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Nuclear genome size and genomic distribution of ribosomal DNA in *Musa* and *Ensete* (Musaceae): taxonomic implications

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Abstract. Nuclear DNA content and genomic distributions of 5S and 45S rDNA were examined in nineteen diploid accessions of the genus *Musa* representing its four sections *Eumusa*, *Rhodochlamys*, *Callimusa* and *Australimusa*, and in *Ensete gilletii*, which was the outgroup in this study. In the *Eumusa* (x = 11), 2C DNA content ranged from 1.130 to 1.377 pg, *M. balbisiana* having the lowest DNA content of all sections. *M. beccarii* (x = 9), a representative of *Callimusa*, had the highest 2C nuclear DNA content (1.561 pg). Species belonging to *Rhodochlamys* (x = 11) and *Australimusa* (x = 10) had 2C DNA contents ranging from 1.191 to 1.299 pg and from 1.435 to 1.547 pg, respectively. *E. gilletii* (x = 9) had 2C DNA content of 1.210 pg. The number of 5S rDNA loci in *Musa* varied from 4 to 8 per diploid cell. While different numbers of 5S rDNA loci were observed within *Eumusa* and *Rhodochlamys*, four 5S rDNA loci were observed in all accessions of *Australimusa*. *M. beccarii (Callimusa)* and *E. gilletii* contained 5S rRNA gene clusters on five and six chromosomes, respectively. The number of 45S rDNA loci was conserved within individual sections. Hierarchical cluster analysis of genome size, number of chromosomes and 45S rDNA sites suggested a close relationship between *Rhodochlamys* and *Eumusa*; *Australimusa* was clearly separated as were *M. beccarii* and *E. gilletii*. Within the *Eumusa-Rhodochlamys* group, *M. balbisiana*, *M. schizocarpa* and *M. ornata* formed distinct subgroups, clearly separated from the accessions of *M. acuminata*, *M. mannii*, *M. laterita* and *M. velutina*, which formed a tight subgroup. The results expand the knowledge of genome size and genomic distribution of ribosomal DNA in *Musa* and *Ensete*. They aid in clarification of the taxonomical classification of *Musa* and show a need to supplement the analyses on the DNA sequence level with cytogenetic studies.

Bananas and plantains (*Musa* ssp.), hereafter called bananas, are the world's largest perennial herbs grown in tropical and subtropical regions. The production of bananas, which exceeded 100 million tons in 2002 (http://www.fao.org), ranks them the fourth among the most important food crops. About 90% of the production is consumed locally, being an important nutrition source for hundreds millions of people in developing countries. Cultivated bananas are seed sterile diploid, triploid or tetraploid clones containing various combinations of the A and B genomes coming from two diploid species of *Musa*, *M. acuminata* and *M. balbisiana* (Simmonds and Shepherd, 1955). Recently banana production has been threatened by viral and fungal diseases, nematodes and pests (Robinson, 1996). One way to overcome these problems is to search for resistance traits in species evolutionarily related to *M. acuminata* and *M. balbisiana*. However, despite the socio-economical importance of bananas, only the genomes of *M. acuminata* and *M. balbisiana* have been studied to a limited extent and little is known about the nuclear genomes of other species of *Musa*.

The taxonomy of *Musa*, which comprise about 50 species, has never been fully resolved and remains a subject of debate.

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Table 1. List of *Musa* and *Ensete* species and cultivars used in this study

Accession name	ITC code[a]	Species/Group	Section	Subspecies/Subgroup
Calcutta 4	0249	acuminata	*Eumusa*	*burmanicoides*
Galeo	0259	AA[b]	*Eumusa*	
Pisang Mas	0653	AA[b]	*Eumusa*	sucrier
Musa acuminata ssp. *banksii*	0896	acuminata	*Eumusa*	*banksii*
Guyod	0299	AA[b]	*Eumusa*	
Musa balbisiana type Cameroun	0246	balbisiana	*Eumusa*	
Honduras	0247	balbisiana	*Eumusa*	
Musa schizocarpa	0890	schizocarpa	*Eumusa*	
Musa laterita	0627	laterita	*Rhodochlamys*	*laterita*
Musa velutina	0011	velutina	*Rhodochlamys*	*velutina*
Musa velutina	0638	velutina	*Rhodochlamys*	*velutina*
Musa mannii	1411	sanguinea	*Rhodochlamys*	*sanguinea*
Kluai Bou	0528	ornata	*Rhodochlamys*	*ornata*
Musa ornata	0637	ornata	*Rhodochlamys*	*ornata*
Musa beccarii	1070	beccarii	*Callimusa*	*beccarii*
Musa peekelii ssp. *peekelii*	0917	peekelii	*Australimusa*	*peekelii*
Musa textilis	0539	textilis	*Australimusa*	*textilis*
Musa maclayi type Hung Si	0614	maclayi	*Australimusa*	
Kawaputa	0927	fe'i	*Australimusa*	
Ensete gilletii	1398	gilletii	related genus	

[a] ITC code: code assigned by the INIBAP Transit Centre (Leuven).
[b] AA: edible diploid *acuminata* cultivar.

Based on plant phenotype and chromosome number, the genus has traditionally been divided into four sections as proposed by Cheesman (1947): *Eumusa* (x = 11), *Rhodochlamys* (x = 11), *Callimusa* (x = 10) and *Australimusa* (x = 10). This classification was followed by subsequent authors; however its validity has been questioned, at least for some sections, and the inclusion of some newly described species appeared problematic (Simmonds, 1962; Shepherd, 1999; De Langhe, 2000). For example, Argent (1976) created a separate section *Ingentimusa* for *M. ingens* (x = 7). Recently, traditional approaches based on the analysis of morphology and chromosome number were supplemented by analyses at the DNA sequence level, such as ribosomal gene spacer length (Lanaud et al., 1992), RFLP (Gawel et al., 1992; Jarret et al., 1992) and AFLP (Wong et al., 2002; Ude et al., 2002a, b). These studies revealed shortcomings of the current *Musa* classification, and Wong et al. (2002) proposed the grouping of *Musa* species into only two sections, joining *Australimusa* and *Callimusa*, and *Eumusa* and *Rhodochlamys*.

Despite recent introduction of the molecular tools, they have been applied to a larger range of *Musa* species than the cytogenetic methods. Nuclear DNA content is one of the basic characteristics commonly used in taxonomic studies of higher plants (Bennett et al., 2000). Until now, genome size has been determined for about 3,500 angiosperm species (Hanson et al., 2003). To our knowledge, nuclear DNA content was estimated in only six species of *Musa* (Doležel et al., 1994; Lysák et al., 1999; D'Hont et al., 1999; Asif et al., 2001; Kamaté et al., 2001). All studies were performed using flow cytometry, which has been shown to be a rapid and reliable method for nuclear DNA content determination in plants (Doležel, 1991). Most of the estimates were focused on *M. acuminata* and *M. balbisiana*; the two main progenitors of cultivated banana varieties. Genome size of some triploid (Lysák et al., 1999; Kamaté et al., 2001) and tetraploid (Kamaté et al., 2001) clones was also determined. Clearly, there is an urgent need to expand the knowledge to other species of *Musa*.

A karyotype, which is characterized by the number and morphology of chromosomes, is an important characteristic of a species. Unfortunately, chromosome studies in *Musa* have been hampered by the small chromosome size and lack of suitable chromosome landmarks. Until now, it was not possible to identify individual chromosomes of *Musa*. Ribosomal RNA (rRNA) genes (45S rDNA comprising the 18S–5.8S–26S rRNA genes and intergenic spacers, and 5S rDNA comprising 5S rDNA gene and spacer) are found universally in plants, with multiple copies of coding sequences and spacers organized in tandemly repeated units localized at a few discrete chromosomal sites. This facilitates their visualization and Doleželová et al. (1998) and Osuji et al. (1998) showed that fluorescence in situ hybridization (FISH) with probes for ribosomal DNA may be used to identify a subset of chromosomes in *Musa*. Until now, only *M. acuminata* and *M. balbisiana* and their hybrids were studied with this technique. It was found that while diploid species possessed two 45S rDNA loci (one pair of chromosomes), the number of 5S rDNA loci could vary from four to six (Doleželová et al., 1998; Osuji et al., 1998; Valárik et al., 2002).

This study was undertaken to determine nuclear genome size and genomic distribution of 5S and 45S rDNA in a set of *Musa* species representing its four traditional accepted sections, with the aim to expand the number of species where these characteristics are known, and aid in clarification of the relationship between the species and sections of *Musa*.

Material and methods

Plant material

One *Ensete* and nineteen *Musa* species and clones (hitherto referred to as accessions) were obtained from the INIBAP Transit Centre (ITC), Katholieke Universiteit Leuven (Belgium) as in vitro rooted plantlets (Table 1). *Ensete*, belonging to the second genus of the family Musaceae, was included as reference taxa for comparison with *Musa*. Seedlings were transferred to soil and grown in a heated greenhouse. Soybean plants (*Glycine max* L. cv.

Polanka) were grown in a greenhouse from seeds obtained from Sempra, Uherský Ostroh.

Determination of genome size

Approximately 50 mg of midrib was cut from a young *Musa* leaf and transferred to a glass Petri dish. About 10 mg of a young leaf of soybean (*Glycine max* L. cv. Polanka) with 2C = 2.5 pg DNA (Doležel et al., 1994) was added and served as an internal reference standard. The tissues were chopped simultaneously in 1 ml of Otto I buffer (0.1 M citric acid, 0.5 % v/v Tween 20; Otto, 1990). The crude suspension of isolated nuclei was filtered through a 50-µm nylon mesh. Nuclei were then pelleted (300 *g*, 5 min), resuspended in 200 µl Otto I and incubated for 1 h at room temperature. Finally, 600 µl of Otto II buffer (0.4 M Na_2HPO_4; Otto, 1990), supplemented with 50 µg/ml RNase and 50 µg/ml propidium iodide (PI), was added. Samples were analysed using Partec PAS flow cytometer (Partec GmbH, Münster, Germany) equipped with 488-nm argon laser. The gain of the instrument was adjusted so that the peak representing soybean G_1 nuclei appeared approximately on channel 200 on a histogram of relative fluorescence intensity when using a 512-channel scale. About 5,000 nuclei were analysed at rate 10–25 nuclei/s. Three plants were measured per accession. Analysis of each plant was repeated three times on different days. Nuclear DNA content was calculated from individual measurements following the formula:

$$\text{2C nuclear DNA content} = \frac{2.5 \times \text{G}_1 \text{ peak mean of } \textit{Musa}}{\text{G}_1 \text{ peak mean of } \textit{Glycine}}$$

Mean nuclear DNA content was then calculated for each plant. Genome size, which represents one copy of nuclear genetic information (equal to 1C), was further determined considering 1 pg DNA equal to 0.978×10^9 bp (Doležel et al., 2003).

Localization of rDNA loci

FISH probe for 45S rDNA was obtained by labelling a *Radka*1 DNA clone containing the 26S rRNA gene (Valárik et al., 2002) with digoxigenin-11-dUTP or biotin-16-dUTP (Roche). 5S rDNA probe *(Radka 2)* was prepared from 400 bp insert of a part of the 5S rRNA gene (Valárik et al., 2002). Both probes were labelled by PCR using M13 direct and reverse primers.

Metaphase spreads were prepared according to Doleželová et al. (1998). The slides were treated with 100 mg/ml RNase in a 2× SSC solution at 37 °C for 1 h in a humid chamber, washed 3 × 5 min in 2× SSC at room temperature. After two washes in 2× SSC the slides were treated in 4 % paraformaldehyde for 10 min at room temperature, washed in 2× SSC, dehydrated in ethanol series, and air dried. Prior to hybridization, the probes were mixed in a solution containing 50 % formamide, 10 % dextran sulphate, 0.12 % SDS in 2× SSC and 5 ng/µl salmon sperm DNA. 1 µl of probe in 30 µl hybridization mixture per slide was used. The hybridization mixture was denatured at 70 °C for 10 min and incubated on ice for 10–15 min before being added to the preparations. The chromosomes together with the probes were denatured at 70 °C for 5 min and the hybridization was performed overnight at 37 °C in a humid chamber. The slides were then washed in 2× SSC at 42 °C and rinsed in a stringent washing solution of 20 % formamide in 0.1× SSC at 42 °C for 10 min, followed by several washes in 2× SSC and 4× SSC (0.2 % Tween). The sites of digoxigenin- and biotin-labelled probe hybridization were detected using anti-digoxigenin fluorescein (Roche) and streptavidin conjugated to Cy3 (Sigma), respectively. Finally, the preparations were counterstained with DAPI (0.2 µg/ml) and mounted in Vectashield antifade solution (Vector Laboratories).

The preparations were evaluated using an Olympus BX60 microscope equipped with optical filter sets appropriate for DAPI, fluorescein and Cy3 fluorescence. The images of DAPI, fluorescein and Cy3 fluorescence were acquired separately with a b/w CCD camera, which was interfaced to a PC running the ISIS software (Metasystems, Altlussheim, Germany). The images were superimposed after contrast and background optimization.

Statistical analysis

Statistical analysis was performed using the NCSS 97 statistical software (Statistical Solutions Ltd., Cork, Ireland). One-way ANOVA and a Bonferroni's (All–Pairwise) multiple comparison test were applied to analyze variation in nuclear DNA content. The significance level $\alpha = 0.01$ was used. Hierarchical cluster analysis was used to determine relationship among diploid *Musa* accessions, with 2C nuclear DNA content, number of chromosomes and number of 45S rDNA sites as the variables. The unweighted pair-group linkage type was used for clustering with Manhattan distance method and standard deviation used for scaling.

Results

Flow cytometric analysis of propidium iodide-stained nuclei resulted in histograms of relative nuclear DNA content with two dominant peaks corresponding to G_0/G_1 nuclei of *Musa* and *Glycine*, respectively (Fig. 1). 2C nuclear DNA content was determined based on the ratio of G_0/G_1 peak positions and ranged from 1.130 to 1.561 pg in accessions representing the genus *Musa*. *E. gilletii*, which was the outgroup in this work, had 2C DNA content of 1.210 pg (Table 2). Within the section *Eumusa*, the lowest nuclear DNA content was found in both accessions of *M. balbisiana* (2C = 1.130 and 1.133 pg). The highest DNA content was found in *M. schizocarpa* (2C = 1.377 pg). An intermediate 2C DNA content (1.224–1.266 pg) was observed in *M. acuminata*. The differences between the three species of *Eumusa* were statistically significant. Although the differences between the accessions of *M. acuminata* were small (max. 3.4 %), some of them were statistically significant as well (Table 2).

Smaller interspecific variation of 2C DNA content was observed within the section *Rhodochlamys* (1.191–1.299 pg) but differences between some species were still statistically significant. The smallest range of nuclear DNA content variation (7.8 %) was found between the species of *Australimusa*, with 2C value ranging from 1.435 to 1.547 pg. The highest 2C nuclear DNA content in this study (1.561 pg) was found in *M. beccarii*, the only representative of the section *Callimusa* in this study. Bonferroni's multiple comparison test revealed 10 groups distinguishable according to relative nuclear DNA content (Table 2), three of them being represented by only one accession *(M. schizocarpa, M. ornata* and *M. textilis)*. Five groups comprised representatives of at least two different sections; two of them involved accessions belonging to *Musa* and *Ensete*.

FISH with the probe for 45S rDNA revealed distinct hybridization sites on one pair of nucleolar organizing chromosomes in all accessions of *Eumusa* and *Australimusa* (Table 2, Figs. 2a, b, d). In *Eumusa*, the sites of hybridization coincided with secondary constrictions of both chromosomes of the homologue pair. On the other hand, secondary constrictions were not detectable on one of the homologues in all four accessions of *Australimusa*, indicating only one active nucleolar organizer region (Fig. 2d). A variable number of 45S rDNA sites was observed in the section *Rhodochlamys* (Fig. 2c). While three accessions possessed two sites (one chromosome pair), two accessions representing *M. ornata* were characterized by four 45S loci (two chromosome pairs). However, two additional loci were detected as very weak hybridization signals. They were located in the terminal position and did not coincide with secondary constrictions. *M. beccarii* was characterized by six sites of 45S rDNA genes (three chromosome pairs) (Fig. 2e). Among the six strong hybridization clusters, only two coincided with the secondary constriction. The highest number (four pairs) of 45S rDNA loci was observed in *E. gilletii*. The intensity of the

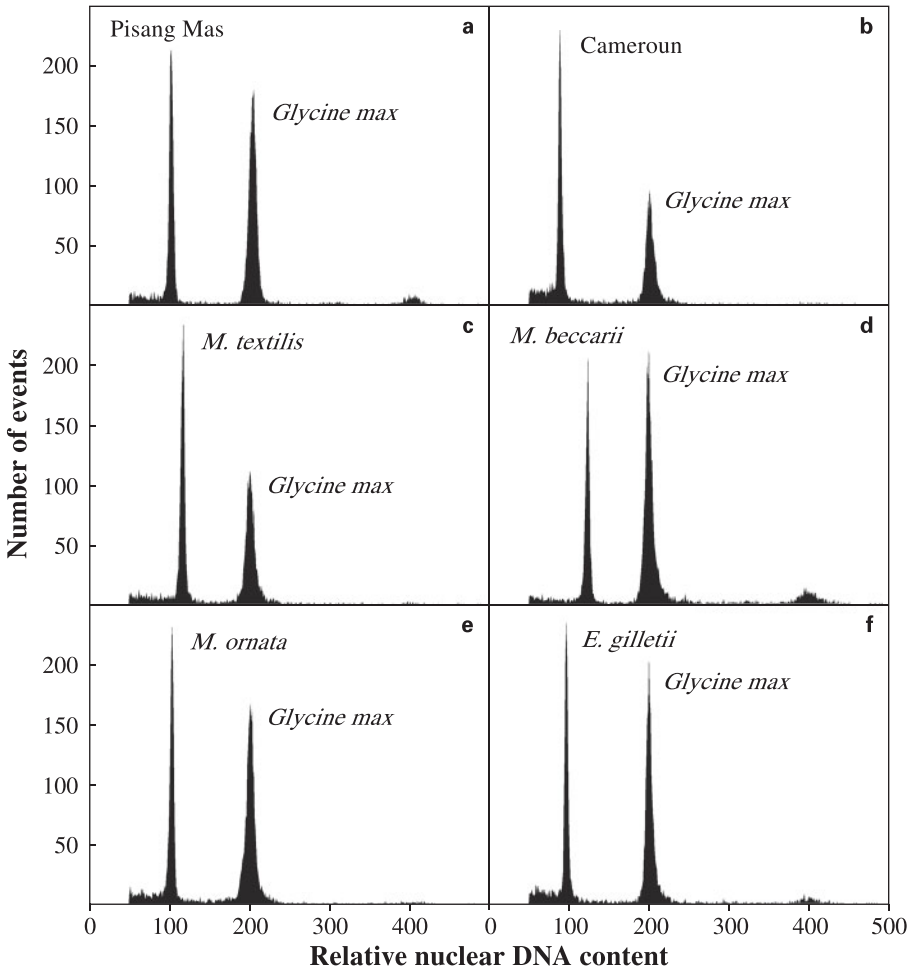

Fig. 1. Histograms of relative nuclear DNA content obtained after flow cytometric analysis of propidium iodide stained nuclei isolated from accessions of *Musa* (a–e) and *Ensete* (f). G_0/G_1 peaks of unknown sample and *Glycine max* (2C = 2.500 pg), which served as internal reference standard, are clearly visible. 2C nuclear DNA content was determined based on the ratio of G_0/G_1 peak positions. (a) "Pisang Mas" (2C = 1.243 pg); (b) "Cameroun" (2C = 1.130 pg); (c) *M. textilis* (2C = 1.435 pg); (d) *M. beccarii* (2C = 1.561 pg) and (e) *M. ornata* (2C = 1.299 pg); (f) *E. gilletii* (2C = 1.210 pg).

Table 2. DNA content, genome size, Bonferroni's groups, and numbers of chromosomes, 5S and 45S rDNA sites

Accession name	ITC code[a]	2C nuclear DNA content [pg]		Mean genome size [Mbp/1C][b]	Bonferroni's DNA content grouping[c]										Number of chromosomes	No. of 5S rDNA sites	No. of 45S rDNA sites
		Mean	± SD														
Calcutta 4	0249	1.226	0.004	627			C	D							22	8	2
Galeo	0259	1.224	0.007	626			C	D							22	6	2
Pisang Mas	0653	1.243	0.010	635				D	E						22	5	2
Musa acuminata ssp. *banksii*	0896	1.263	0.004	646					E						22	6	2
Guyod	0299	1.266	0.008	647					E						22	6	2
Musa balbisiana type Cameroun	0246	1.130	0.009	578	A										22	4	2
Honduras	0247	1.133	0.002	579	A										22	4	2
Musa schizocarpa	0890	1.377	0.005	704							G				22	6	2
Musa laterita	0627	1.221	0.011	624			C	D							22	4	2
Musa velutina	0011	1.242	0.009	635				D	E						xxx	xxx	xxx
Musa velutina	0638	xxx	xxx	xxx											22	6	2
Musa mannii	1411	1.269	0.005	649					E						22	4	2
Kluai Bou	0528	1.191	0.005	609		B									22	4	4
Musa ornata	0637	1.299	0.009	664						F					22	4	4
Musa beccarii	1070	1.561	0.007	798										J	18	5	6
Musa peekelii ssp. *peekelii*	0917	1.547	0.006	791										J	20	4	2
Musa textilis	0539	1.435	0.008	734								H			20	4	2
Musa maclayi type Hung Si	0614	1.476	0.007	755									I		20	4	2
Kawaputa	0927	1.498	0.006	766									I		20	4	2
Ensete gilletii	1389	1.210	0.007	619		B	C								18	6	8

[a] ITC code: code assigned by the INIBAP Transit Centre (Leuven).
[b] Mean genome size: one copy of nuclear genome (1C); 1 pg = 0.978×10^9 bp (Doležel et al., 2003).
[c] Statistical analysis was performed using mean values calculated for individual plants (n = 3) and significance level $\alpha = 0.01$. DNA content is not significantly different within each class identified by the same letter.

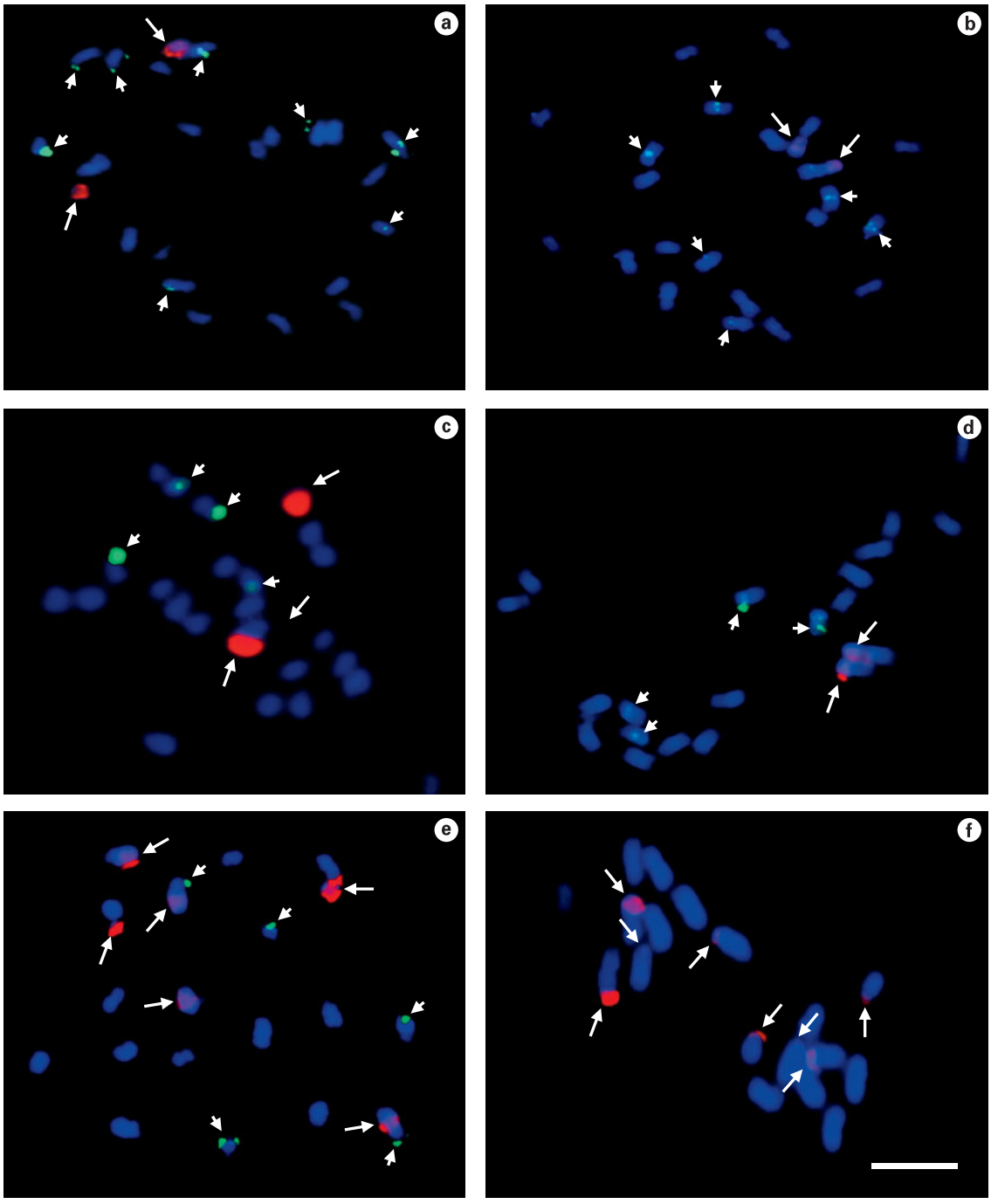

Fig. 2. Genomic distribution of 5S and 45S rDNA determined on mitotic metaphase chromosomes of *Musa* and *Ensete* after FISH with labelled probes. The probes were detected either with fluorescein (yellow-green colour) or Cy3 (red colour). Chromosomes were counterstained with DAPI (blue colour). 45S and 5S rDNA loci are labelled by long arrows and short arrows, respectively. (a) "Calcutta 4"; (b) *M. schizocarpa*; (c) *M. laterita*; (d) *M. textilis*; (e) *M. beccarii*; and (f) *E. gilletii* (only 45S rDNA probe was used). Bar = 5 μm.

signals on different chromosome pairs differed, indicating a difference in the copy number of the 18S–5.8S–26S rRNA genes (Fig. 2f).

A significantly larger variation was observed in the number of 5S rDNA loci (Table 2). In the *Eumusa* section, the number of 5S rDNA sites ranged from four to eight, five loci were observed in the seed sterile clone "Pisang Mas". All *Rhodochlamys* accessions comprised two pairs of chromosomes bearing 5S rRNA genes except *M. velutina*, which had three chromosome pairs bearing 5S rDNA. In this case, two sites were major and four sites were minor, with significantly lower copy number. In contrast to a large variation in the number of 5S rDNA loci in

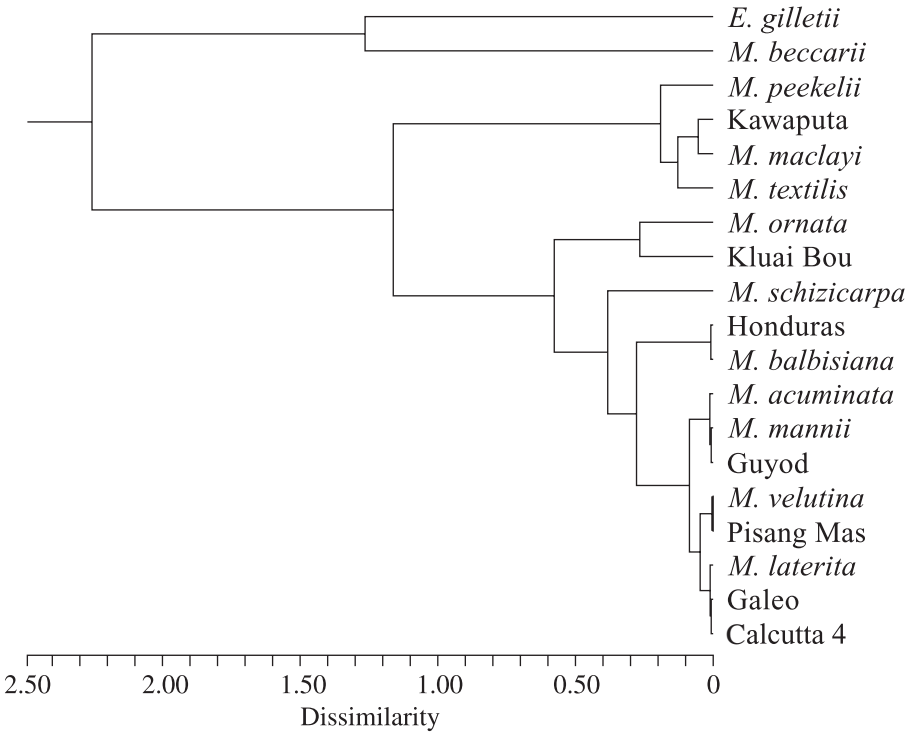

Fig. 3. Dendrogram representing genetic relationship between *Musa* species and clones based on genome size, number of chromosomes and number of 45S rDNA loci. *E. gilletii*, which belongs to the genus *Ensete* of the same family Musaceae, was used as outgroup.

other sections, all *Australimusa* accessions possessed four 5S rDNA sites (Fig. 2d). *M. beccarii (Callimusa)* and *E. gilletii* contained 5S rRNA gene clusters on five and six chromosomes, respectively. Two of the five 5S rDNA sites in *M. beccarii* were localized at terminal positions of chromosomes with interstitially localized signals of 45S rDNA (Fig. 2e).

Hierarchical cluster analysis of genome size, chromosome number and the number of 45S rDNA loci separated the 19 *Musa* accessions and one *Ensete* accession into four distinct clusters (Fig. 3). Because genome size and rDNA analyses were not available for both accessions of *M. velutina*, the data were pooled to facilitate the analysis. Due to large variation in the genomic distribution, 5S rDNA was excluded from this analysis. The accessions of *Eumusa* and *Rhodochlamys* formed a single cluster; the second cluster represented *Australimusa*, while *M. beccarii* and *E. gilletii* were separated as individual species. Within the *Eumusa-Rhodochlamys* group, *M. balbisiana*, *M. shizocarpa* and *M. ornata* formed distinct subgoups, clearly separated from the accessions of *M. acuminata*, *M. mannii*, *M. laterita* and *M. velutina*, which formed a tight subgroup. The two wild *acuminata* accessions were at the extremes of this cluster: "*M. acuminata*", which belongs to the geographically southernmost subspecies *banksii*, and "Calcutta 4", a variety of the northernmost subspecies *burmannica*.

Discussion

Our findings on genomic distribution of rDNA loci and genome size significantly expand the number of *Musa* species where these key characteristics of the nuclear genome are known. This work includes representatives of all *Musa* sections and provides the first global perspective of the whole genus. One of the most interesting observations is the absence of variation in the number of 45S rDNA loci within individual sections (Table 2). With the exception of *M. acuminata* and *M. balbisiana*, this seems to be true also for the number of the 5S rDNA loci.

There is a general agreement that *M. acuminata* is a complex of subspecies. The diversity in genome size and genomic distribution of 5S loci observed within *M. acuminata* is on line with the previous studies on rDNA distribution (Doleželová et al., 1998; Osuji et al., 1998; Valárik et al., 2002), genome size (Doležel et al., 1994; Lysák et al., 1999; Kamaté et al., 2001) and variation at DNA level (Ude et al., 2002a). Hybridization between subspecies differing in the number of 5S rDNA sites could explain the origin of clonally propagated and seed sterile diploid clones with odd numbers of 5S rDNA sites observed previously (Doleželová et al., 1998; Osuji et al., 1998) and in this study. Until now, a consensus on the number of *acuminata* subspecies has not been achieved (Simmonds, 1962; Ude et al., 2002a). Among others, the subspecies rank of *M. acuminata* ssp. *banksii* (Simmonds, 1962) has been questioned and Argent (1976) argued for the species rank. Subsequent analysis of *Alu* sequences placed *banksii* out of the cluster formed by other accessions of *M. acuminata* (Baurens et al., 1998). Our analyses support these observations. It is noteworthy that the largest difference in genome size observed within *M. acuminata* between ssp. *banksii* and "Calcutta 4", a variety of the northernmost subspecies *burmannica*, correlates with the geographical distance of their areas of distribution.

Recent AFLP studies clearly discriminate *M. balbisiana* from other species of *Eumusa* (Ude et al., 2002a; Wong et al., 2002). Its globular seed form stands alone in the section (Simmonds, 1962) and its resistance to several physical stresses is remarkable (Shepherd, 1999). This particular place in the section is confirmed by the present study (Fig. 3). The nuclear genome size of the two accessions of *M. balbisiana* differed significantly from any species within *Eumusa* (Table 2). *M. balbisiana* seems to have the smallest genome of all *Musa* species and the present data, together with those of Lysák et al. (1999), suggest its negligible intraspecific variation in genome size. On the other hand, the species is not homogenous with respect to genomic distribution of 5S rDNA, and accessions with either four or six loci were described previously (Doleželová et al., 1998; Osuji et al., 1998) and in this study. Recently, larger than expected morphological variation and variation at DNA sequence level was observed within *M. balbisiana* (Lanaud et al., 1992; Sotto and Rabara, 2000; Ude et al., 2002a). Based on the AFLP analysis, Ude et al. (2002a) divided *M. balbisiana* accessions into two groups. Interestingly, one of the groups contained "Singapuri" and "Butohan", which possess the same number of 5S loci (Doleželová et al., 1998; Osuji et al., 1998). It is tempting to speculate that the separation of *M. balbisiana* into two groups reflects the number of 5S loci.

M. schizocarpa has traditionally been placed within the section *Eumusa* (Simmonds, 1962). The present estimate of its nuclear genome size reveals that the genome is the largest within *Eumusa* and significantly different from other *Eumusa* species. While our results separate this species from other *Eumusa* species (Fig. 3), the AFLP analysis of Ude et al. (2002b) and inter-*Alu* genomic profiling of Baurens et al. (1998) showed close affinity of *M. schizocarpa* and the *M. acuminata* subspecies complex. The fact that the number of rDNA loci in *M. schizocarpa* as observed in this study is not different from other *Eumusa* species indicates a similarity between the two species.

Several authors noted a close relationship between the species of *Rhodochlamys* and *Eumusa* at both the morphological and the molecular level (Simmonds, 1962; Gawel et al., 1992; Jarret and Gawel, 1995; Shepherd, 1999; De Langhe, 2000), and Wong et al. (2002) suggested merging them into one section. With one exception, our results support this view (Fig. 3). Nuclear genome sizes of *M. velutina*, *M. laterita* and *M. mannii* overlap with those of the studied *M. acuminata* wild species and edible clones, and the numbers of rDNA loci fall within the same range. As for the two accessions of *M. ornata* their genome sizes are significantly different from any other *Eumusa* species (Table 2) and, perhaps more importantly, the number of 45S rDNA loci differs from any other *Rhodochlamys* and *Eumusa* species. Thus the merge of the two sections should be considered at a species level. The reason why the two accessions of *M. ornata* appeared as outliers is not clear. The possibility of incorrect classification should not be overlooked since Häkkinen and Sharrock (2002) point to the fact that *M. ornata* is often confused with *M. rosacea* (also classified within *Rhodochlamys*). On the other hand, Shepherd (1999) described fertile hybrids between *M. ornata* and *M. flaviflora*, which did not show any meiotic disturbances, and speculated on a hybrid origin of *M. ornata* after crossing *M. flaviflora* with *M. velutina*. As in any other similar study, the issue of the material may be questioned. To avoid any doubts and to permit reanalysis of the same accessions in the future, we have used materials obtained from the INIBAP Transit Centre.

Shepherd (1999) concluded that *Australimusa* is a recent group and that its species are isolated by geographic rather than by reproductive barriers, as is the case with the *M. acuminata* subspecies. The AFLP study of Ude et al. (2002b) revealed a low heterogeneity within the section. Our results are on line with these views. At the same time, the group is clearly discriminated from other *Musa* sections by the genome size, which does not overlap with that of *Eumusa* and *Rhodochlamys*. Small variation in genome size within the section, and the absence of variation in rDNA loci, make it difficult to speculate on the relationships between individual species of *Australimusa* and edible fe'i bananas, whose origin remains unclear (Jarret et al., 1992; Sharrock, 2001). However, if only genome size is considered, the fe'i banana analysed in this work ("Kawaputa"), seems to be closer to *M. maclayi* than to other species, which would support the view of Simmonds (1956) and du Montcel (1990) that *M. maclayi* is a wild ancestor of fe'i bananas.

Based on small differences at the DNA sequence level, it has been suggested that the sections *Callimusa* and *Autralimusa* be merged (Gawel et al., 1992; Wong et al., 2002). *M. beccarii* has been included in the section *Callimusa* (Daniels et al., 2001), although not by all authors (Simmonds, 1962). If *M. beccarii* is considered as belonging to *Callimusa*, our results do not support the notion of similarity between the two sections. Genome size of *M. beccarii* is the only affirmative parameter, as it is similar to that of the *Australimusa* species. The number of chromosomes and the number of 45S rDNA loci differ dramatically. Unfortunately, only one species of *Callimusa* could be analysed in this work. The chromosome number of *M. beccarii* ($2n = 18$), which differs from 2n = 20 reported for *Callimusa* (Simmonds, 1962) opens the possibility that other species of this section would have produced different data. Whatever the outcome of future studies will be, our results indicate that *M. beccarii* should not be included in a *Callimusa-Australimusa* merger. This conclusion contradicts the AFLP study of Wong (2002), which indicated a close relationship between *M. beccarii* and *M. textilis*. Earlier results of Gawel et al. (1992) obtained with RFLP analysis placed *M. beccarii* within the *M. acuminata* subspecies complex, which is at odds with our results as well. On the other hand, the number of chromosomes and rDNA sites indicates a similarity with *E. gilletii*, which was used as an outgroup in this study. This is in line with Simmonds (1962), who hypothesized that the lower chromosome numbers of *M. beccarii* and *E. gilletii* reflected their ancient origin, and considered them relics of the evolution of Musaceae.

During the last ten years, there has been a shift towards using molecular tools to assess the genetic difference and propose a new classification of genus *Musa*. Our results show that these studies should be completed by cytogenetic analyses. Although only a subset of *Musa* species could be analyzed, this study provides novel data on nuclear genome size and genomic distribution of rDNA loci. Localization of rDNA provided the first chromosome landmarks, which may be used to identify

specific chromosomes or groups of chromosomes. Higher number of chromosome-specific cytogenetic markers will facilitate the analysis of *Musa* karyotype in detail and reveal structural chromosome changes that accompanied the evolution and speciation of *Musa* and shed light on its phylogenesis.

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