgard® Tissue (Biomatrica) http://www.biomatrica.com/media/dnagard_tissue/DNAgard-Tissue-preserves-plant-DNA.pdf and one silica dried. Fresh leaves were sent to the MGC-Olomouc through fast courier upon return of the team to Port Moresby. Back-ups were used for DNA extraction when necessary, i.e., when fresh leaves arrived at MGC that were too damaged.

Ploidy level estimation

Ploidy level of accessions for which fresh leaves were available was estimated by flow cytometry according to Doležel et al. (1997, 2007). About 30 mg of young leaf tissue was chopped with a razor blade in a Petri dish containing 500 µL Otto I solution (0.1 M citric acid, 0.5% v/v Tween 20). Crude homogenate was filtered through a 50 µm nylon mesh. Chicken red blood cell nuclei (CRBC), prepared according to Galbraith et al. (1998), were added to the suspension of banana nuclei as an internal standard. After 30 min incubation at room temperature, 1 mL Otto II solution (0.4 M Na_2HPO_4) (Otto 1990) supplemented with 2 µg/mL DAPI (4,6-diamidino-2-phenylindole). The samples were analysed using Sysmex-Partec CyFlow flow cytometer (Görlitz, Germany). The gain of the instrument was adjusted so that the peak of the CRBC nuclei was positioned approximately on channel 100 on a histogram of relative fluorescence intensity when using a 512-channel scale. The ploidy level of each banana accession was then determined by comparing peak positions of CRBC and *Musa* nuclei. The ratio between relative DAPI fluorescence intensity of CRBC nuclei and G1-phase nuclei of the accessions from the *Musa* section is $\sim$ 0.5 for diploid plants and $\sim$ 0.75 for triploid plants (Doležel et al. 1997; Christelová et al. 2017).

SSR genotyping

Leaf tissues were used for DNA extraction using NucleoSpin Plant II kit (Macherey–Nagel, Düren, Germany). Genotyping based on 19 SSR loci was performed according to Christelová et al. (2011). Briefly, all SSR loci were amplified from each DNA sample by PCR with locus-specific M13-tailed primer pair and fluorescently labelled M13 universal primer. PCR conditions were set as follows (in total volume of 20 µL): PCR reaction buffer (10 mM Tris-HCl, pH 8; 50 mM KCl; 0.1% Triton-X100; 1.5 mM $MgCl_2$), 200 µM dNTPs, 1 U Taq polymerase (NEB), 8 pmol of the M13-tailed locus-specific forward primer, 10 pmol of the locus-specific reverse primer and 6 pmol of fluorescently labelled M13-universal primer. Four different fluorescent dyes (6-FAM, VIC, NED PET) for the M13 primer were used to allow for multiplexed fragment analysis of resulting PCR products. PCR was done in 35 cycles (94 °C/45 s, Ta/60 s, 72 °C/60 s) preceded by a denaturation step (94 °C/5 min) and followed by a final extension step (72 °C/5 min). Locus specific annealing temperature (Ta) followed Christelová et al. (2011). PCR products were purified by ethanol/sodium acetate precipitation and two independent PCR runs were done for each DNA sample.

Subsequently, purified PCR products were diluted 40-fold in Hi-Di formamide, mixed with internal standard GeneScan™– 500 LIZ size standard (Applied Biosystems, Foster City, CA, USA), denatured for 5 min at 95 °C and loaded onto capillary electrophoresis DNA analyzer (ABI 3730xl, Applied Biosystems, USA). Default module settings were used for electrophoretic separation and signal detection. Resulting raw data were processed by GeneMarker® v.1.75 (Softgenetics, State College, PA, USA) software to call alleles at individual SSR loci.

SSR data analysis

Due to the co-dominant nature of SSR markers and to the presence of several ploidy levels, the whole AROB dataset was coded as a binary presence (1) and absence (0) matrix and merged to a core dataset (CS) from Christelová et al. (2017). The CS used here is composed of 583 accessions with robust classification. Both the *Musa* Sect. (545 accessions) and the *Callimusa* Sect. (38 accessions) were represented.

The total number of alleles in the combined dataset was evaluated, the allelic patterns of the CS and the AROB datasets were compared and private alleles, i.e. alleles present in the AROB dataset but not in the CS, were identified. For the purpose of this paper, we then considered cultivated bananas from both sections separately.

The computer program DARwin 6 (Perrier and Jacquemoud-Collet 2006; Perrier et al. 2003) was then used to calculate dissimilarity values between pairs of accessions within a joint CS-AROB dataset using the Dice index. For this purpose we filtered the dataset on missing data and the marker mMaCIR164 which didn't amplify 24% of the samples was excluded. Equally, 23 accessions exhibiting more than 20% of missing data were discarded from the set. All 23 accessions belonged to the *Callimusa* section (five cultivated Fe'i and 18 wild species). The CS was then pruned to avoid bias due to redundancies between identical genotypes or numerous closely related accessions. Notably, only six accessions of the Plantains Group were kept out of the 113 in the initial set. The pruned CS comprised 357 accessions from the section *Musa* including cultivated varieties and wild direct ancestors and 38 accessions belonging to the section *Callimusa*. We identified identical genotypes within the AROB dataset and between the AROB and the pruned CS datasets (dissimilarity = 0). Using the method proposed by Douhovnikoff and Dodd (2003) and a graph of the distribution of dissimilarity values with a step of 0.02 produced by DARwin 6 (S1 file), we also identified a dissimilarity threshold below which the genetic variation observed between accessions is considered to result either from genotyping errors or from the accumulation of mutations during the clonal propagation of a unique original genotype. This dissimilarity threshold was determined at 0.1 (S1 file) and allowed the identification of Genotype Groups (GGs) clustering around the AROB accessions and composed of nearly identical genotypes.

Due to high rates of missing data in the *Callimusa* section, we decided to only consider the edible bananas from the *Musa* section and their closely associated wild relatives. Using DARwin 6 and given the known history of cultivated bananas, we built a weighted Neighbor-Joining (NJ) tree under the topological constraint of a NJ tree built on the diploid accessions. The accessions for which ploidy was not measured were considered polyploids.

Results

Collection

In total and based on their morphology, the expedition collected 61 accessions probably not conserved in NARI, including three wild specimens belonging to the species *M. bukensis* (AROB002 and AROB008) and *M. maclayi* subsp. *maclayi* var. *erecta* (AROB013) (Table 1). Among the 58 cultivated accessions collected, four duplicate pairs that were not at the same developmental stage at the time of collection are suspected: AROB042 "Asi"/AROB045 "Glenda's dwarf", AROB043 "Sausage banana"/AROB061 "Sausage banana", AROB029 "Kourai"/AROB031 "Kourai" and AROB051"Limot"/AROB052 "Poso-olohi". In a few cases, names of the banana varieties were not known and the team named the accessions after the place of collection or after the owner of the variety.

Out of the cultivated banana varieties for which uses were documented, eight were used as ornamental among which a specimen belonging to the species *M. ornata* Roxb., 23 were preferentially used as cooking varieties, 12 were either cooked or used as dessert types, and nine were used preferentially as dessert varieties. We noted that among the cooking accessions collected, one was variegated, AROB055 "Tambra". In total, nine accessions were named "wild banana" in local languages. Three of them were actually the wild accessions collected while the six others were found in cultivation, often near houses, and were classified as Fe'i bananas based on their morphology. However, among these Fe'i, two (AROB010 "Bia Kaura" and AROB030 "Korai 2") were reported by farmers to bear a few seeds in the fruits and to have been collected from the wild. None of these accessions was flowering at the time of the prospection so it was not possible to strictly assign them to one of the local wild species.

The cultivated varieties collected were classified as AA (28), AAA (7), AAB (14) and two potential tetraploids (4x) were identified: AROB027 "Buka", morphologically close to the Pisang Awak Group (ABB), and AROB056 "Kalmagol", similar to the Silk Group (AAB). In addition to the two potential tetraploids, the team was not able to propose robust classifications for nine accessions. Four of them were red or reddish ornamental plants: AROB001 "Flower banana", AROB006 "Nono 1", AROB041 "Glenda's Red" and AROB043 "Sausage Banana". The other were edible types: AROB009 "Bukatawawe", which was morphologically similar to ITC0605 "Japaraka n°2" (AA) but taller and therefore suspected to be AAA, AROB032 "Toitoi", evoking an AAB but with dark green young fruits, AROB038 "Sinsiruai", somewhat Maoli-Popoulu like and therefore suspected to be AAB, AROB057 "Sepik", a very tall plant with a large bunch and a very unusual round and obtuse purple/yellow male bud and AROB061 "Sausage Banana" with fruits said to be red and sausage-like in appearance but were not observed.

Detailed results of the prospection are presented in Table 1 and pictures are available in Sachter-Smith et al. (2017).

Ploidy

Out of the 61 fresh leaf samples collected and sent to the MGC, 48 arrived in a good state to be used for ploidy measurement using flow cytometry. Overall, the results obtained were consistent with the classification determined based on the accessions' morphology and allowed to refine a number of cases for which the team had doubts, notably confirming the tetraploid status of AROB027 "Buka" and AROB056 "Kalmagol". Out of the 48 accessions, 34 were diploid. However, we noted that five of the seven Fe'i accessions collected and the accession AROB013 *M. maclayi* subsp. *maclayi* var. *erecta* exhibited peak ratios with internal standard slightly higher than expected for a regular diploid. Ploidy for these samples should ultimately be checked by chromosome counting but these results are not surprising for *Callimusa* accessions. It was shown that despite a lower number of chromosomes (x = 10), their genomes are larger than in the *Musa* section (x = 11) (Bartoš et al. 2005, Čížková et al. 2015).

SSR genotyping

Number of alleles

Out of the 61 samples sent to the MGC for genotyping, DNA extraction failed for AROB004 "Wiau". We therefore obtained genotyping results for 60 accessions. Considering the *Musa* and *Callimusa* sections together, a total of 207 alleles was found in the AROB

Table 1 Names, meaning of the names, place of collection and uses of the accessions collected in the Autonomous Region of Bougainville

Date of collection	Accession Code	Nomenclature		Meaning of name	Place of collection (Local Level Government)	Uses
		Botanical name[a]	Name (genomic composition)			
21/10/2016	AROB001	*Musa*	"Flower banana"	Flower banana	Toboroi (Arawa)	Ornamental (red plant)
21/10/2016	AROB002	*Musa bukensis*	"Kaura"	Wild banana	Kurai (Arawa)	Dried pseudostem can be used to tie things (as for other bananas)
21/10/2016	AROB003	*Musa*	"Mero Mero" (AA)	Young men	Kurai (Arawa)	Cooking
21/10/2016	AROB004	*Musa*	"Wiau" (AA)	No meaning	Kurai (Arawa)	Cooking and dessert
21/10/2016	AROB005	*Musa*	"Duma"[b] (AA)	Place of collection	Duma (Arawa)	Cooking
21/10/2016	AROB006	*Musa*	"Nono 1"	Breast	Duma (Arawa)	Ornamental (variegated red plant); edible fruits but not tasty
22/10/2016	AROB007	*Musa* Iholena Group	"Navente 2" (AAB)	A part of something	Kurai (Arawa)	Cooking and dessert
22/10/2016	AROB008	*Musa bukensis*	"Kamura"	Wild banana with dark pseudostem	Roreinang (Arawa)	Purple sap used as a dye for mats
22/10/2016	AROB009	*Musa*	"Bukatawawe" (AAA)	Something that was fought over	Roreinang (Arawa)	NR
22/10/2016	AROB010	*Musa* Fe'i Group	"Bia Kaura"	Wild banana	Tunaniya, Aropa (Arawa)	Ornamental, edible but with few seeds
22/10/2016	AROB011	*Musa*	"Navente 1" (AAB)	Refers to a part of men's body	Tunaniya, Aropa (Arawa)	Cooking, sap used to heal bites of centipede
22/10/2016	AROB012	*Musa ornata*	"Flower banana"	Flower banana	Tunaniya, Aropa (Arawa)	Ornamental
22/10/2016	AROB013	*Musa maclayi*	"Kaura"	Wild banana	Tunaniya, Aropa (Arawa)	Young leaves used as plates during Custom ceremonies, sap formally drunk to "be strong"
23/10/2016	AROB014	*Musa* Cavendish Group	"Tamoa" (AAA)	Samoa	Tarumi, Koromera (Arawa)	Dessert
24/10/2016	AROB015	*Musa*	"Laguai"[b] (AAA)	Place of collection	Laguai, road to Kangu beach (Buin)	NR
24/10/2016	AROB016	*Musa*	"Nape'e" (AAA)	Not known	Kararu (Buin)	Cooking or dessert; used for custom ceremonies
24/10/2016	AROB017	*Musa*	"Banawa" (AAA)	No meaning	Kararu (Buin)	Cooking or dessert, preferred dessert
24/10/2016	AROB018	*Musa*	"Tomea" (AA)	NR	Kararu (Buin)	Cooking
24/10/2016	AROB019	*Musa*	"Tavilo" (AA)	No meaning	Kararu (Buin)	Cooking
24/10/2016	AROB020	*Musa*	"Kararu 1"[b] (AAB)	Place of collection	Kararu (Buin)	Cooking or dessert
24/10/2016	AROB021	*Musa*	"Abau" (AA)	Abau district	Kararu (Buin)	Cooking
24/10/2016	AROB022	*Musa*	"Kararu 2"[b] (AA)	Place of collection	Kararu (Buin)	Cooking
25/10/2016	AROB023	*Musa*	"Morou"[b] (AA)	Place of collection	Morou, road from Buin to Siwai (Buin)	Cooking

Table 1 continued

Date of collection	Accession Code	Nomenclature		Meaning of name	Place of collection (Local Level Government)	Uses
		Botanical name[a]	Name (genomic composition)			
25/10/2016	AROB024	*Musa*	"Seven Kina" (AAA)	Seven Kinas	Aku (Buin)	Cooking
25/10/2016	AROB025	*Musa* Maoli-Popoulu Group	"Taiop" (AAB)	Taiop island	Likui (Siwai)	Cooking and dessert
25/10/2016	AROB026	*Musa* Fe'i Group	"Kaurai"	Wild banana	Likui (Siwai)	Cooking and dessert
25/10/2016	AROB027	*Musa*	"Buka" (AABB)	Buka island	Likui (Siwai)	Dessert or cooking for pigs
25/10/2016	AROB028	*Musa*	"Popondetta" (AA)	Popondetta town	Likui (Siwai)	Cooking or dessert
25/10/2016	AROB029	*Musa* Fe'i Group	"Korai 1"	Wild banana	Kapana (Siwai)	Cooking (when ripe), fruit also used to cure kidney problems
25/10/2016	AROB030	*Musa* Fe'i Group	"Korai 2"	Wild banana	Kapana (Siwai)	To feed pigs, could have seeded fruits
26/10/2016	AROB031	*Musa* Fe'i Group	"Kourai"	Wild banana	Siwai district office (Siwai)	Cooking or dessert
26/10/2016	AROB032	*Musa*	"Toitoi"[b]	Place of collection	Toitoi (Siwai)	NR
26/10/2016	AROB033	*Musa*	"Papua" (AA)	Papua	Toitoi (Siwai)	Cooking
26/10/2016	AROB034	*Musa*	"Nesuri" (AA)	NR	Mamagota (Siwai)	Cooking
26/10/2016	AROB035	*Musa*	"Talasea" (AA)	Talasea province	Mamagota (Siwai)	Cooking
26/10/2016	AROB036	*Musa*	"Mopere" (AA)	Ripe	Rasu (Siwai)	Dessert
26/10/2016	AROB037	*Musa*	"Baby banana" (AA)	Baby banana	Sinsiruai (Bana)	NR
26/10/2016	AROB038	*Musa*	"Sinsiruai"[b] (AA)	Place of collection	Sinsiruai (Bana)	Cooking
27/10/2016	AROB039	*Musa* Iholena Group	"Kibirori" (AAB)	No meaning	Arawa town, Arawa-Panguna road (Arawa)	Dessert
27/10/2016	AROB040	*Musa*	"Navotavu" (AA)	Can feed a whole family	Rorobana primary school (Arawa)	Cooking (liked for Tamatama[c] preparation) and dessert
27/10/2016	AROB041	*Musa*	"Glenda's Red"[b]	Named after provider	Arawa town (Arawa)	Ornamental variegated red banana, no fruits
28/10/2016	AROB042	*Musa*	"Asi" (AA)	Place of origin (Asitavi school)	Arawa town (Arawa)	Ornamental, fruit edible cooked
28/10/2016	AROB043	*Musa*	"Sausage banana" (AA)	Sausage banana	Arawa town (Arawa)	Ornamental, cooking or dessert banana
28/10/2016	AROB044	*Musa*	"Arawa"[b] (AAB)	Place of collection	Arawa town (Arawa)	Cooking and dessert
28/10/2016	AROB045	*Musa*	"Glenda's dwarf"[b] (AA)	Named after provider	Arawa town (Arawa)	Ornamental, dessert
28/10/2016	AROB046	*Musa*	"Itonia" (AA)	Not known	Arawa town (Arawa)	Cooking, preparation of Tamatama[c] when unripe

Table 1 continued

Date of collection	Accession Code	Nomenclature		Meaning of name	Place of collection (Local Level Government)	Uses
		Botanical name[a]	Name (genomic composition)			
28/10/2016	AROB047	*Musa*	"Tobaung" (AA)	Not known	Arawa town (Arawa)	Dessert
28/10/2016	AROB048	*Musa*	"Kaesi" (AA)	Not known	Arikua (Wakunai)	Cooking
28/10/2016	AROB049	*Musa*	"Nono 2" (AA)	Breast	Arikua (Wakunai)	Dessert
28/10/2016	AROB050	*Musa*	"Sesévé" (AA)	No meaning	Teohoa n°2 (Tinputz)	Cooking
29/10/2016	AROB051	*Musa* Fe'i Group	"Limot"	Upright	Gonatun (Buka)	Cooking (boiled or roasted when ripe), medicine against stomach aches
29/10/2016	AROB052	*Musa* Fe'i Group	"Poso-olohi"	Wild banana	Hanahan (Buka)	Cooking, preferred roasted
29/10/2016	AROB053	*Musa*	"Poso Huhu 1" (AA)	Banana cook above the fire	Mapiri (Buka)	Cooking, preferred roasted
29/10/2016	AROB054	*Musa*	"Poso Huhu 2" (AA)	Banana cook above the fire	Holu (Buka)	Cooking
29/10/2016	AROB055	*Musa*	"Tambra" (AA)	No meaning	Holu (Buka)	Cooking, preferred roasted
30/10/2016	AROB056	*Musa*	"Kalmagol"	No meaning	Bobobow, Novah (Buka)	Dessert
30/10/2016	AROB057	*Musa*	"Sepik" (AAA)	Sepik province	Monlus, Hagus (Buka)	Cooking and dessert
30/10/2016	AROB058	*Musa*	"Korukapi" (AAA)	Cut from a shell	Sing (Buka)	Dessert
30/10/2016	AROB059	*Musa*	"Goum" (AA)	No meaning	Sing (Buka)	Cooking (when ripe) or dessert
30/10/2016	AROB060	*Musa*	"Bubun" (AAB)	No meaning, banana from ancient time	Sohano island (Buka)	Cooking (roasted, fried, boiled)
30/10/2016	AROB061	*Musa*	"Sausage banana" (AA)	Sausage banana	Sohano island (Buka)	NR

[a]For landraces following the International Code of Nomenclature for Cultivated Plants, [b]Given by collecting team, [c]Dish made of pounded cooked bananas and/or taro with coconut milk

NR not recorded

dataset while 353 alleles are present in the combined AROB—CS dataset. Out of the ten alleles only observed in the AROB accessions and not in the CS, seven were found within the *Callimusa* section and three in the *Musa* section (Fig. 2). In the *Musa* section, two of the new alleles were found in the triploid AAA AROB057 "Sepik" while the third one was found in the triploid AAA AROB017 "Banawa". In the *Callimusa* section, new alleles were found in both the wild species and the Fe'i that were collected.

Identical genotypes and Genotype Groups (GGs)

Out of 60 accessions genotyped, we identified 46 different genotypes or Genotype Groups (GGs), among which 35 were not present in the CS. Six pairs of strictly identical genotypes were identified within the AROB accessions collected (Tab. 2). In two cases, the genotyping confirmed what was suspected in the field (AROB042 "Asi"/AROB045 "Glenda's Dwarf" and AROB051 "Limot"/AROB052 "Poso-olohi") but in four cases it highlighted similarities that were

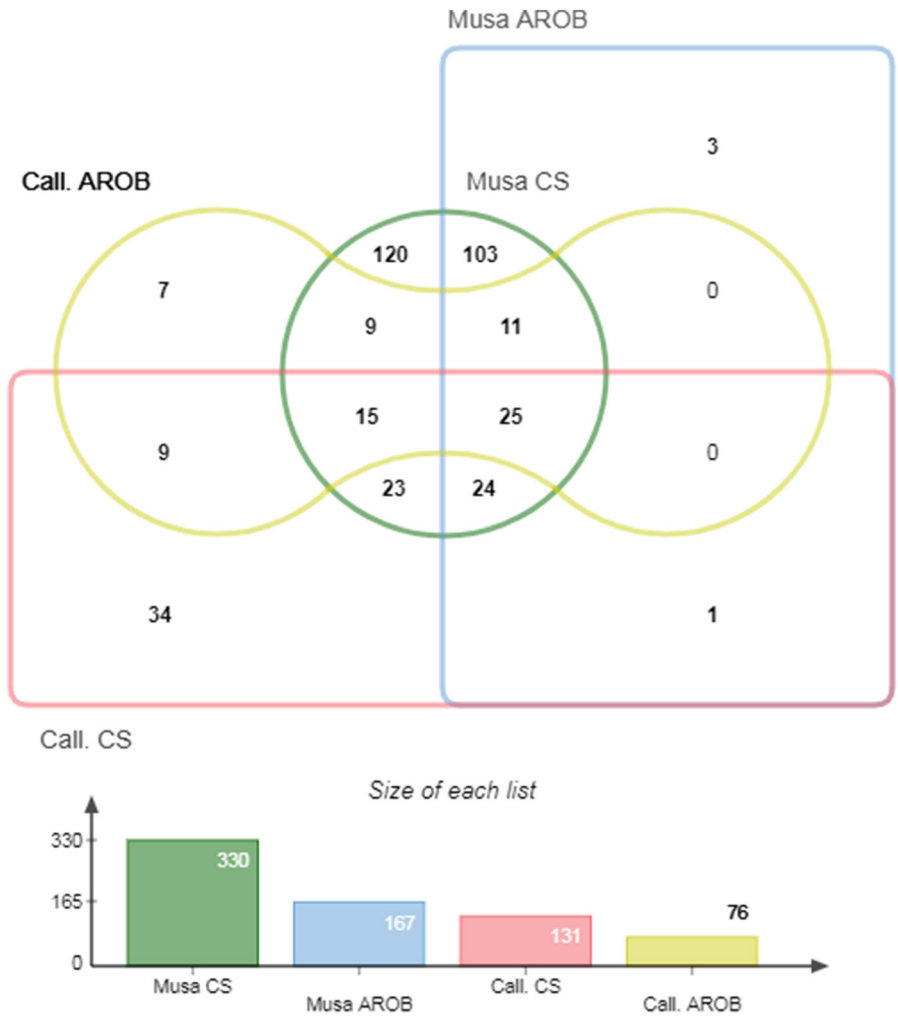

Fig. 2 Distribution of all distinct alleles across 19 SSR loci for the *Musa* and *Callimusa* sections of the Core Set (CS) and of the Autonomous Region of Bougainville (AROB). Overlapping areas denote shared alleles between categories. Venn diagram constructed with the tool developed by Bardou et al. (2014)

not detected at collection (AROB018 "Tomea"/AROB033 "Papua", AROB021 "Abau"/AROB028 "Popondetta", AROB023 "Morou"/AROB055 "Tambra" and AROB059 "Goum"/AROB061 "Sausage Banana"). In addition, we identified two AROB genotypes that were strictly identical to ITC accessions: AROB009 "Bukatawawe" classified as AAA has the same genotype as ITC0372 "Hungtu" (AAA) while AROB028 "Popondeta" and AROB021 "Abau", both classified as AA, have genotypes identical to ITC1013 "Sena" (AA). We then identified Genotype Groups (GGs) composed of genotypes with pairwise dissimilarities < 0.1. Eleven GGs involving AROB accessions were identified including some corresponding to known Groups of *Musa* (Cavendish, Maoli-Popoulu and Iholena). Six GGs involved both AROB and ITC accessions and two were composed of AROB accessions only. We also noted that the Fe'i bananas AROB010 "Bia Kaura", AROB026 "Kauraï", AROB029 "Koraï 1" and AROB031 "Kourai" were part of the same GG.

Diversity clustering

The NJ tree built with the edible accessions of the *Musa* section and their close ancestors provided a better image of the diversity collected in Bougainville (Fig. 3). While the clustering of many accessions within the AA from PNG or their link to the AAB Pacific Groups Maoli-Popoulu and Iholena was not surprising, we noted that AROB024 "Seven Kina" (AAA) was linked to the East African Mutika/Lujugira (AAA) while AROB057 "Sepik" (AAA) was located within a wide cluster of various AA and AAA mainly

Table 2 Flow cytometry results, genotyping clustering and proposed classification for the accessions collected in the Autonomous Region of Bougainville

Accession Codes	Names	Ploidy measurement (flow cytometry)	Identical genotypes	Genotype Group (Dis < 0.1)	Clustering (tree location)	Proposed classification	
						Morphology	Molecular
AROB001	"Flower banana"	NM	–	–	With AS	?	AS?
AROB002	"Kaura"	NM	–	–	NA	*Musa bukensis*	*Callimusa*
AROB003	"Mero Mero"	NM	–	ITC0984 "Yanun Yefan"	In AA PNG	AA	AA
AROB004	*"Wiau"*	NM	*Not genotyped*	*not genotyped*	*not genotyped*	AA	AA
AROB005	"Duma"[a]	NM	–	–	In AA PNG	AA	AA
AROB006	"Nono 1"	NM	–	–	With AS	?	AS?
AROB007	"Navente 2"	NM	–	Iholena Group ("Iholena" S11, ITC0909 "Kupulik", AROB060 "Bubun")	With AAB Iholena Group	AAB	Iholena Group AAB
AROB008	"Kamura"	NM	–	–	NA	*Musa bukensis*	*Callimusa*
AROB009	"Bukatawawe"	3x	ITC0372 "Hungtu"	ITC0884 "Awondaeke"	In AA and AAA PNG/ East Indonesia	AAA?	AAA
AROB010	"Bia Kaura"	2x	–	AROB026 "Kaurai", AROB031 "Kourai"	NA	Fe'i	*Callimusa*
AROB011	"Navente 1"	NM	–	–	Near AAB Iholena Group	AAB	AAB
AROB012	"Flower banana"	NM	–	–	NA	*Musa ornata*	*Musa ornata*
AROB013	"Kaura"	$2X^{\alpha}$	–	–	NA	*Musa maclayi*	*Callimusa*
AROB014	"Tamoa"	3x	–	Cavendish Group	With AAA Cavendish Group	Cavendish Group AAA	Cavendish Group AAA
AROB015	"Laguai"[a]	3x	–	–	In AA PNG	AAA	AAA
AROB016	"Nape'e"	2x	–	–	In AA PNG	AA	AA
AROB017	"Banawa"	3x	–	–	In AA PNG	AAA	AAA
AROB018	"Tomea"	2x	AROB033 "Papua"	ITC0589 "Gulum"	In AA PNG	AA	AA
AROB019	"Tavilo"	2x	–	AROB023 "Morou"[a], AROB046 "Itonia", AROB055 "Tambra"	In AA PNG	AA	AA

Table 2 continued

Accession Codes	Names	Ploidy measurement (flow cytometry)	Identical genotypes	Genotype Group (Dis < 0.1)	Clustering (tree location)	Proposed classification	
						Morphology	Molecular
AROB020	"Kararu 1"[a]	NM	–	–	Near AAB Laknau Group	AAB	AAB
AROB021	"Abau"	2x	AROB028 "Popondetta" - ITC1013 "Sena"	–	In AA PNG	AA	AA
AROB022	"Kararu 2"[a]	NM	–	–	In AA PNG	AA	AA
AROB023	"Morou"[a]	2x	AROB055 "Tambra"	AROB019 "Tavilo", AROB046 "Itonia"	In AA PNG	AA	AA
AROB024	"Seven Kina"	3x	–	–	Linked to AAA Mutika/ Lujugira Group	AAA	AAA
AROB025	"Taiop"	3x	–	Maoli - Popoulu Group (ITC1169 "Mai'a popo'ulu moa", "Huamoa" S19, "Eke ula" S22, "Manini Koae", "Maoli" S4, ITC0335 "Popoulou")	In AAB Maoli - Popoulu Group	Maoli - Popoulu Group AAB	Maoli - Popoulu Group AAB
AROB026	"Kaurai"	2x	–	AROB029 "Korai 1", AROB031 "Kourai"	NA	Fe'i Group	*Callimusa*
AROB027	"Buka"	4x	–	–	Near ABB Pisang Awak Group	ABB or 4X	AABB?
AROB028	"Popondetta"	2x	AROB021 "Abau" - ITC1013 "Sena"	–	In AA PNG	AA	AA
AROB029	"Korai 1"	$2X^{\alpha}$	–	AROB026 "Kaurai"	NA	Fe'i Group	*Callimusa*
AROB030	"Korai 2"	$2X^{\alpha}$	–	–	NA	Fe'i Group or local wild	*Callimusa*
AROB031	"Kourai"	$2X^{\alpha}$	–	AROB026 "Kaurai", AROB010 "Bia Kaura"	NA	Fe'i Group	*Callimusa*
AROB032	"Toitoi"[a]	3x	–	–	with AS	AAB?	AAS
AROB033	"Papua"	2x	AROB018 "Tomea"	ITC0589 "Gulum"	In AA PNG	AA	AA

Table 2 continued

Accession Codes	Names	Ploidy measurement (flow cytometry)	Identical genotypes	Genotype Group (Dis < 0.1)	Clustering (tree location)	Proposed classification	
						Morphology	Molecular
AROB034	"Nesuri"	2x	–	AROB037 "Baby Banana"	In AA PNG	AA	AA
AROB035	"Talasea"	2x	–	–	In AA PNG	AA	AA
AROB036	"Mopere"	2x	–	ITC0778 "Gorop"	In AA PNG	AA	AA
AROB037	"Baby banana"	2x	–	AROB034 "Nesuri"	In AA PNG	AA	AA
AROB038	"Sinsiruai"[a]	2x	–	–	In AA PNG	AAB?	AA
AROB039	"Kibirori"	3x	–	Iholena Group ("Kapua" S27, "Iholena" S11, ITC0825 "Uzakan")	With AAB Iholena Group	Iholena Group AAB	Iholena Group AAB
AROB040	"Navotavu"	2x	–	–	In AA PNG	AA	AA
AROB041	"Glenda's Red"[a]	2x	–	–	With AS	?	AS
AROB042	"Asi"	2x	AROB045 "Glenda's dwarf"	–	In AA PNG	AA	AA
AROB043	"Sausage banana"	2x	–	–	In AA PNG	?	AA
AROB044	"Arawa"[a]	3x	–	–	Linked to AAB Iholena Group and to unclassified AAB from PNG and East Indonesia	AAB	AAB
AROB045	"Glenda's dwarf"[a]	2x	AROB042 "Asi"	–	In AA PNG	AA	AA
AROB046	"Itonia"	2x	–	AROB019 "Tavilo", AROB023 "Morou"[a], AROB055 "Tambra"	In AA PNG	AA	AA
AROB047	"Tobaung"	2x	–	–	In AA PNG	AA	AA
AROB048	"Kaesi"	2x	–	ITC0603 "Somani", ITC0809 "Maleb", ITC0849 "Sepi", AROB053 "Poso Huhu 1"	In AA PNG	AA	AA
AROB049	"Nono 2"	2x	–	–	In AA PNG	AA	AA
AROB050	"Sesévé"	2x	–	ITC0819 "Uyam"	In AA PNG	AA	AA

Table 2 continued

Accession Codes	Names	Ploidy measurement (flow cytometry)	Identical genotypes	Genotype Group (Dis < 0.1)	Clustering (tree location)	Proposed classification	
						Morphology	Molecular
AROB051	"Limot"	$2X^{\alpha}$	AROB052 "Poso-olohi"	–	NA	Fe'i Group	*Callimusa*
AROB052	"Poso-olohi"	$2X^{\alpha}$	AROB051 "Limot"	–	NA	Fe'i Group	*Callimusa*
AROB053	"Poso Huhu 1"	2x	–	ITC0603 "Somani", ITC1245 "Papat", AROB048 "Kaesi"	In AA PNG	AA	AA
AROB054	"Poso Huhu 2"	NM	–	–	In AA PNG	AA	AA
AROB055	"Tambra"	2x	AROB023 "Morou"[a]	AROB019 "Tavilo", AROB046 "Itonia"	In AA PNG	AA	AA
AROB056	"Kalmagol"	4x	–	–	Near AB Kunnan Group and AAB Silk Group	4x (AABB?)	AABB?
AROB057	"Sepik"	3x	–	–	Within various AA and AAA from SE Asia, not branched to any cluster	AAA?	AAA
AROB058	"Korukapi"	3x	–	–	In AA PNG	AAA	AAA
AROB059	"Goum"	2x	AROB061 "Sausage banana"	–	In AA PNG	AA	AA
AROB060	"Bubun"	3x	–	Iholena Group ("Iholena" S11, ITC0909 "Kupulik", AROB007 "Navente 2")	Near AAB Iholena Group	AAB	AAB
AROB061	"Sausage banana"	2x	AROB059 "Goum"	–	In AA PNG	?	AA

Genotype Groups correspond to clusters of accessions for which pairwise dissimilarity values are below 0.1

[a]Given by collecting team, *NM* not measured, $2X^{\alpha}$: the peak ratio between sample and internal standard was slightly higher than expected for a diploid, *NA* not applicable

from SE Asia, but was not directly branching on any accession from the CS. One accession, AROB020 "Kararu", clustered near the Laknau Group (AAB) from the Philippines. We also noticed that AROB015 "Laguai", AROB017 "Banawa" and AROB058 "Korukapi" (all triploid AAA) clustered within the AA PNG Group originally composed of AA only. The tetraploids accession AROB027 "Buka" and

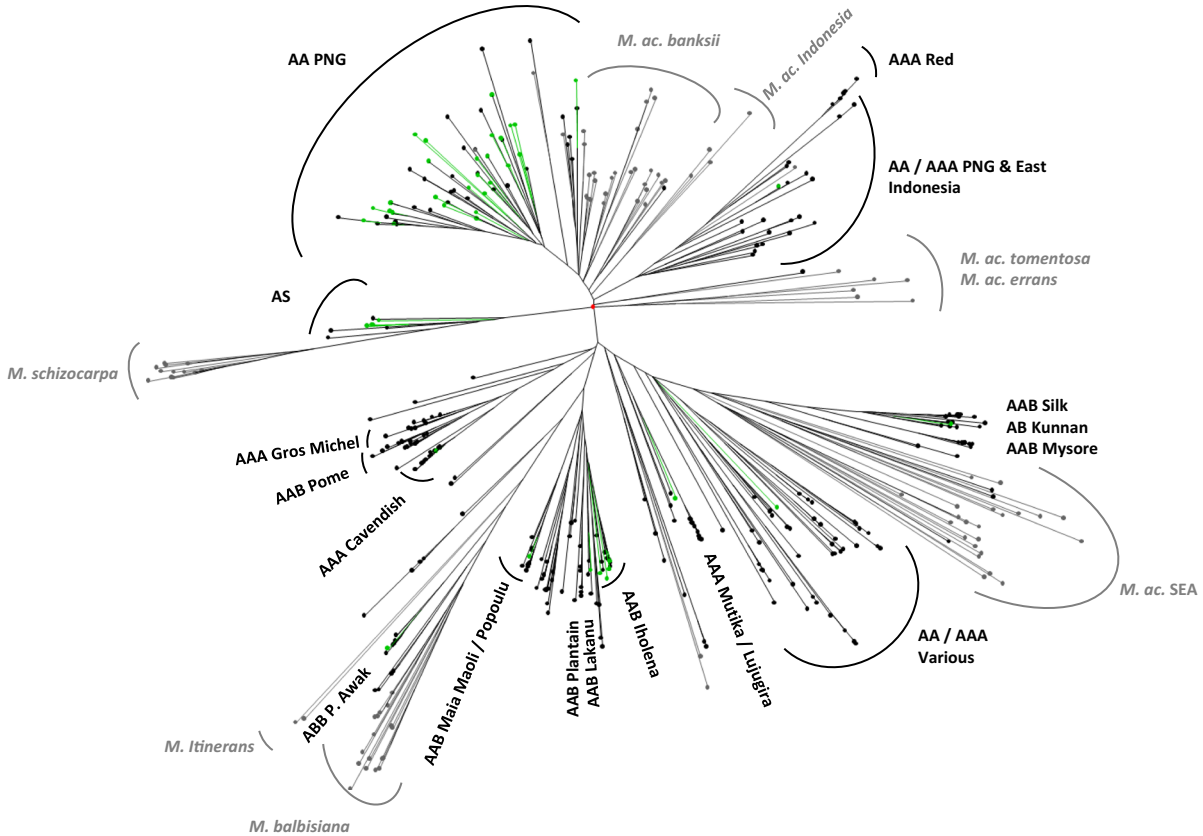

Fig. 3 Weighted NJ tree of the pruned CS and the AROB dataset (*Musa* section only) built under the constraint of the diploid accessions. Grey colour represents wild specimens. AROB accessions are coloured in green

AROB056 "Kalmagol" clustered within the ABB Pisang Awak Group and with the AAB Silk/AB Kunnan Groups, respectively. Four accessions branched on *M. schizocarpa* and clustered with two natural hybrids between *M. acuminata ssp. banksii* and *M. schizocarpa* (ITC0859 and ITC0822 "Sosi") and a cultivated variety classified as AS, ITC0822 "Tonton Kepa". Among these accessions, three are ornamentals (AROB001 "Flower Banana", AROB006 "Nono 1" and AROB041 "Glenda's Red"). The fourth one is AROB032, a triploid edible accession, named "Toitoi" by the team and originally classified as potential AAB.

Refining the classification of doubtful accessions

Combining the observations on the morphology, ploidy estimation and SSR genotyping we confirmed the classification determined in the field for quite a number of accessions and we were able to refine the classifications for the accessions for which we had doubts (Table 2). Due to its clustering with the ABB Pisang Awak Group, the genomic composition AABB is proposed for the tetraploid AROB027 "Buka". Despite its clustering with Silk AAB, we propose to stick to Allen's classification AABB for AROB056 "Kalmagol" which looked similar to "Kalamagol" AABB collected in Bougainville in the 1960's (Rosales et al. 1999). AROB009 "Bukatawawe" was classified as triploid by flow cytometry and therefore is confirmed AAA. AROB032 "Toitoi" was classified as triploid and branched on *M. schizocarpa* therefore suggesting a genomic composition of AAS. This is consistent with the colour of young fruits observed that is similar to *M. schizocarpa*. If confirmed, AROB032 "Toitoi" would be, at our knowledge, the first recorded triploid composed of an S genome. AROB001 "Flower Banana", AROB006 "Nono 1" and AROB041 "Glenda's Red" also clustered near these hybrids, but their morphology was so different

that further investigation is necessary to confirm the AS classification. AROB038 "Sinsiruai" and AROB061 "Sausage banana" were diploid and clustered within the AA from PNG and were therefore both classified as AA. As AROB057 "Sepik" was triploid and clustered within a group of AA/AAA accessions, we classified it as AAA. However, due to its peculiar morphology and to the two new alleles discovered in its genotype, we cannot exclude introgression from a genepool that is not present in the CS.

Discussion

The collecting mission to AROB was a fruitful exploration. Despite the four extensive collecting missions achieved in PNG in the 1980′s, many new cultivars were discovered.

Coupling ploidy estimation and SSR genotyping using the standardized platform for molecular characterization of *Musa* germplasm (Christelová et al. 2011) with the field prospections was very useful for different purposes. First, the joint analysis of the CS and of the AROB accessions helped in refining or confirming the classification of the AROB accessions by complementing the observations made in the field. The most appropriate stage for banana cultivars description and identification is when the first fruits are ripe (TAG 2010) but it was not always possible on the field to find plants at this particular stage of development. Second, for the same reason and also due to G x E interactions, the formal identification of synonyms/duplicates was not always possible. The molecular characterization of the collected accessions allowed detecting potential duplicates within the newly collected accessions but also within the joint CS-AROB datasets. It also allowed identifying nearly identical genotypes deriving from clonal diversification. Due to the accumulation of mutations and epigenetic changes, strictly identical genotypes do not always have strictly identical phenotypes, and therefore do not correspond to the same varieties, but they give a good estimation of the genetic diversity that was collected.

The only limitation we found using this set of markers is for the *Callimusa* accessions, which appear genetically very similar with the SSR markers used. The high rates of missing data observed overall in this section correspond to non-amplifying loci. It suggests high rates of null alleles for some of the markers used or the total absence of the site for some others such as the marker mMaCIR164 which was missing in all accessions of the section *Callimusa*. The transferability rate of SSR markers between species within genera in monocots was estimated 60% in average, of which only 40% are expected to be polymorphic (Barbará et al. 2007). As the SSR markers used here were developed from the *Musa* species *M. acuminata* and *M. balbisiana* (Crouch et al. 1998; Lagoda et al. 1998; Hippolyte et al. 2010) that belong both to the *Musa* section, the *Callimusa* species may be too genetically distant. Recently, SNPs called from the mapping of *Callimusa* reads on the *M. acuminata* reference genome were not accurate (Y. Hueber and M. Rouard pers. com.) and may also reflect high levels of differentiation. A set of markers developed specifically for targeted *Callimusa* species would be likely to lead to different results. Therefore, the interpretation of the results on the wild and cultivated *Callimusa* genotypes in this study should be made cautiously.

The clustering of some of the AROB accessions within the CS was particularly interesting with regard to the diversification history of cultivated bananas. The emergence of triploid cultivars ensued from sexual diversification through the occurrence of unbalanced meiosis leading to unreduced gametes within edible diploids (De Langhe et al. 2009; Perrier et al. 2011). For example, the AAA Cavendish clones resulted from a natural cross between two AA landraces from the Mlali Group (2n gamete donor) and "Khai Nai On" (n gamete donor) (Carreel et al. 2002; Raboin et al. 2005; Perrier et al. 2009; Hippolyte et al. 2012). In this context, the positions of the three AAA AROB015 "Laguai", AROB017 "Banawa" and AROB058 "Korukapi" in the diversity tree are also of particular interest as they are the only polyploids observed within a wide cluster of edible AA collected in PNG and closely related to *M. acuminata* subsp. *banksii* (F.Muell.) N.W.Simmonds, the Papuan subspecies of *M. acuminata*. Until now, only diploids have been reported in this genetic cluster (Christelová et al. 2017; Sardos et al. 2016b) and this pattern suggests that these triploids likely resulted from natural sexual crosses within Papuan edible diploid bananas. The two tetraploid accessions collected, AROB027 "Buka" and AROB056 "Kalmagol", are also of particular interest. Their locations in the tree and their morphologies suggest the Groups Pisang

Awak (ABB) and Silk (AAB) or Kunnan (AB) as parents, respectively. None of these triploid Groups is native to the region but the Pisang Awak Group is grown worldwide. In PNG, it is notably used to produce a local alcohol named Jungle Juice. Seeds are known to be quite common in cultivars from the Pisang Awak Group in their centre of origin, Malaysia (Simmonds 1966). Given the triploid status of this Group, viable progeny would be likely resulting from the natural cross of an unreduced gamete (3n) from the mother plant with a haploid pollen grain (n gamete) from a diploid plant. The tetraploid plant obtained from such a cross could be similar in morphology to its 3x parent, such as AROB027 "Buka", and may be wrongly classified as part of the Pisang Awak Group. This may be the origin of a debate regarding the ploidy level of the Pisang Awak Group, as some tetraploids have been reported within this Group (Pillay et al. 2006).

The occurrence of a tetraploid linked to the Silk Group (AAB) and Kunnan Group (AB), which are both from India, is more surprising. Even though AROB056 "Kalmagol" was more frequently planted than AROB027 "Buka", we didn't note an abundance of Silk locally, as only a single plant was observed in the town of Arawa (Sachter-Smith et al. 2017) and no diploid AB was recorded. Therefore, this tetraploid was likely introduced to the island. Allen already collected the variety under the name "Kalamagol" in the 1960′s (Rosales et al. 1999) showing it was already there 50 years ago. Given the thousands of Indian indentured labourers who were brought to the Pacific, mainly to Fiji, by the British Empire in the 19th century, AROB056 "Kalmagol" may have reached Bougainville from India via Fiji. Interestingly, "Kalamagol" sounds similar to "Kalaimagal" which is a feminine name of Indian origin.

Due to high levels of sterility, many banana varieties are clonal selections from a single clone. For example, the more than 150 known Plantain varieties are considered to be derived from the clonal diversification of a single original plant (Noyer et al. 2005). Comparing the AROB accessions and the CS, we identified six pairs and a triplet of identical genotypes. If it strongly suggests that these pairs/triplet are duplicates, it is not the case for the pair AROB023 "Morou"/AROB055 "Tambra". AROB055 "Tambra" is indeed variegated, that is to say that its leaves and fruits exhibit white stripes, while AROB023 "Morou" is not (Sachter-Smith et al. 2017). Variegation in plants is due to the partial fixation of a mutation in the chlorophyll-synthesis pathway (Marcotrigiano 1997). It is therefore likely that AROB055 "Tambra" ensues from the clonal diversification of AROB023 "Morou" and was intentionally selected by farmers for its attractive morphology. Such a case was already documented for cassava in the Pacific archipelago of Vanuatu, located further south of the Solomon Islands, where farmers capture all the variations of an initial genotype, independently of whether these variations might be agronomically useful (Caillon and Lanouguère-Bruneau 2005; Sardos et al. 2008). Here, we therefore documented in banana the fixation of an obvious mutation followed by the intentional and likely local selection of the variant by farmers. If variegated cassava is mostly used as an ornamental plant, we observed variegated bananas sold in the market in Buka town, attesting the potential value of growing such a variety.

Conclusion

The results of the collecting mission presented in this paper confirmed PNG as an important hotspot for banana genetic diversity as new genotypes, and new alleles, were collected despite the extensive collecting missions performed in the country in the past. The results of the SSR genotyping achieved concomitantly with the mission allowed highlighting not only the diversity cultivated in the AROB but also the different diversification processes at work in the region despite the crop's vegetative mode of propagation. The maintenance of crop evolution under farmer management is key to the successful establishment of in-situ and on -farm conservation initiatives (Bellon et al. 2017). Our results suggest the occurrence of gene flow, the accumulation of mutations and the introduction of new varieties in the AROB. Even though further studies should be undertaken in the future to fully characterize and understand these processes, we already have good insights that AROB and, by extension, PNG is an excellent candidate for the

establishment of on -farm conservation programmes for *Musa*.

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