Abstract The AFLP technique was used to assess the genetic diversity and sectional relationships in 39 accessions representing the four main sections of the genus *Musa*. Eight AFLP + 3 primer pairs produced 260 polymorphic bands that were used in cluster and PCO analysis. A wide range of variability was observed among the species within the sections of the genus *Musa*. AFLP data was useful in separating the different sections of the genus as well as differentiating the different genomic groups of section *Eumusa*. Section *Rhodochlamys* \((x = 11)\) appeared as a distinct entity and clustered closely with the *Musa acuminata* Colla complex of section *Eumusa* that has the same basic chromosome number. This relationship is congruent with previous studies. However, unlike previous proposals that questioned the identity of *Rhodochlamys* as a separate taxonomic unit, PCO analysis of the AFLP data showed that it is a distinct entity. *Musa laterita* Cheesman (*Rhodochlamys*) and *Musa schizocarpa* Simmonds clustered with the *M. acuminata* complex suggesting that they may be sources of useful genes for the improvement of the cultivated bananas. *Callimusa* formed a distinct unit and was closer to *Australimusa* than to the other sections. Although both sections share the same basic chromosome number of \(x = 10\) these sections are genetically distinct.

Keywords *Musa* · Sectional relationships · AFLP · Diversity · Genetic distance

Introduction

The genus *Musa* is composed of 30–40 species and is usually divided into five sections: *Australimusa*, *Callimusa*, *Eumusa*, *Ingentimusa* and *Rhodochlamys* (Cheesman 1947; Simmonds 1966; Stover and Simmonds 1987) on the basis of the basic chromosome numbers and the orientation and arrangement of flowers. The sections *Australimusa* and *Callimusa* comprise about six species each with a basic chromosome number of \(x = 10\), while *Eumusa* and *Rhodochlamys* contain 15 and 6 species, respectively, with \(x = 11\) (Stover and Simmonds 1987; Karamura 1999). *Musa ingens* Simmonds is the only member in the section *Ingentimusa* with \(x = 14\) (Argent 1976).

The section *Eumusa* is the largest, most diversified and most ancient group. It includes the diploid wild ancestors of modern bananas, *Musa acuminata* (AA) and *Musa balbisiana* (BB) Colla, which contributed the A and B genomes, respectively, to the edible bananas (Simmonds 1962). *M. acuminata* originated in Malaysia and evolved into a complex of diploid (AA) subspecies, which have been classified in several ways by taxonomists (Simmonds and Shepherd 1955; Shepherd 1988; Tezenas du Montcel 1988). *M. balbisiana* originated in India and, contrary to general opinion, recent reports on diversity within the species have indicated a wide morphological variation (Sotto and Rabara 2000). However, despite the existence of genetic variation (Shepherd 1988; Hari 1989) there is no record of subspecies classification in *M. balbisiana*.

Intra- and inter-specific hybridization of *M. acuminate* and *M. balbisiana*, in conjunction with chromosome restitution, gave rise to: (1) autoploids and homogenom-ic hybrids which comprise mainly the AAA dessert and East African highland and beer bananas, and (2) allopol-oids and heterogenomic hybrids comprising the plantains (AAB) and the cooking bananas (ABB). Many other genome groups also exist naturally or were produced by human intervention.

*Rhodochlamys* comprises several species that can hybridize with those of *Eumusa* due to weak reproductive
isolation between these sections. For example, *Musa flaviflora* Simmonds (Eumusa) and *Musa ornata* Roxb. (Rhodochlamys) hybridize easily to produce very vigorous plants (Shepherd 1988). It is thought that *M. ornata* itself may be a secondary species derived from a natural hybrid of *Musa velutina* Wendl. & Drude (Rhodochlamys) and *M. flaviflora* (Shepherd 1988). *Musa laterita* (Rhodochlamys) also hybridizes naturally with some subspecies of *M. acuminata* (Shepherd 1988).

The section *Australimusa* contains species that appear to be more geographic than genetic isolates in nature (Shepherd 1988). In contrast, species in *Callimusa* shows more differentiation and reproductive isolation than those of *Australimusa*.

The separation of species into sections and the delimitation of sectional boundaries in *Musa* have been done mainly on the basis of morphological differences, geographical distribution and hybridization studies (Simmonds 1966; Argent 1976; Swennen and Vyulsteke 1987; Ortiz 1997; Osuji et al. 1997; Karamura 1999). Morphological traits, however, are subject to genotype × environment interaction which reduces their discriminatory power for germplasm classification, particularly when accessions only display subtle differences, as commonly found within the *M. acuminata* complex (Simmonds 1962; Tezenas du Montcel 1988). Moreover, allopatric species that are distinguishable from each other because of geographical isolation, can hybridize under greenhouse conditions or when placed in the same geographical area. The success of hybridization within and between the sections of *Musa* raises questions about the genetic identity of the species and the reproductive isolating mechanisms between them.

Shepherd (1988) reported that *Australimusa* and *Callimusa* are morphologically and reproductively very different. However, he identified distinct morphological differences between *Eumusa* and *Rhodochlamys*, despite the absence of strong reproductive barriers between them. Simmonds and Weatherup (1990), rejected the section *Ingentimusa* indicating that *M. ingens* should not be placed in a separate section. They suggested that the genus *Musa* be divided into four sections (*Australimusa, Callimusa, Eumusa* and *Rhodochlamys*) and showed that there are two divergent groups within *Rhodochlamys*.

Recent developments in DNA marker technology provide alternative means of assessing genetic diversity and phylogenetic relationships (Staub and Serquen 1996; Saghai Maroof et al. 1997). DNA markers are more abundant than morphological markers and are largely unaffected by environmental influences, which makes them attractive for genetic analyses of plant populations. In particular, DNA marker systems based on the polymerase chain reaction (PCR) are well suited to applications in plant breeding (Rafalski and Tingey 1993) and are being used increasingly for genetic analyses in *Musa* (Jarret et al. 1993; Bhat and Jarret 1995; Kaemmer et al. 1997; Crouch et al. 1998a, b; Tenkouano et al. 1999; Pillay et al. 2001). Among these systems, the amplified fragment length polymorphism (AFLP) technique has the extra advantage of combining the speed of the PCR with the precision of the restriction fragment length polymorphisms (RFLPs) (Vos et al. 1995; Powell et al. 1996). Despite its potential, the AFLP technique has not been used for genetic analysis of the wild diploid accessions of the genus *Musa* (Engelborghs and Swennen 1999).

The objectives of the present study were to use AFLP analysis (1) to determine genetic relationships between a sample of *Musa* species, and (2) to assess the extent of genetic variation within the sections of *Musa*.

### Materials and methods

#### Plant material

Forty accessions (Table 1) from the germplasm collection of the International Institute of Tropical Agriculture, Onne, Nigeria, were used in this study. The sample included 29 accessions of *Eumusa*, six *Australimusa*, one *Callimusa*, three *Rhodochlamys*, and one accession from the genus *Ensete* represented by *Ensete ventricosum* (Welw.) Cheesman.

#### DNA isolation

Approximately 10 g of leaf tissue from the cigar leaf (youngest unfurled leaf) was collected and transported on ice from the field to the laboratory and subsequently ground in liquid nitrogen with a mortar and pestle. Isolation of total DNA followed the protocol described by Gaweł and Jarret (1991) with a few modifications. The ground tissue was added to an oak ridge tube containing 15 ml of pre-heated extraction buffer (4% of CTAB-hexadecyltrimethylammonium bromide, 100 mM of Tris-HCl, pH 8.0, 1.4 M of NaCl, 20 mM of EDTA, 4 μl/ml of β-mercaptoethanol) and incubated at 65 °C for 30 min. The samples were extracted with 15 ml of chloroform: isooamyl alcohol (24:1, v/v) and centrifuged at 6,000 rpm for 5 min. The upper aqueous phase was transferred to a new tube and extracted as before with chloroform: isoamyl alcohol. The DNA was precipitated by adding a two-thirds volume of cold ethanol and recovered by centrifugation at 6,000 rpm for 5 min. The upper aqueous phase was transferred to a new tube and extracted as before with chloroform: isooamyl alcohol. The DNA was precipitated by adding a two-thirds volume of ice-cold isopropanol and recovered by centrifugation at 6,000 rpm for 5 min. The DNA was dissolved in 600 μl of TE buffer (10 mM Tris, 1.0 mM EDTA, pH 8.0) and treated with 10 μg/ml of RNase for 30 min at room temperature. The purified DNA was precipitated by adding a one-tenth volume of 3 M sodium acetate (NaOAc, pH 6.8), followed by two volumes of cold ethanol and centrifugation at 6,000 rpm for 5 min. The DNA pellet was washed with 70% ethanol, air-dried briefly and re-suspended in 200 μl of TE buffer. DNA concentrations were quantified with a Pharmacia Gene Quant II spectrophotometer (Pharmacia Biotech, England). To confirm the concentration and quality of the DNA samples, a 2-μl sample of the stock DNA solution was run in a 1% agarose gel stained in 1 μg/ml of ethidium bromide solution and compared visually with Lambda DNA standards of known concentration under UV illumination. An aliquot of the isolated DNA for each sample was diluted to 40 ng/ml in TE buffer and stored in a refrigerator (4 °C) for use in subsequent assays, while the stock DNA samples were stored at −20 °C.

#### AFLP procedure

The AFLP procedure was carried out as reported by Vos et al. (1995) with few modifications.

#### DNA digestion and adapter ligation

Approximately 40 ng of DNA was digested, simultaneously, with EcoRI and Msel at 37 °C for 2 h. The restriction digestion was