Curr Genet (1994) 25:265–269

Current Genetics

Maternal inheritance of chloroplast genome and paternal inheritance of mitochondrial genome in bananas (*Musa acuminata*)

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Received: 14 May 1993/Accepted: 16 August 1993

Abstract. Restriction fragment length polymorphisms (RFLPs) were used as markers to determine the transmission of cytoplasmic DNA in diploid banana crosses. Progenies from two controlled crosses were studied with heterologous cytoplasmic probes. This analysis provided evidence for a strong bias towards maternal transmission of chloroplast DNA and paternal transmission of mitochondrial DNA in *Musa acuminata*. These results suggest the existence of two separate mechanisms of organelle transmission and selection, but no model to explain this can be proposed at the present time. Knowledge of the organelle mode of inheritance constitutes an important point for phylogeny analyses in bananas and may offer a powerful tool to confirm hybrid origins.

Key words: *Musa acuminata* – Inheritance – Chloroplast – Mitochondria

Introduction

Bananas are a basic food for millions of people in the inter-tropical area and represent a very important economical crop. Several pathogens seriously threaten banana production and numerous breeding programs have been undertaken in order to create resistant varieties (reviewed by Novak 1992). One requirement of these breeding programs is improved knowledge of the genus *Musa*, i.e., a thorough evaluation of the germplasm and a better understanding of the diversity and evolution of species and subspecies within this genus. Complementary to studies based on morphological and agronomic characters, molecular analyses have been undertaken to evaluate diversity and phylogeny among diploid bananas. These molecular investigations are based on isozymes (Horry 1989) as well as nuclear and cytoplasmic RFLP markers (Gawel and Jarret 1991a, b; Carreel et al., unpublished results). Little is known about the genetic determinism of agronomic characters in bananas and more especially about a possible involvement of the cytoplasmic genomes. De Langhe (1969) reports differences between reciprocal crosses. Information on the mode of inheritance of cytoplasmic organelles in bananas should be of importance for phylogenetic studies as well as for comprehension of the genetic determinism of some agronomic characters. Until recently, plant cytoplasmic organelles were considered to be strictly maternally transmitted to progeny. However recent studies have revealed numerous exceptions to this rule. In particular, chloroplast DNA may be biparentally inherited as in alfalfa (Lee et al. 1988; Masoud et al. 1990) or *Pelargonium* (Metzlaff et al. 1981). Exceptions to the maternal inheritance of mitochondrial DNA are even scarcer but have been reported in coast redwood and incense cedar (Neale et al. 1989; Neale quoted by McCown and Ellis 1989). No analysis of cytoplasmic transmission has been published to-date on bananas. The present study was undertaken in order to determine the inheritance of chloroplast and mitochondrial DNA in *Musa acuminata*. To this end, controlled crosses were studied using heterologous cytoplasmic probes.

Materials and methods

Plant material. Controlled crosses were carried out at the Guadeloupe CIRAD-FLHOR center (F.W.I.). Leaf samples were obtained from parents and progeny of two crosses (female Calcutta 4 × male Banksii; female SF265 × male Banksii). Neither Calcutta 4 nor SF265 bear hermaphrodite flowers, thus excluding any possibility of selfing. Progenies were respectively composed of 25 and nine plants. The validity of the crosses was substantiated by RFLP analysis of progeny compared to parent genotypes using homologous nuclear probes (Fauré et al. 1993). Complete names of parents are given in Table 1. Leaves were harvested in Guadeloupe, sent in an icebox to the CIRAD-BIOTROP laboratory where they were lyophilized and ground with a mechanical mill. The powder thus obtained was conserved in small airtight flasks until DNA extraction.

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Table 1. Plant material

Name	Classification	CIRAD-FLHOR code number
Calcutta 4	*Musa acuminata* ssp. *burmannicoïdes* type Calcutta 4 (diploid, 2n=22, wild non-parthenocarpic banana)	II.04.01.003.001
Banksii	*Musa acuminata* ssp. *banksii* type Banksii (diploid, 2n=22, wild non-parthenocarpic banana)	II.04.01.004.001
SF265 (NBB11/SF265)	*Musa acuminata* (diploid, 2n=22, parthenocarpic cultivar)	II.04.20.004.020

Probes. Chloroplast DNA transmission was studied using two heterologous probes: one of these corresponds to the cytochrome f (Cyt f) gene from pea chloroplast DNA (Willey et al. 1984) and the other corresponds to the Rubisco large subunit gene of spinach. The Rubisco probe was provided by R. Mache (Laboratoire de Biologie Moléculaire Végétale, Grenoble university). Mitochondrial DNA transmission was analyzed using three heterologous probes: the first one corresponds to the ATPase-α gene from sunflower (Recipon 1989), the two others correspond to the cytochrome oxidase subunit I (Cox I) gene from wheat and to the cytochrome oxidase subunit III (Cox III) gene from wheat. Cox I and Cox III probes were provided by B. Lejeune (Laboratoire de Biologie Moléculaire Végétale, Paris XI university).

RFLP methods. Total DNA extractions were performed according to the protocol of Hoisington (1992), but with the extraction buffer of Gawel and Jarret (1991c). Digestions were carried out according to the supplier's recommendations (BRL), but with 2.5 units/μg DNA of restriction enzyme. *Dra*I and *Eco*RV were selected out of ten restriction enzymes that had been surveyed for restriction polymorphism using 13 genomic probes and nine banana clones (Fauré et al. 1993). After DNA restriction, a phenol:chloroform:isoamyl-alcohol (25:24:1) purification step was carried out. Restricted DNA (7.5 μg/lane) was then separated in 0.8%-TAE agarose gels at 1.04 V/cm for 9 h. The gels were depurinated in 0.25 N HCl for 10 min, denatured in 0.4 N NaOH for 30 min, and then blotted onto Hybond N^+ membranes (Amersham). Probes were labelled with ^{32}P-αdCTP using the random primer labelling method of Feinberg and Vogelstein (1983). Incorporation of radioactive nucleotides was checked by chromatography on PEI-cellulose F. Prehybridization, hybridization and washes were performed in a hybridization oven (Appligène). Membranes were placed in glass tubes and the following buffer was added: 6×SSPE, 0.5% SDS, 5×Denhart's, and 25 μg/ml of sheared herring sperm DNA. This buffer was supplemented with dextran sulphate to a final concentration of 8% (w/v) for hybridization. Membranes were then washed at 68 °C for 30 min in each of the following buffers: 2×SSPE, 2×SSPE-0.1% SDS, 0.1×SSPE-0.1% SDS. They were exposed to X-ray film (Kodak X-OMAT) at −80 °C for 4 days with one intensifying screen.

Results

Maternal inheritance of chloroplast DNA

Parental accession DNAs were digested with *Dra*I or *Eco*RV and hybridized with each of the Rubisco and Cyt f probes. No polymorphisms were detected with the Rubisco probe (Table 2). Hybridization of the Cyt f probe onto total DNA of SF265 and Banksii revealed no pattern differences with either restriction enzyme. Calcutta 4 and Banksii could be differentiated by the Cyt f probe hybridized onto DNA digested with *Dra*I. The 25 F_1 individuals obtained from the cross Calcutta 4×Banksii displayed the same pattern as the female parent, Calcutta 4, since they all had a 9.5-kb fragment whereas the Banksii male parent had a 5-kb fragment (Table 2, Fig. 1). Hybrid patterns were not observed.

Paternal inheritance of mitochondrial DNA

Polymorphic patterns were revealed between the parental accessions of the two crosses by the Cox I probe with both enzymes. These parental accessions could also be differentiated by the Cox III probe, but only with *Dra*I. The ATPase-α probe revealed no differences between SF265 and Banksii (Table 3). In each case where a probe differentiated parental accessions of a cross, progeny patterns were identical to that of the male parent (Table 3). For example, when hybridized with the Cox III probe, the female parent Calcutta 4, had a 5.6-kb fragment whereas the Banksii male parent and the 25 F_1 individuals had a 9.2-kb fragment (Fig. 2). Hybrid patterns or patterns identical to that of the female parent were never observed.

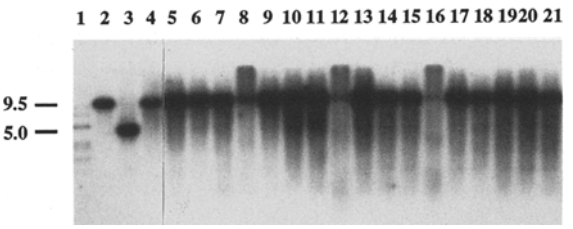

Fig. 1. Maternal inheritance of chloroplast DNA in progeny from *M. acuminata* spp. *burmannicoides* type Calcutta 4×*M. a.* spp. *banksii* type Banksii cross. Total DNA was digested with *Dra*I and hybridized with a Cyt f probe. *Lane 1*, molecular weight marker (Raoul, Appligène®); *lane 2*, female parent Calcutta 4; *lane 3*, male parent Banksii; *lanes 4–21*, progeny of the cross Calcutta 4 × Banksii

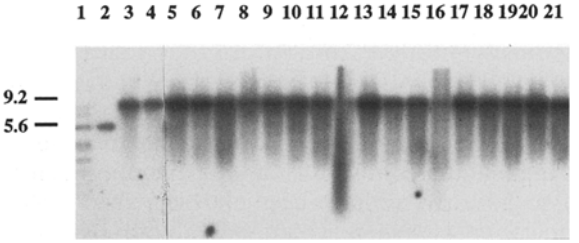

Fig. 2. Paternal inheritance of mitochondrial DNA in progeny from *M. acuminata* spp. *burmannicoides* type Calcutta 4 × *M. a.* spp. *banksii* type Banksii cross. Total DNA was digested with *Dra*I and hybridized with a Cox III probe. *Lane 1*, molecular weight marker (Raoul, Appligène®); *lane 2*, female parent Calcutta 4; *lane 3*, male parent Banksii; *lanes 4–21*, progeny of the cross Calcutta 4 × Banksii

Table 2. Crosses analyzed and sizes (kb) of fragments revealed by chloroplastic probes

Probe	Restriction enzyme	Crosses					
		Calcutta 4	Banksii	Progeny (25)	SF265	Banksii	Progeny (9)
Rubisco	*Dra*I	3.4+1.6	3.4+1.6	3.4+1.6	3.4+1.6	3.4+1.6	3.4+1.6
	*Eco*RV	3.3	3.3	3.3	3.3	3.3	3.3
Cyt f	*Dra*I	9.5	5.0	9.5	5.0	5.0	5.0
	*Eco*RV	14.7	14.7	14.7	14.7	14.7	14.7

Table 3. Crosses analyzed and sizes (kb) of fragments revealed by mitochondrial probes

Probe	Restriction enzyme	Crosses					
		Calcutta 4	Banksii	Progeny (25)	SF265	Banksii	Progeny (9)
Cox I	*Dra*I	10+6.5+4.7+3.9	6.0+2.9	6.0+2.9	14.9+3.9	6.0+2.9	6.0+2.9
	*Eco*RV	10.5+5.0	9.0+3.2	9.0+3.2	14.0+2.2	9.0+3.2	9.0+3.2
Cox III	*Dra*I	5.6	9.2	9.2	13.0+9.2	9.2	9.2
	*Eco*RV	9.5+2.3	9.5+2.3	9.5+2.3	9.5+2.3	9.5+2.3	9.5+2.3
ATPase-α	*Dra*I	8.5+2.6	10.0+5.6	10.0+5.6	10.0+5.6	10.0+5.6	10.0+5.6
	*Eco*RV	3.1	3.5	3.5	3.5	3.5	3.5

Discussion

We studied the inheritance of cytoplasmic genomes in *M. acuminata* using heterologous cytoplasmic probes hybridized onto total DNA. A more direct and precise analysis would have been to compare restriction patterns of cytoplasmic DNAs of parents and progeny. However, cytoplasmic DNA extraction techniques are strenuous and time consuming, require fresh leaves and thus cannot be routinely applied to bananas. Working with total DNA is a commonly-applied procedure and has been proven to give specific hybridization on cytoplasmic genomes (Breiman 1987; Lee et al. 1988; Polans et al. 1990; Gastony and Yatskievych 1992). The strict uniparental inheritance patterns observed in our study strongly suggests that probes actually hybridized with cytoplasmic DNA and not with nuclear DNA. Confusion could also have stemmed from the homologies between chloroplast and mitochondrial genomes that have been reported in several plant species (Stern and Palmer 1984; Lejeune et al. 1988). However, the clear transmission differences observed in bananas effectively exclude the hypothesis that the mitochondrial genome is revealed with chloroplast probes and vice-versa.

The small number of probes used in this study can be assumed to be sufficient to demonstrate the mode of inheritance of cytoplasmic organelles since the genome of these organelles is transmitted as a whole to progenies so that, in a study of inheritance, even a few probes can be regarded as representative of the entire genome. Similar experimental designs have been used to elucidate the inheritance of organelles in *Sequoia sempervirens* (Neale et al. 1989) and chelanthoid ferns (Gastony and Yatskievych 1992).

Two chloroplastic probes were used in order to study the mode of inheritance of chloroplasts in bananas. No polymorphisms were detected with the Rubisco probe. The length of this gene is known to be conserved in angiosperms (Zurawski et al. 1981), but polymorphic patterns could be expected since polymorphisms have already been detected with this probe in bananas (Gawel and Jarret 1991b), *Nicotiana* (Kung et al. 1982), *Oryza* species (Kanno and Hirai 1992), and cocoa (Laurent et al. 1993). The hybridization of the Cyt f heterologous chloroplast probe on total DNA of parental accessions and progeny of a controlled cross of bananas revealed a unique pattern among the progeny. This pattern was identical to that of the female parent, suggesting a maternal transmission of chloroplasts. This result is in general accord with the commonly observed maternal inheritance of the chloroplast genome in plants, where the main exceptions are alfalfa (Lee et al. 1988; Masoud et al. 1990), *Pelargonium* (Metzlaff et al. 1981) and several conifers (Neale et al. 1986; Szmidt et al. 1987; Neale and Sederoff 1989; Neale et al. 1989; Sutton et al. 1991).

Three mitochondrial probes were used in order to study the mode of inheritance of mitochondria in bananas. Surprisingly, the analysis of patterns obtained by the hybridization of these probes revealed the identity of progeny patterns with male parent patterns. This paternal inheritance of mitochondria is very unusual since mitochondrial DNA appears to be strictly maternally inherited in most animals and plants (reviewed by Neale et al. 1989). To-date, such a paternal inheritance of mitochondria has only been reported in some conifers (Neale et al. 1989; Neale quoted by McCown and Ellis 1989). Independent inheritance of chloroplasts and mitochondria has already been observed in loblolly pine (Neale and Sederoff 1989) and spruce (Sutton et al. 1991).

As the size of the progenies analyzed was limited, we cannot confirm a strict maternal inheritance of the chloroplast genome nor a strict paternal transmission of

the mitochondrial genome. The observation of larger progenies and additional crosses may allow us to detect biparental and/or male patterns in the case of chloroplast probes (biparental and/or female patterns in the case of mitochondrial probes). However, our observations provide evidence for a strong bias towards maternal transmission of chloroplasts and paternal transmission of mitochondria in *M. acuminata*.

Once the mode of transmission of cytoplasmic organelles is known, the problem of the mechanism and determinism of this transmission must be examined. The use of DAPI (4′,6-diamidino-2-phenyl-indole) staining in conjunction with epifluorescence microscopy has shown that plastid DNA was not present in the generative or sperm cells of pollen from numerous plant species (Corriveau and Coleman 1988). Exclusion of organelles at this stage seems to be the most common behavior, but they might be eliminated during generative and/or sperm cell maturation, fertilization, or else shortly after fertilization (Sears 1980; van Went 1992). For example, a specific digestion of organelles of paternal (or maternal) origin may be possible in the egg cytoplasm (Bell and Duckett 1976, quoted by Gastony and Yatskievych 1992). A nuclear control of organelle transmission has been demonstrated in *Pelargonium* (Tilney-Bassett and Birky 1981) and alfalfa (Masoud et al. 1990). There may also be an unequal input of organelles from the gametes of each parent and the least frequent may be diluted out in subsequent cell divisions (Schumann and Hancock 1989). This last phenomenon, in particular, may explain the cases of biparental inheritance.

Transmission determinism cannot be accounted for at the moment in bananas. None of the hypotheses on the determinism of parental transmission mentioned in the literature can be rejected, but the independent inheritance of chloroplasts and mitochondria observed in our study suggests that two separate mechanisms of organelle transmission and selection exist in *M. acuminata* since paternal chloroplasts and maternal mitochondria must be eliminated. Analysis of additional crosses with larger progenies and the use of different and complementary techniques, such as DAPI staining and electron microscopical observations of gametes and gametophytes, should help to gain a better understanding of organelle inheritance in bananas.

The particular mode of inheritance of cytoplasmic organelles observed in bananas offers interesting possibilities for the analysis of *Musa acuminata* genetics. The chloroplast genome being maternally inherited and the mitochondrial genome being paternally inherited, the use of cytoplasmic probes may offer a powerful tool to confirm hybrid origins as well as to distinguish the contributions of maternal and paternal parents in hybrids. Such a parentage test would be relatively simple to implement and could be applied as an early test on young plants.

Knowing the particular mode of transmission of organelles in bananas may also prove of importance in diversity and phylogeny studies on bananas. One such example can be drawn from the present study. SF265 is a cultivar supposed to be derived from the subspecies *M. acuminata banksii* (Tezenas du Montcel personal communication). Parents of the cross SF265 × Banksii should therefore share at least one close common ancestor. This has been confirmed by the relatively low level of polymorphism revealed between SF265 and Banksii by homologous nuclear probes (Fauré et al. 1993). We were able to differentiate the mitochondrial genome of these two accessions whereas no difference between their chloroplast genome could be detected. Even taking into account that only a small part of their chloroplast genome has been analyzed here, this observation suggests that SF265 and Banksii may indeed share a common maternal ancestor.

It is clearly important to acquire more information on the structure of the genetic variability of the genus *Musa* using cytoplasmic markers. This should assist in gaining a better understanding of the evolution of wild and cultivated bananas and of the relationships between diploid and triploid bananas.

Acknowledgements. We thank Dr. A. D'Hont for helpful comments and discussions. We acknowledge colleagues that have kindly sent clones of genes isolated in their laboratories.

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Communicated by C. J. Leaver