PROCEEDINGS

OF THE

26th ANNUAL MEETING

July 29 to August 4, 1990

Mayaguez, Puerto Rico

Published by:

Caribbean Food Crops Society

with the cooperation of the USDA-ARS-TARS

Mayaguez, Puerto Rico

DETECTION OF CYTOPLASMIC VARIABILITY IN *Musa* USING CHLOROPLAST DNA RFLPs

N. Gawel and R.L. Jarret

Department of Horticulture, University of Georgia
Georgia Station, 1109 Experiment Street, Griffin, GA and
USDA-ARS, Regional Plant Introduction Station
1109 Experiment Street, Griffin, GA 30223 USA

ABSTRACT

Concerns over yield declines in bananas and plantains due to the spread of Black Sigatoka disease in *Musa* have drawn attention to the collection of *Musa* germplasm and its use in conventional and biotechnological improvement programs. This report demonstrates the use of chloroplast DNA (cpDNA) restriction fragment length polymorphisms (RFLPs) for differentiating cytoplasms of various *Musa* clones. DNA was extracted from lyophilized leaf blade tissue and digested with either *Eco* R1, *Hind* III, *Bam* H1 or *Pst* I. Southern blots onto nylon membranes were probed using radioactively labeled heterologous orchid and lettuce cpDNA fragments. Among the 14 *Musa* clones examined, a single *M. balbisiana* and four *M. acuminata*-type cytoplasms were differentiated. The ability to distinguish between cytoplasms and to place plants within a cytoplasmic grouping demonstrates the usefulness of RFLP technology in evaluating diversity and determining the ancestry of *Musa* clones.

INTRODUCTION

The genus *Musa* is a vital source of food and fiber in many parts of the world; over 100 million people depend upon bananas and plantains as their principal source of carbohydrates (Rowe, 1981). Yields of bananas and plantains have declined due to the spread of the leaf spot disease Black Sigatoka caused by *Mycosphaerella fijiensis*. As a result, attention has become focused on the collection and conservation of *Musa* germplasm. In order to ensure that available funding is utilized effectively for germplasm conservation, it is essential that genetic diversity in this genus be accurately identified.

Bananas and plantains (except the Fe'i bananas) are derived from *Musa acuminata*, Colla. or hybrids of this species with *M. balbisiana*, Colla. Initial attempts at taxonomic classification of individual clones were unclear and often ambiguous. Simmonds and Shepherd (1955) developed a categorization system based upon the numerical scoring of morphological characteristics which reflects the genetic contribution of both species, and ploidy level. Although this system has been used successfully to group

morphologically distinct clones, the classification of some types is disputed. This confusion may be due either to the high incidence of somatic mutations within specific clones (Vuylsteke et al., 1988) or the confounding influence of cytoplasmic effects on the whole plant phenotype (Jarret, 1986). Maternal effects have been observed in synthetic Musa hybrids (Simmons, 1966; De Langhe, 1969).

Efforts to efficiently conserve Musa germplasm depend upon an accurate knowledge of the degree of genetic relatedness between clones and the range of diversity present. In lieu of analysis of morphological characteristics, laboratory techniques are becoming increasingly popular. Isozymes have been useful in grouping a limited number of dessert and cooking-type clones (Bonner et al., 1974; Jarret and Litz, 1986). However, the genomic constitution of specific clones within groups remains unclear (Rivera, 1983; Jarret, 1986). We report here the use of heterologous chloroplast DNA probes to detect cytoplasmic diversity in the cultivated Musa. In addition, we suggest that these, and related techniques, are likely to yield important data relative to the evolution of bananas and plantains.

MATERIALS AND METHODS

Blade tissue from furled or recently-expanded leaves was harvested, weighed, frozen and lyophilized. Total DNA was extracted essentially as described by Murray and Thompson (1980). Lyophilized leaf tissue (500 mg) was ground in liquid N and added to 15 ml of extraction buffer containing 2% (w/v) hexadecyltrimethylammonium bromide (CTAB), 1.4M NaCl, 20 mM EDTA and 1% v/v) mercaptoethanol. The mixture was inverted to mix, and incubated at 65C for 30 min. Following incubation, two chloroform:isoamyl alcohol (24:1, v/v) extractions were performed: an equal volume of chloroform:isoamyl alcohol (24:1, v/v) was added, the mixture was gently shaken for 15 minutes at room temperature, centrifuged at 5,000 x g for 5 min and the aqueous phase filtered through 2 layers of miracloth (CalBiochem). To the aqueous phase was added an equal volume of ice-cold isopropanol. The precipitated DNA was removed using a glass hook, washed in 70% ethanol and resuspended in TE buffer (Saghai-Maroof et al., 1984). Samples were treated with RNAse (10 ug/ml for 30 min. at room temperature) and precipitated with ethanol (Maniatis et al., 1982). DNA pellets were resuspended in 200 to 500 ul TE. DNA yields ranged from 100 to 500 ug (O.D. 260) per gram of fresh weight of leaf tissue.

DNA extracts (8 ug) were digested with 20 to 30 U of restriction enzyme (Eco RI, Hind III, Pst I or Bam HI) for 12 hours at 37C. Fragments were separated on 0.8% (w/v) agarose gels at 2 V/cm for 12 hours in TAE buffer. Fragments were transferred to nylon membranes (Biotrans, ICN) according to Southern (1975) and baked in a vacuum oven at 80C for 1 hour.

Membranes were pre-hybridized for 3-4 hours in 5X SSC, 50 mM NaH_2PO_4, 5X Denhart's, 2.5 mM EDTA, 0.4% SDS, 5% dextran sulfate and 0.001% sonicated and denatured salmon sperm DNA at 65C according to the membrane manufacture's recommendations. Denatured ^{32}P-labeled probe was added to the pre-hybridization mixture and allowed to hybridize overnight at 65C. Probe DNA was random primer labeled (Bethesda Research Laboratories). Membranes were washed for 20 min. each in 2X SSC, 0.1% SDS and IX SSC, 0.1% SDS; wrapped in plastic wrap, and exposed to X-ray film for 1 to 2 days at -20C with intensifying screens.

Probes utilized in this study were isolated from the lettuce (Lactuca sativa) chloroplast genome and included: J2, a 9.9kb sequence from the 16S rRNA gene; J4, a 1.8 kb fragment of the 23S rRNA gene; and J12, a 10.6 kb fragment containing the large subunit of the RUBP carboxylase gene. Probes 6b, 10a and 16 are 6.7kb, 5.7kb and 4.0kb fragments (respectively) isolated from an orchid (Oncidium excavatum) chloroplast DNA library. Entire plasmids (containing probe insert) were labeled and used as probes.

Plant materials were obtained from Dr. Phil Rowe, Fundacion Hondureana de Investigacion Agricola (FHIA), La Lima, Honduras; Dr. Randy Pleotz, University of Florida Homestead; and through the International Network for the Improvement of Bananas and Plantains (INIBAP) germplasm transit center at Katholieke Universiteit Leuven (KUL), Belgium. The clones selected for study represent the extremes in morphological diversity within each of the recognized genome/ploidy level groupings.

RESULTS

Ploidy level and genomic grouping of the plants examined are listed in Table 1. Various probe/enzyme combinations were useful in delineating cytoplasmic differences among the clones examined. For all enzymes, all probes differentiated M. balbisiana from the M. acuminata cytoplasms. In all, five distinct cytoplasms were identifiable among the accessions analyzed. These are grouped as shown in Fig. 1 after analysis using Phylogenetic Analysis Using Parsimony (PAUP) software (Swofford, 1985).

Probe 6b (Bam HI digest) differentiated all five cytoplasms. In addition to differentiating between M. acuminata and M. balbisiana cytoplasms, this probe/enzyme combination detected additional variation between the M. acuminata-derived clones cv. Igitsiri (AAA), a triploid African beer banana; II-357, a diploid M. acuminata ssp. malaccensis wild-type and the dessert banana cv. Grande Naine (AAA). Beer-types are generally morphologically indistinguishable from similar types used for cooking (Simmonds, 1966).

Table 1. *Musa* clones examined for cytoplasmic diversity.

Clone	Species	Genome	Comments
Grande Naine	*M. acuminata*	AAA	Desert banana
Igitsiri	*M. acuminata*	AAA	Beer banana
II-357	*M. acuminata* ssp. *malaccensis*	AA	Wild diploid
Ihitisim	*M. acuminata* x *M. balbisiana*	AAB	Horn plantain
Bae Ako Ukom	*M. acuminata* x *M. balbisiana*	AAB	Horn plantain
Bobby Tannap	*M. acuminata* x *M. balbisiana*	AAB	French plantain
Pisang Raja	*M. acuminata* x *M. balbisiana*	AAB	Indonesian
Pisang Awak	*M. acuminata* x *M. balbisiana*	ABB	Indochinese
Pelipita	*M. acuminata* x *M. balbisiana*	ABB	'Saba' group
Ice Cream	*M. acuminata* x *M. balbisiana*	ABB	Hawaiian
Chato	*M. acuminata* x *M. balbisiana*	ABB	Bluggoe
Cardaba	*M. acuminata* x *M. balbisiana*	ABB	'Saba' group
Saba	*M. acuminata* x *M. balbisiana*	ABB?	'Saba' group
I-63	*M. balbisiana*	BB	Wild diploid

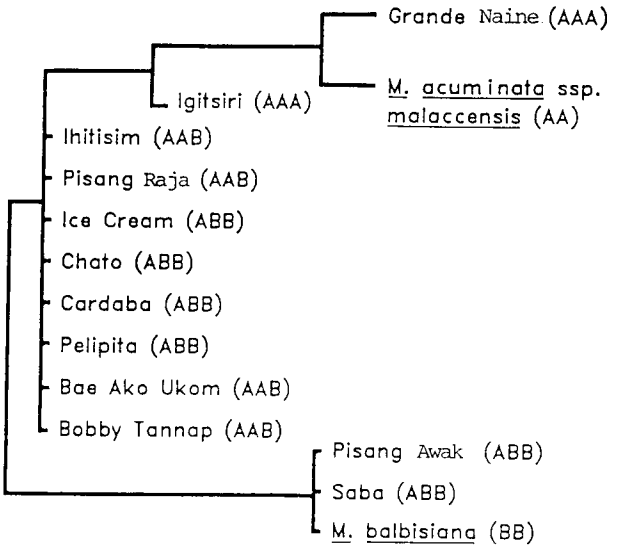

Fig. 1 Unrooted tree showing cytoplasmic groupings of *Musa* clones examined (Consistency index = 0.950).

ø

The cytoplasm of both the plantain (AAB) cvs. Bae Ako Ukom, Ihitisim, Bobby Tannap and Pisang Rajah were indistinguishable from the cooking-types (ABB) cvs. Chato, Pelipita and Cardaba, although these groups are believed to be genetically quite distinct from one another.

The cytoplasms of cvs. Pisang Awak and Saba were indistinguishable from the wild, seeded M. balbisiana. This is in contrast to another member of the Saba group (Allen, 1965), cv. Cardaba and other ABB types. Saba and Cardaba have been classified as ABB, however their cytoplasms are distinct as detected by probe 10a (Bam I digest).

DISCUSSION AND CONCLUSIONS

Most clones examined could not be differentiated from one another based on their cpDNA RFLPs using this limited number of probe/enzyme combinations. This reflects the highly conserved nature of the chloroplast genome. However, the data also show cytoplasmic differences between the M. acuminata-derived cultivars, thus, these differences are suggestive of a divergence of ancestry. Also, the data indicate that variation within group AAA is greater than between groups AAB and ABB. These results may reflect the extreme diversity within M. acuminata (Simmonds, 1966) and perhaps the occurrence of a common maternal parent.

Isozyme evidence (Jarret and Litz, 1986) suggests that cv. Saba is an M. acuminata x M. balbisiana triploid. However, this clone has also been classified as triploid M. balbisiana (Rivera, 1983). These results clearly show that cv. Saba is distinct from other members of the Saba subgroup examined to date. In addition, the data presented suggest that there are multiple evolutionary origins for clones within this subgroup. While these data do not indicate the genomic group within which these clones should be placed, utilization of cpDNA RFLPs would enable a rapid means to determine the relationship of Pisang Awak with other clones within the Pisang Awak subgroup, other ABB subgroups and putative sports. Research in progress, utilizing nuclear DNA RFLPs, should allow for their unambiguous genomic classification.

The analysis of organelle and nuclear DNA RFLPs, in combination, can be used not only to detect and quantify genetic diversity in Musa germplasm, but also may be helpful in reconstructing the mechanism of the ancestral hybridizations or mutations which gave rise to today's cultivars.

ACKNOWLEDGMENTS

This research was funded by the International Board for Plant Genetic Resources (IBPGR) whose support is gratefully acknowledged. We also wish to express appreciation to the International Network for the Improvement of Bananas and Plantains (INIBAP-Montpellier), Mr. Jos Schoofs (KUL-Leuven), Dr. R. Ploetz

(IFAS-Homestead) and Dr. P. Rowe (F.H.I.A.) La Lima, Honduras for the provision of plant material for this study, and to Drs. M. Chase (University of North Carolina, Durham) and R. Jansen, University of Connecticut, Storrs) for the provision of DNA probes.

REFERENCES

Allen, P.H. 1965. Annotated list of Philippine Musaceae. Philip Agric 49:320-411.

Bonner, J.W., Warner, R.M., and Brewbaker, J.L. 1974. A chemosystematic study of Musà cultivars. HortScience 9:325-328.

De Langhe, E. 1969. Bananas, Musa spp. In: F.P. Ferwerda and F. Wit (eds.). Outlines of Perennial Crops Breeding in the Tropics. Wageningen, Netherlands, Landbouwhogeschool. pp. 53-78.

Jarret, R.L. 1986. Determining the interspecific origins of clones within the 'Saba' cooking banana complex. HortScience 21:1433-1435.

Jarret, R.L and Litz, R.E. 1986. Isozymes as genetic markers in bananas and plantains. Euphytica 35:539-547.

Maniatis, T., Fritsch, E.F., and Sambrook, J. (eds.). 1982. Molecular cloning: A laboratory manual. Cold Spring Harbor Laboratory, NY.

Murray, M.G., and Thompson, F.W. 1980. Rapid isolation of high molecular weight plant DNA. Nuc. Acids. Res. 8:4321-4325.

Rivera, R.N. 1983. Protein and isozyme banding patterns among Philippine cooking bananas and their wild parents (Musa species). Paradisiaca 6:7-12.

Rowe, P., 1981. Breeding an intractable crop. Bananas. In: K.O. Rachie & J.M. Lyman (eds.), Genetic Engineering for Crop Improvement. Working Papers. The Rockefeller Foundation, Washington, DC. pp. 66-84.

Saghai-Maroof, M.A., Soliman, K.M., Jorgensen, R.A., and Allard, R.W. 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc. Natl. Acad. Sci. (USA) 81:8014-8018.

Simmonds, N.W. 1962. The evolution of the bananas. Longman, London.

Simmonds, N.W. 1966. Bananas, 2nd. ed., Longman, London.

Simmonds, N.W., and Shepherd, K. 1955. The taxonomy and origins of the cultivated bananas. J. Linn. Soc. London, Bot. 55:302-312.

Southern, E. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98:503-517.

Swofford, D.L. 1985. PAUP-Phylogenetic analysis using parsimony, version 2.4.0. Illinois Natural History Survey, Champaign, IL.

Timothy, D.H., Levings III, C.S. Pring, D.R., Conde, M.F., and Kermicle, J.L. 1979. Organelle DNA variation and systematic relationships in the genus Zea: Teosinte. Proc. Natl. Acad. Sci. (USA) 76:4220-4224.

Vuylsteke, D., Swennen, R., Wilson, G., and De Langhe, E. 1988. Phenotypic variation among in-vitro propagated plantain (Musa sp. cultivar 'AAB'). Scient. Horticult. 36:79-88.