Heredity **68** (1992) 147–156

Received 26 February 1991

Variation of ribosomal gene spacer length among wild and cultivated banana

C. LANAUD,* H. TEZENAS du MONTCEL, M. P. JOLIVOT, J. C. GLASZMANN & D. GONZALEZ DE LEON

*BIOTROP, CIRAD, BP 5035, 34032 Montpellier Cédex, et *IRFA, c/o, INIBAP, 7bd de la Lironde, 34980 Montferrier-sur-Lez, France*

The diversity of rDNA spacer length (IGS) of 107 wild and cultivated clones of banana belonging to different genetic groups, was studied using a sulphonated wheat rDNA probe. The length of the rDNA unit was between 10 and 12.6 kb and was highly variable. Fifteen different types of IGS could be observed. IGS diversity within the *acuminata* complex was greater among the diploid cultivars than among the triploid cultivars. Variation was also found among BB genotypes and confirms the diversity of this species. As opposed to previous studies, based on enzymatic or polyphenolic markers, there was no obvious relation between the rDNA structure and the classification into genetic groups. More particularly, the IGS variations did not differentiate between the A and B genomes. This analysis, however, indicates a relationship between the geographical origin and the IGS structure of Thai and Indonesian clones.

Keywords: banana, genetic diversity, intergenic spacer (IGS), rDNA, RFLP.

Introduction

Banana is a vegetatively propagated plant which originates from South East Asia. Its genetic organization is based on two diploid species *Musa acuminata* and *Musa balbisiana*, which possess two different genomes, A and B, respectively. Wild, seminiferous and usually non-parthenocarpic genotypes are still encountered in these two species (Fig. 1). Some parthenocarpic AA diploid cultivars can also be found in Asia and the Pacific but most cultivated bananas are triploid and are consumed as a vegetable or dessert (Simmonds, 1966; Champion, 1967). *Musa acuminata* has diversified into several sub-species. Shepherd (1987) studied the cytology of hybrids between these sub-species and identified seven regional groups. They differ from each other by the presence of one or several translocations, or by one inversion. All the diploid cultivars studied so far have been shown to be heterozygous for one or more translocations (Dodds, 1943; Dodds & Simmonds, 1946, 1948; Wilson, 1946; Hutchinson, 1966; Shepherd, 1987; Dessauw, 1989). Some of these cultivars may represent hybrids between the regional groups.

The genetic origin of triploid cultivars is not well known. It probably involved several diploid cultivars which were partly sterile and produced non-reduced gametes. The taxonomy of triploid cultivars and classification into genetic groups was first studied by Simmonds & Shepherd (1955) using 15 morphological characters that differentiate *Musa acuminata* from *Musa balbisiana*. The probable genetic origin and classification of the cultivars are drafted on Fig. 2 (Tézenas du Montcel, 1989).

Enzymatic and polyphenolic markers were applied by Jarret *et al.* (1986a, b), Jarret (1987), Horry & Jay (1988) and Horry (1989) to the study of genetic diversity and to shed new light on the extent of variability among wild and cultivated genotypes and the possible origin of diploid and triploid cultivars.

Nevertheless, many uncertainties remain. In a study of genetic diversity of bananas using RFLP markers, we analysed the variation of ribosomal RNA gene spacer length. The structure of RNA genes (rDNA) has been described in many plant species (Hemleben *et al.*, 1988). The rDNA genes are organized in tandemly repeated units ranging from 250 to 22,000 copies per genome (Rogers & Bendich, 1987). Each unit contains coding sequences which give rise to 18, 5.8 and 25 S after cleavage of an rRNA precursor, and a non-coding intergenic spacer (IGS) which contains signals for transcription initiation and termination. The rDNA encoding regions tend to be highly conserved between different species and genera (Appels & Dvorak, 1982)

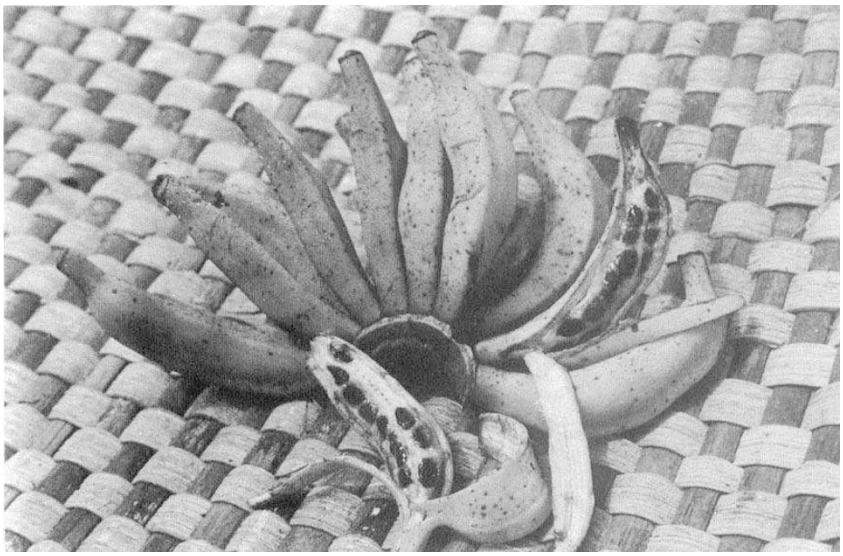

Fig. 1 A diploid and fertile wild banana clone.

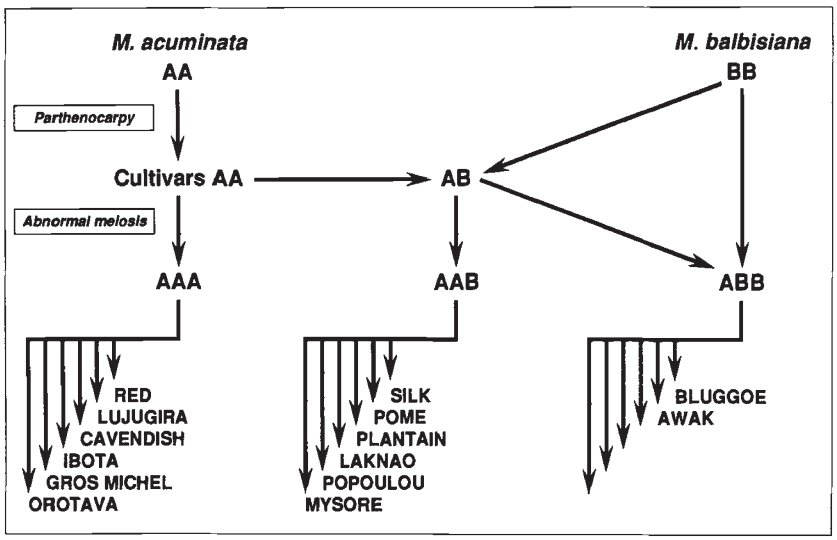

Fig. 2 Possible origin of the main genetic groups and sub-groups of the bananas (revised by Tezenas du Montcel, 1989).

while the IGS usually shows extensive intra and inter-specific variation.

rDNA varies in three features: the number of copies of each type of unit, IGS and the conserved region. These three features do not evolve in a similar way. The copy number may evolve rapidly, faster than the IGS, and changes may occur even among the somatic cells of an individual (Rogers & Bendich, 1987) or under stress (Cullis, 1981, 1986). The IGS evolves faster than the coding region (Appels & Honeycutt, 1986; Zimmer *et al.*, 1988).

Each type of variation involves different mechanisms of evolution. Variation in the conserved region of rDNA results from accumulation of point mutations and has been used for phylogenetic studies (e.g. in *Fusarium* spp., Guadet *et al.*, 1989). This kind of variation may correspond to ancestral differentiation, similar to that revealed by isozyme markers. IGS variation may be interpreted otherwise. The IGS is made of variable numbers of small repeated sub-units. In many plants, variation in IGS is related to the number of subrepeat-units that the spacer contains (Appels & Dvorak, 1982) which can be generated by unequal crossovers between tandem arrays of IGS units (Coen *et al.*, 1982; Rogers *et al.*, 1986). This IGS length is potentially useful as a marker of diversity and evolution between species; in this paper, we present some of its variation among wild and cultivated bananas.

Materials and methods

Plant materials

The materials surveyed consisted of 107 banana clones tentatively classified in different genetic groups and 'sub-groups' at IRFA (Institut de Recherches pour les Fruits et Agrumes) (CIRAD/IRFA, 1986, 1987, 1988/89) (Table 1). They are representative of the IRFA banana collection based in Guadeloupe (French West Indies) and of accessions more recently collected from Thailand and Indonesia (Tézenas du Montcel, 1985).

RFLP procedures

DNA was extracted from leaves according to the method of Dellaporta *et al.* (1983) modified by Cordesse *et al.* (1990). DNA was digested by *Eco*RI or *Bam*HI, or both of these enzymes, and then separated by gel electrophoresis in 0.8 per cent agarose with a 40 mM Tris-acetate pH 7.5, 2 mM EDTA buffer. DNA was then transferred to a Nytran nylon membrane and hybridized with the rDNA probe of wheat PTA 71 (Gerlach & Bedbrook, 1979). The probe was labelled by sulphonation of cytosine residues using an Orgenic's kit. Detection of hybridizations was done using antibodies against sulphonated cytosine and the alkaline phosphatase linked antibody reaction as described by Lebacq *et al.* (1988).

Analysis of rDNA diversity

A synthetic visualization of rDNA diversity was obtained using a modification of the principal component analysis, developed by Benzecri (1973), termed Factor Analysis of Correspondences (FAC). Each type of spacer was defined as a character with presence and absence as the two possible states for each clone and without taking into account the variation in IGS copy number. In this analysis, the FAC identifies several independent axes which are linear combinations of the characters and reflect the largest part of the variation. Each clone is located along these axes.

Results

EcoRI and BamHI restriction map of rDNA unit

Restriction patterns obtained after digestion by *Eco*RI, *Bam*HI, and after a double digestion by these two restriction enzymes are shown in Fig. 3. The interpretation, based on the simplest cases, led to the model proposed in Fig. 4; in this model, the two *Eco*RI sites (Ec) and the two *Bam*HI sites (Ba) were located in the conserved part of the rDNA unit. These sites would result in the two fragment lengths observed, i.e. 1 and 2.9 kb, within the conserved region as shown in Fig. 4.

Even in the case of partial digestion, such as that shown in lane 2 of Fig. 3, the results could be interpreted within the framework of our model without ambiguities.

The length of the entire rDNA unit was confirmed after digestion of total banana DNA by *Eco*RV, which has a unique restriction site in the rDNA unit (data not shown).

Molecular diversity of rDNA spacer length

Distribution of spacer length among the genetic groups. Fifteen different spacer lengths were observed in the clones studied. The spacer lengths varied from 6.1 to 8.7 kb. Up to five, and in one case six spacer lengths could be observed in several genotypes.

Examples of banding patterns are shown in Fig. 5. The results are presented in Table 1 in terms of each genetic group.

In wild diploid species (AAw and BBw), the mean number of spacers present in a single banana clone was 2.2 and 2.1; this average was 3.1 in diploid cultivars, and varied between 2.9 and 3.4 among the groups of triploid cultivars (Table 1).

The various rDNA spacers showed distinctive distributions between wild and cultivated genotypes or between genetic groups (Table 1).

Type 3 was commonly present in all cultivars groups, absent from the wild AA group and in one wild BB accession.

Types 5 and 6 were found in wild and cultivated AA diploids. They were completely absent from the AAA and ABB groups, while type 6 only was present in AAB cultivars and AAAB cultivars.

Types 1, 2, 5 and 13 were present, albeit infrequently, in the AA diploids only.

Type 14 was present only in seven of the 13 triploid cultivars, three of which were dwarf mutants.

Type 15 was found only in Pisang Cici, a wild AA diploid.

Synthetic analysis of genetic diversity. The FAC was performed on the following matrix: 107 banana clones × 30 characters that correspond to the presence or absence of the 15 different spacer lengths observed. A better representation of the total diversity was obtained after excluding from the active variables four characters that corresponded to very rare spacer types.

The first two axes accounted for 33 per cent of the total diversity. The characters that contribute mostly to the construction of the first axis are the spacer types 6, 7 and 9, and to a lesser extent 13 and 14. Those that

Table 1 Variation in the number of different types of IGS found in each genetic group

Genetic group	Provisional classification	Number of individuals scored for rDNA	Mean number of spacer types per individual	Number of spacer types in the genetic group	Number of individuals having each type of spacer														
					Spacer type	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
AAw	Malaccensis	3				0	0	0	0	0	2	1	1	2	0	0	0	0	0	0
	Siamea	3				0	0	0	0	1	0	1	1	1	2	1	0	0	0	0
	Microcarpa	4				0	2	0	2	0	1	0	1	1	0	0	0	1	0	0
	Miscellaneous	6				2	0	0	0	2	0	2	1	2	2	0	3	0	0	1
	Total AAw	16	2.2	13		2	2	0	2	3	3	4	4	6	4	1	3	1	0	1
AAcv	Malaccensis der.	2				0	0	0	0	0	0	2	0	2	0	0	0	0	0	0
	Microcarpa der.	3				0	1	0	1	0	1	1	2	3	0	0	1	0	0	0
	Banksii der.	4				0	0	4	0	0	0	0	1	0	0	0	1	0	0	0
	Miscellaneous	15				0	0	9	5	3	3	6	6	5	7	5	3	3	0	0
	Total AAcv	24	3.1	12		0	1	13	6	3	4	9	9	10	7	5	5	3	0	0
BBw		7	2.1	5		0	0	1	0	0	2	0	3	6	3	0	0	0	0	0
AB		1	—	4		0	0	0	1	0	0	1	0	1	0	1	0	0	0	0
AAA	Cavendish	4				0	0	0	0	0	0	1	5	3	1	5	0	0	1	0
	Gros Michel	2				0	0	2	0	0	0	1	1	0	0	1	0	0	0	0
	Red	5				0	0	4	1	0	0	4	4	1	0	1	0	0	0	0
	Orotava	2				0	0	1	0	0	0	0	0	2	0	0	0	0	0	0
	Ibota	5				0	0	5	0	0	0	5	5	5	0	0	0	0	0	0
	Miscellaneous	9				0	0	4	0	0	0	3	6	5	3	1	3	0	1	0
	Total AAA	27	3.0	7		0	0	16	1	0	0	14	21	16	4	8	3	0	2	0
AAB	Plantain	7				0	0	7	0	0	6	1	0	7	0	0	0	0	1	0
	Popoulou	3				0	0	3	0	0	0	0	0	3	0	0	0	0	0	0
	Silk	2				0	0	2	0	0	0	2	1	1	0	0	0	0	2	0
	Laknau	3				0	0	1	3	0	0	1	3	4	0	0	2	0	0	0
	Miscellaneous	6				0	0	4	0	0	1	0	1	5	0	2	0	0	2	0
	Total ABB	21	2.9	9		0	0	17	3	0	7	4	5	20	0	2	2	0	5	0
ABB	Bluggoe	2				0	0	2	0	0	0	2	1	0	1	0	0	0	1	0
	Miscellaneous	5				0	0	3	2	0	0	1	3	4	1	0	2	0	1	0
	Total ABB	7	3.4	8		0	0	5	2	0	0	3	4	4	2	0	2	0	2	0
AAAB		3	3.0	3		0	0	3	0	0	3	0	0	3	0	0	0	0	0	0
ABBB		1	—	1		0	0	0	0	0	0	0	0	1	0	0	0	0	0	0

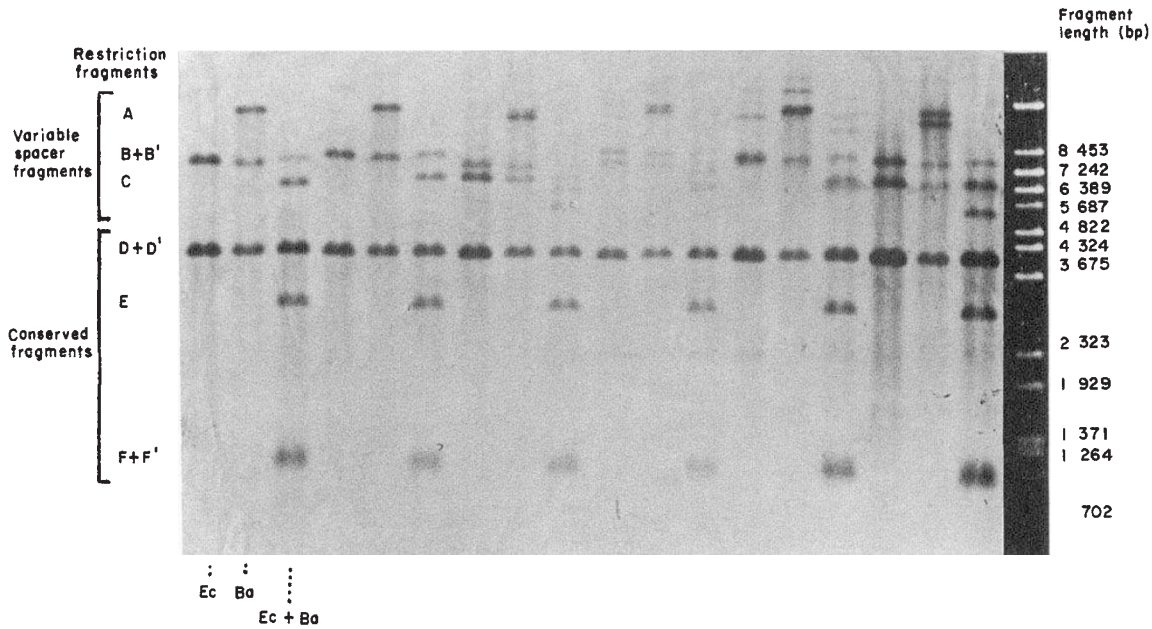

Fig. 3 Total DNA from six different clones digested by *Eco*RI (Ec) (lanes 1, 4, 7, 10, 13, 16), *Bam*HI (Ba) (lanes 2, 5, 8, 3, 14, 17) and *Eco*RI + *Bam*HI (Ec + Ba) (lanes 3, 6, 9, 12, 15, 18), and hybridized with a wheat rDNA probe.

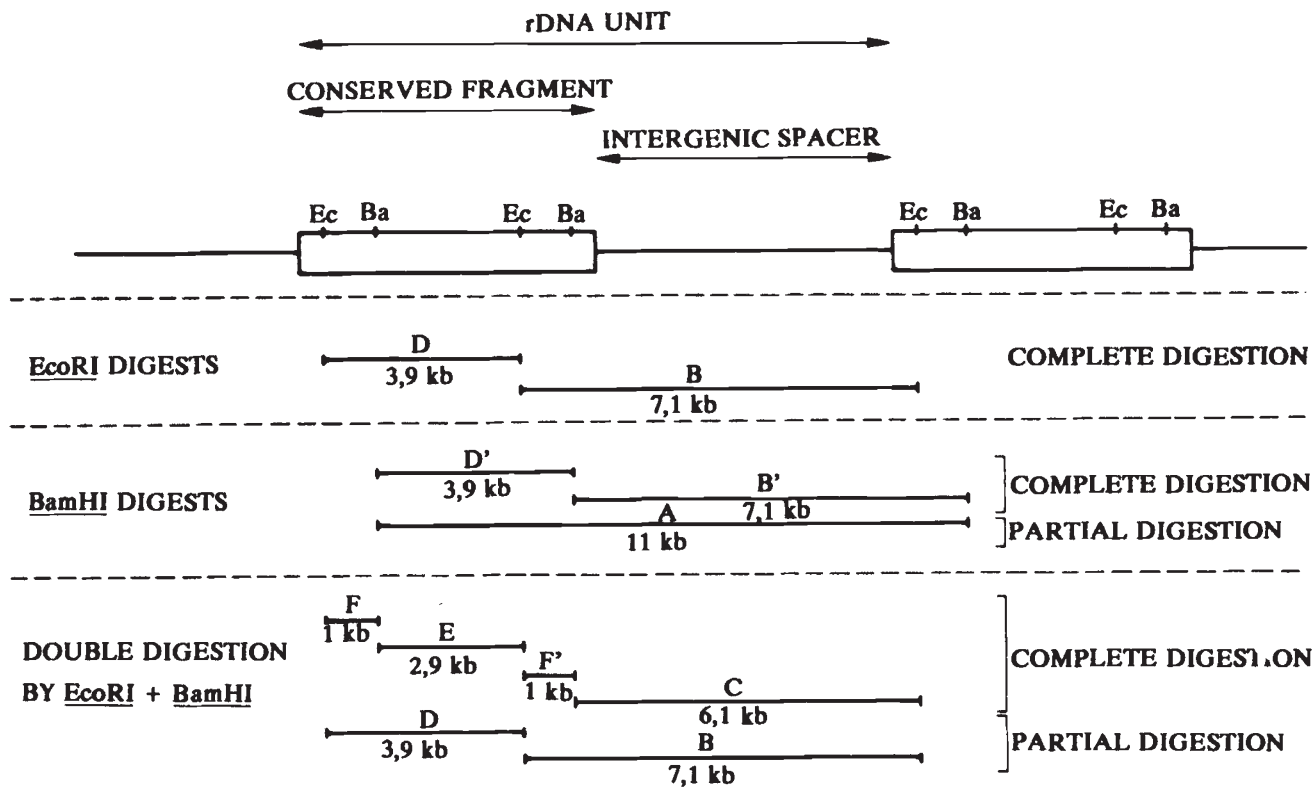

Fig. 4 Interpretation of the location of restriction sites on rDNA unit. This diagram is based on the results obtained for the clone shown on lanes 1–3 on Fig. 3. Ec = *Eco*RI restriction site, Ba = *Bam*HI restriction site. A, B, B′, C, D, D′, E, F, F′ = restriction fragments obtained after enzyme digestion.

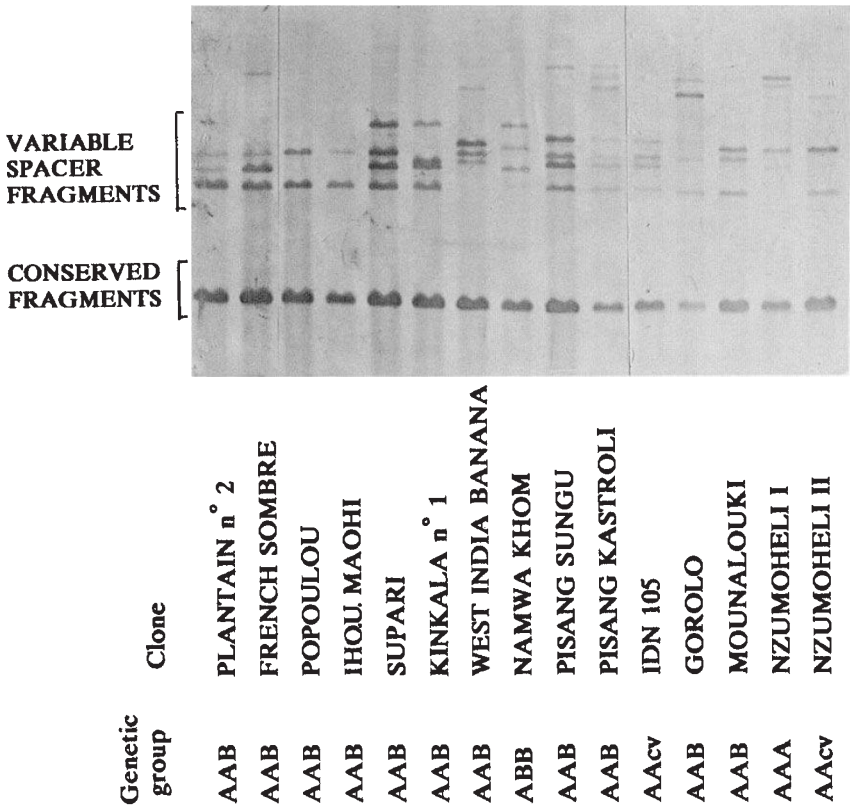

Fig. 5 Polymorphism of rDNA in banana cultivars. A sulphonated probe of wheat rDNA was used to reveal the diversity.

contribute mostly to the construction of the second axis are spacers 3, 4, 8 and 12, and to a lesser extent 10 and 11.

In this study the extent and organization of diversity is first compared with the tentative genetic group and sub-group classification (Fig. 6). They are then analysed according to the assumed geographical origin of the clones (Fig. 7a and b).

There was no obvious relation between the rDNA structure revealed by this analysis and the classification into genetic groups (AA, BB, AAA, AAB, ABB). More particularly, this analysis does not differentiate between the A and B genomes. Thus, most groups were scattered all over the first FAC plane. Only the AAA cultivars had a narrower distribution on the left side of the plane. Additional observations about each genetic group are as follows.

Among the wild bananas, the pool of *Musa balbisiana*, usually considered as homogeneous, displayed some variability, even though the number of clones studied was low. Only two were identical to one another. The *Musa acuminata* wild genotypes were also highly diversified even within sub-species such as *malaccensis* or *siamea*.

The variability of the diploid cultivars encompassed the total diversity revealed across the collection of clones studied.

Among triploid cultivars, some sub-groups, such as 'Red', 'Cavendish', 'Silk', 'Laknau', 'Bluggoe' displayed within sub-group diversity. By contrast, the five clones of the 'Ibota' sub-group were similar in spite of their varied origins. Moreover, the three cultivars of the 'Popoulou' sub-group showed no detectable differences in rDNA structure in spite of important morphological differences between them.

In the Plantain sub-group, all the cultivars that originated from Africa (including the Comores accession) contained the same types of IGS but differed from the only plantain cultivar originating from Indonesia (Pisang Tandok).

If we consider the geographical origin of the clones, additional information may be obtained.

The distribution of the clones from Indonesia and Thailand on the first FAC plane is given in Fig. 7a and b respectively. Approximately half of the material surveyed had these two geographical origins. The distribution of each of these two sets of clones on the first FAC plane was apparently non-random. Above the first axis, both sets of clones intersected, but below it, they tended to have a disjunct distribution. Thus, the Indonesian clones were concentrated on the lower left quarter of the plane, while the Thai clones occupied the lower right quarter, irrespective of the genetic group to which each belonged. One exception was the

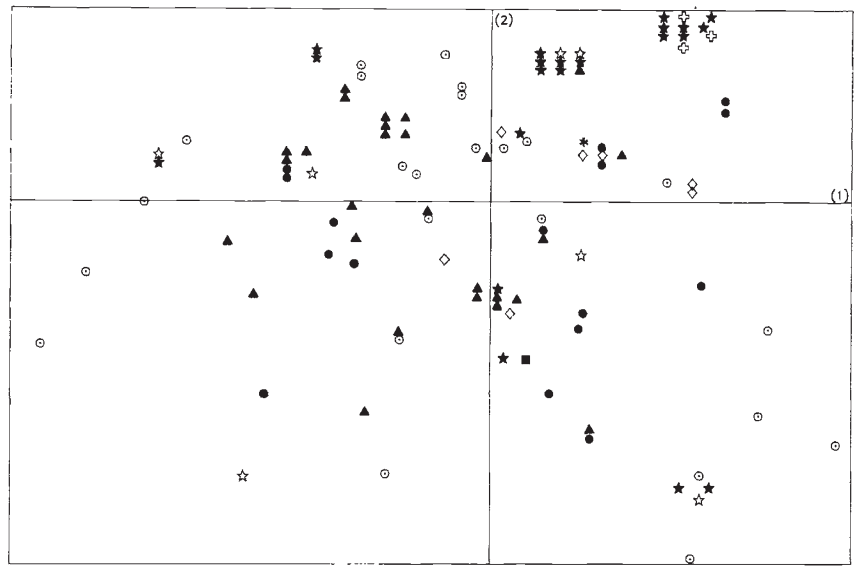

Fig. 6 Diversity of the 107 clones of banana belonging to the different genetic groups represented on the first two axes of the FAC. (●) AAs, (⊙) AAcv, (◇) BB, (■) AB, (▲) AAA, (★) AAB, (✯) ABB, (✱) ABBB, (✣)AAAB.

AA cultivar, THA052, considered to be a Thai cultivar, but which was included in the Indonesian pool of clones.

Note that the *zebrina* accession, which was found in Martinique (FWI), but originated from Indonesia (Simmonds, 1962; Horry, 1989) fell among the Indonesian accessions on the first FAC plane; however only two of the *siamea* accessions fell among the Thai clones. *M. acuminata siamea* is known to have originated from Thailand.

Discussion

The analysis of 107 banana clones revealed that the rDNA spacer was highly variable. The length of the rDNA unit was between 10 and 12.6 kb, and was thus similar to that found in many other plant species (Hemleben *et al.*, 1988). The 15 different types of IGS length observed differed by about 100–400 bp. Variation in IGS length is generally considered to be a reflection of the variation in the number of sub-repeat units located within the IGS; these subrepeat units range from 100 to 350 bp (Appels & Dvorak, 1982; Rogers & Bendich, 1987). Our results were therefore consistent with these observations.

Several types of IGS can be encountered in a single individual. The clones SUPARI and KINKALA number 1, for example, show four different spacer types each (lanes 5 and 6 on Fig. 5). Cytological studies have shown the hybrid nature of all the diploid cultivars that have been analysed. This may explain the larger mean number of types of spacer found within AAcv clones (3.1) as compared to that found in AAw accessions (2.2) (Table 1).

Within the *acuminata* complex (AA and AAA), IGS diversity was greater among the diploid than among triploid cultivars. This confirms the hypothesis of a loss of genetic variability during the domestication process as was recently put forward by Horry & Jay (1988) and Horry (1989) on the basis of isozyme and polyphenolic marker data. In the BB group, eight different types of spacers were encountered despite the small number of clones analysed. Both these observations and those of Horry (1989) demonstrate that *M. balbisiana* is far from being a genetically homogeneous species.

The extensive polymorphism of rDNA detected between and within individuals may be related to the vegetative propagation of banana clones. This would allow the maintenance of new recombinant forms; hybrid forms, giving rise to sterile and parthenocarpic clones, have been preferentially conserved during domestication (Simmonds, 1962). Moreover, barriers to genetic exchange, due to the presence of translocations in hybrid clones, may have limited the relative uniformity of rDNA types that would be expected after successive generations of crossing.

As opposed to enzymatic or polyphenolic markers (Jarret & Litz, 1986a; Horry & Jay, 1988; Horry, 1989), variation in rDNA did not distinguish between the A and the B genomes but seemed to reveal a relationship between the geographical origin and the IGS structure of the clones. This could be related to differences in their respective modes of evolution. IGS rDNA is generally considered to be a non-transcribed region. However, it has been shown that it may be associated with transcriptional activity in some organisms such as *Xenopus laevis* (Moss, 1983; Reeder *et al.*, 1983). In maize, for example, it has been suggested that

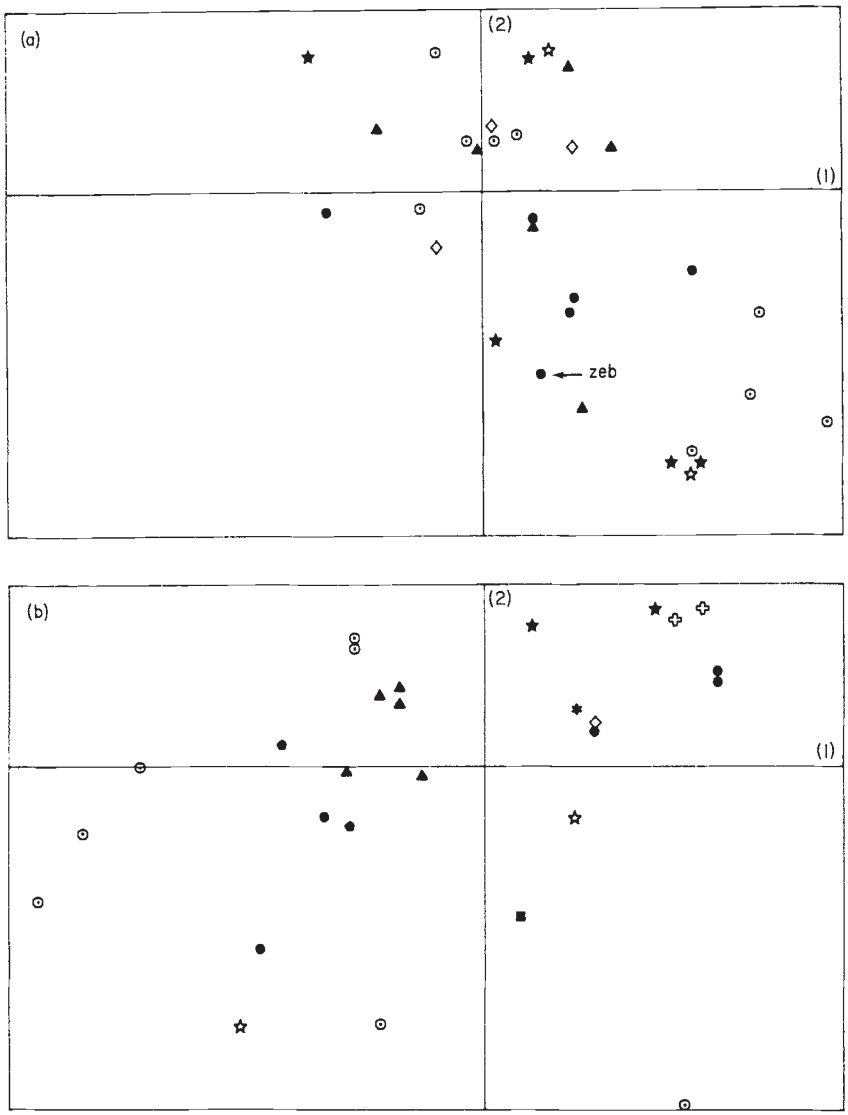

Fig. 7 Distribution of banana clones originating from Indonesia (a) and Thailand (b) on the first two axes of the FAC. Key as for Fig. 6.

the spacer sub-units may play an important role in rDNA transcription (McMullen *et al.*, 1986; Toloczyki & Feix, 1986). Variation in IGS length has been related to several selection factors: change in the rDNA IGS composition has been reported during cycles of selection in various plant species such as barley (Saghai-Maroof *et al.*, 1984) and maize in which there was an increase in the frequency of the larger IGS types (Rocheford *et al.*, 1990). In *Drosophila melanogaster*, it has been suggested that the presence of longer spacer elements may favour a greater rDNA transcriptional activity (Cluster *et al.*, 1987). rDNA variation has also been correlated with ecological factors. In wild populations of *Triticum dicoccoides* from Israel, Flavell *et al.* (1986) demonstrated that rDNA polymorphism is correlated with climatic factors; the variance in the total number of the different fragments was explained by the combination of three temperature variables. In 18 wild populations of barley from Israel and Iran, Saighai-Maroof *et al.* (1990) found a correlation between allele frequencies of two rDNA loci and nine factors of the physical environment, indicating the different adaptative properties of the genotypes marked by the spacer length variants. In rice, Cordesse *et al.* (1990) also mention an association between ecotypes and rDNA variations: short IGS types were found in the Japonica group and longer ones in the Indica group. The variation within the group Japonica is also noteworthy. This group was found to be homogeneous on the basis of 15 isozyme loci (Glaszmann, 1988) and RFLPs using 10 probes and five enzyme/probe combinations, (Wang & Tanksley, 1989). However, marked

differences in rDNA structure were reported between the tropical forms and the temperate forms of this group (Sano & Sano, 1989). In sugarcane, a similar tendency has been observed within the most variable species *Saccharum spontaneum* in which Indian forms and more meridional ones can be distinguished with rDNA, as opposed to the randomly distributed isozyme variation (Glaszmann *et al.*, 1989, 1990).

In our study, the FAC suggested that there may be a relationship between geographical origin and rDNA variation in the case of the Indonesian and the Thai clones. However, another hypothesis could also explain the organization of this diversity in acordance with the hypothesis of multiple places of independent domestication of Simmonds (1962). Indonesian and Thai clones belonging to different genetic groups could have originated from different ancestors that have each marked the present geographical groups.

In our results, and those obtained in rice and in sugarcane, isozyme classification did not correspond to IGS rDNA classification. rDNA may evolve faster than other genomic sequences as a result of mechanisms in addition to the accumulation of point mutations. This raises the question of whether IGS variation may reflect more recent changes than those revealed by isozyme or other RFLP markers.

Acknowledgements

We thank IRFA for providing us the materials surveyed, and X. Perrier and C. Dubois for helping us with the biometrical analysis. We are grateful to Dr M. Delseny for his useful comments.

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