ESTABLISHING GENETIC DIVERSITY OF RWANDA HIGHLAND BANANA USING RANDOM AMPLIFIED POLYMORPHIC DNA MARKERS

by

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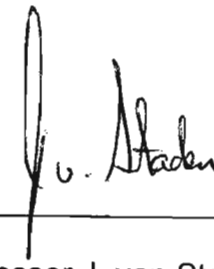
PREFACE

I hereby declare that the experimental work described in this thesis started in the Institut des Sciences Agronomiques du Rwanda and continued in the Research Centre for Plant Growth and Development under the supervision of Professor J van Staden.

These studies have not been submitted in any form for any degree or diploma to any University. Where use has been made of the work of others, it is duly acknowledged in the text.

Antoine Nsabimana

I declare the above to be correct.



Professor J. van Staden
(SUPERVISOR)

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Abstract

The characterization of the banana germplasm collection from Rubona - Rwanda was investigated using morphological and cytological characteristics of the genomic groups. Genetic diversity was assessed using Random Amplified Polymorphic DNA analysis. The survey was conducted to evaluate the distribution of banana cultivars in the four major growing regions of Rwanda.

A total of 90 accessions from the National Banana Germplasm Collection at Rubona Rwanda were characterized and six characters of the fingers (length, width, weight, green life, post green life and length/width ratio) were subjected to principal component analysis (PCA). The cooking and beer clones were separated. The cooking clones were further grouped into three clone sets: Musakala, Nakabululu, and one that constitutes Nakitembe and Nfuuka clone sets. The AAB genomic group was separated from AAA, AB and ABB genomic groups.

The results from the survey showed that East African Highland bananas are the most important genotype group in the four major banana growing regions of Rwanda ranging between 60 - 90% of banana mats counted. Several new Highland banana cultivars were recorded, such as 'Intokatoke', 'Igihuna', 'Ingenge', 'Ingaju', 'Icyerwa', 'Mitoki', 'Madamu', 'Inkokobora', 'Intokekazi', 'Bugoyi', 'Ishoki'. Amongst these cultivars, some were classified as cooking and others as brewing bananas. However, in the National Banana Germplasm Collection at Rubona - Rwanda, the uses of these cultivars are recorded differently therefore increasing the need for agro-morphological characterization.

The assessment of ploidy level of accessions from the National Banana Germplasm Collection at Rubona – Rwanda, by flow cytometry showed misclassification of some accessions such as 'Pomme', 'Kamaramasenge', 'Gisubi kayinja', 'Gisubi kagongo', and 'Dibis' which were classified as diploid, diploid, triploid, and tetraploid respectively. They

were found to be triploid, triploid, triploid, diploid and triploid. All these bananas were recently introduced into Rwanda, while the endemic Highland bananas were triploid.

The genomic group and genetic similarities of 49 accessions were investigated using Random Amplified Polymorphic DNA markers. The genomic group of bananas assessed were established using OPA-18 (PILLAY *et al.,* 2000) and OPG-17 primers. These primers showed bands 441 and 443 base pairs (bp) respectively for the accessions having only the B genome. Whilst they were absent for the accessions having an A genome. The genetic similarity was estimated via a Simple Matching coefficient which showed the lowest value 0.46 measured between 'Ingumba' and 'Ishika 'and the highest value of 0.85 between 'Kirayenda' and 'Inyabukuwe'. The data of matrix of coefficient of similarity was subjected to cluster analysis with unweighted pair group method with arithmetic average (UPGMA). Each accession was clearly separated demonstrating the usefulness of RAPDs in analysis of genetic diversity. The results of this study are very important to the Curator of the banana germplasm collection in Eastern Central Africa and for the future breeding of this crop.

Publications from this thesis

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Abbreviations

µg	Microgram
µl	Microlitre
µm	Micrometre
A	*Musa acuminata* subspecies acuminata genomic group
A_{260}	Absorbance at 260 nm
A_{280}	Absorbance at 280 nm
A_{320}	Absorbance at 320 nm
B	*Musa acuminata* subspecies balbisiana genomic group
bp	Base pair
cm	Centimetre
cv	Cultivar
dATP	Deoxyadenosine triphosphate
dCTP	Deoxycytidine triphosphate
dGTP	Deoxyguanosine triphosphate
DH_2O	Distilled water
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleoside triphosphate
DTT	Dithiothreitol
dTTP	Deoxythimidine triphosphate
EDTA	Ethylene diamine tetraacetic acid
g	Gram
h	Hour
HPLC	High-performance liquid chromatography
kb	Kilobase pairs
l	Litre
M	Molar
mM	Millimolar

mg	Milligram
min	Minute
ml	Millilitre
mm	Millimetre
ng	Nanograms
nm	Nanometre
OD	Optical density
PCR	Polymerase chain reaction
PVPP	Polyvinylpolypyrollidone
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length Polymorphism
sec	Second
TAE	Tris/Acetate EDTA
TE	Tris/EDTA
Tris	2-amino-2(hydroxymethyl)-propane-1,3-diol
Tris-Cl	Tris hydrochloride
UPGM	unweighted pair group method with arithmetic average
v/v	Volume per volume
v	Volt
w/v	Weight per volume

Chapter 1

General introduction

1.1 Background

Bananas are one of the most important food crops in the world (INIBAP, 1999; ROUT *et al.,* 2000; FAO, 2001; PANIS and THINH, 2001). There are many diverse types, classified according to use of the end products, such as dessert, cooking, roasting and beer bananas (SAGI *et al.,* 1998). Dessert bananas are consumed raw at ripeness and are usually identified by their sweet taste when ripe. Cooking bananas, referred to as plantains, are consumed when cooked (SWENNEN and VUYLSTEKE, 1987). However, there are two types of cooking bananas. The first type is cooked when the fruits are green and provides a starchy staple product nutritionally similar to the Irish potato (SIMMONDS, 1966; STOVER and SIMMONDS, 1987). These bananas can be allowed to ripen and then eaten as dessert bananas, therefore they cannot be called true plantains (KARAMURA, 1998). The second group of cooking bananas are unpalatable raw even when the fruits are ripe and therefore require cooking before being consumed. These can be considered as true plantains (SWENNEN and VUYLSTEKE, 1987). Some of the plantains are roasted, especially when ripe. The beer bananas are bananas whose pulp is bitter and astringent and cannot be eaten, raw or cooked. However, juice and alcoholic beverages can be made from this type of banana (SEBASIGARI, 1987).

World banana production is being estimated at 88 million metric tones from over 100 countries throughout the tropical and subtropical regions of the world, grown on an area of approximately 10 million hectares (INIBAP, 1999; FAO, 2001). Only around 13% of banana and plantain crops are exported. The dessert banana is a major export crop of the Latin American and Caribbean countries, where the Cavendish bananas are mainly grown (DANIELLS, 1990; INIBAP, 1999). The remaining bananas are consumed in the

producing countries, where it is estimated that one half of the production is eaten raw as dessert fruits and the other half cooked to provide a starchy staple (INIBAP, 1994).

Bananas constitute the third most important starchy staple in the world after cassava and sweet potatoes (Table 1.1). It is estimated that over 100 million people in sub-Saharan Africa subsist on this crop (INIBAP, 1994; 1999). In Africa, plantains are cultivated from the lowlands of Guinea and Liberia in West Africa to the central basin of the Democratic Republic of Congo where 20% of the world's plantains are grown (FAO, 2001). In East Africa, the cooking bananas alone account for 70% of the bananas produced in this region and 54% of the total world output of cooking bananas. The region is also the main consumer of bananas (INIBAP, 1999; FAO, 2001).

Table 1.1 Production levels of some major starchy staples (million metric tones) (INIBAP, 1999)

Region	Cassava	Sweet potato	Yam	Bananas and plantain
Africa	72.8	6.9	29.1	30
Asia	48.4	1.1	0.2	25
Latin America and the Caribbean	31.1	115.5	0.7	33
World	152.3	124.5	30.1	88

All banana types reduce soil erosion on steep slopes, where the foliage assists in arresting rain drops and protecting the soil from excessive exposure. Due to the large amount of biomass (pseudostems and leaves) that can be used for mulching, bananas are a principal source of mulch for maintaining and improving soil fertility, humidity and aeration (RUSHIRUMUHIRWA, 1997; KANGASNIEMI, 1998).

1.2 Morphology

Bananas are the largest of the herbaceous plants, consisting of a branched, underground stem (rhizome or corm) with roots and vegetative buds, and an erect pseudostem composed of tightly-packed leaf bases (Figure 1.2.1).

The root system is adventitious and the primary roots usually arise in groups of three or four from the fleshy rhizome. Secondary and tertiary roots develop from each primary root, becoming progressively thinner and shorter than the primary roots (ROBINSON, 1996; JONES, 1999).

The corm, also called a rhizome, is the true stem of the plant and is partly or fully underground with short internodes, covered externally by closely packed leaf scars. The corm has an apical growing point, which gives rise to the pseudostem (WARDLAW, 1961).

The pseudostem consists of compact leaf sheaths, which grow directly from the top of the corm, and supports the aerial stem that carries the inflorescence. The leaf sheaths are circular, at first completely enclosing the aerial stem but later the free margins of the sheaths are forced apart by the growth of the new leaves enclosed within the pseudostem (PURSEGLOVE, 1972; SAMSON, 1980; 1986; KARAMURA and KARAMURA, 1995).

The basal growing point at the heart of the pseudostem is transformed into the inflorescence. The inflorescence is a complex spike consisting of a stout peduncle on which the flowers are arranged in nodal clusters. Initially the female and male flowers are morphologically indistinguishable. When the spike has extended, the basal (proximal) nodes bear the female flowers while the upper (distal) nodes contain male flowers. Between them are hermaphrodite flowers that do not develop into edible fruits (PURSEGLOVE, 1972; KARAMURA and KARAMURA, 1995; ROBINSON, 1996; JONES, 1999).

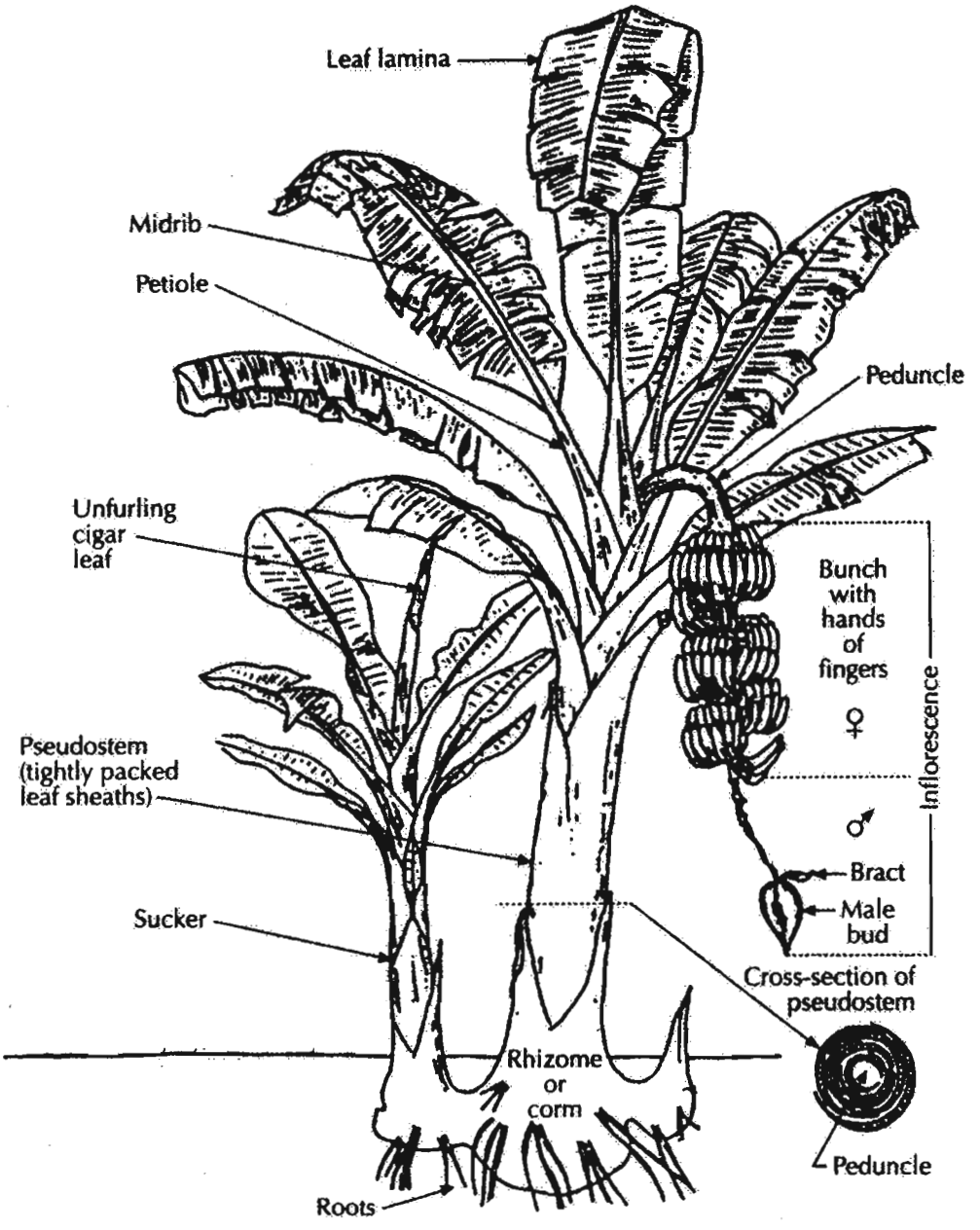

Figure 1.2.1: Diagrammatic representation of a fruiting banana plant with suckers (CHAMPION, 1963).

1.3 Classification

Bananas are monocotyledonous, perennial plants belonging to the order Zingibirales and the family Musaceae. Musaceae contains only two genera: *Musa* and *Ensete*. The genus *Ensete* contains a total of six species (SIMMONDS, 1962). The distinguishing characteristics of *Ensete* and *Musa* are listed in Table 1.3.1.

Table 1.3.1 Distinguishing characters of *Ensete* and *Musa* (VALMAYOR *et al.*, 2002)

Characters	*Ensete*	*Musa*
Growth habit	Single stem, monocarpic	Free suckering
Shape of pseudostem	Enlarged base	Cylindrical
Seed	Big, smooth nearly globose	Small, irregular
Bracts covering basal/female flowers	Persistent	Dehiscent
Basic chromosome number	9	10 – 14

The genus *Musa* is complex, comprising the five sections Eumusa, Rhodochlamys, Callimusa, Australimusa and Ingentimusa (SIMMONDS and WEATHERUP, 1990a; VALMAYOR *et al.*, 2002). The genus *Musa* contains 30-40 species. The section Eumusa is the largest and most diversified in the genus (PURSEGLOVE, 1972). Some of the known species and subspecies of the sections are listed in Table 1.3.2.

Table1.3.2 Classification of genus *Musa* (DE LANGHE, 1969)

Section	General characteristics	Species	Number of chromosomes
Australimusa	Short stature Inflorescence erect Seeds flattened	*M. textilis* Née *M. peekelii* Lauterbach *M. angustigemme* Simmonds *M. maclayi* Mueller. *M. bukensis* Argent *M. jackeyi* Hill *M. tolodensis* Cheesman	2n = 20
Callimusa	Short stature Inflorescence erect Seeds cylindric	*M. coccinea* Andrews *M. gracilis* Holttum *M. borneensis* beccari	2n = 20
Rhodochlamys	Slender, rather short to very short stature Inflorescence erect (plant ornamental)	*M. velutina* Wendl.et Drude *M. sanguinea* Hode *M. ornata* Roxb *M. laterita* Cheesman.	2n = 22
Eumusa	Plant of more diverse stature and more robust. Horizontal-pendulous fruit bunches	*M. acuminata* Colla *ssp.malaccensis* Simmonds *ssp.microcarpa* Simmonds *ssp.burmannica* Simmonds *ssp.burmannicoides* De Langhe & Devreux	2n = 22

Table1.3.2 Classification of genus *Musa* (DE LANGHE, 1969) (continued)

Section	General characteristics	Species	Numbers of Chromosomes
Eumusa	Plant of more diverse stature and more robust. Horizontal-pendulous fruit bunches	*M. acuminata* Colla *ssp.Siamea* Simmonds *ssp.Banksii*(F.Muel) *ssp.Errans* Allen *ssp.Zebrina* Simmonds *M. flaviflora* Simmonds *M. balbisiana* Colla *M. itinerans* Cheesman *M. basjoo* Siebold *M. schizocarpa* Simmonds *M. nagensium* Prain *M. ochraecea* Shepheld *M. truncata (Ridl.)* Shepherd	2n = 22
Ingentimusa		*M. ingens* Simmonds	2n = 14, 18

1.4 Evolution and distribution of wild banana

Based on morphological, geographical and cytological characteristics, SIMMONDS (1962) postulated that the main centre of diversity of bananas is the Assam-Burma-Thailand area. This region is the centre of origin where the *Musaceae* developed into two lines, one of which became *Ensete*, the other became *Musa*. The *Ensete* line spread widely in Asia and Africa and differentiated into few species, while *Musa* spread only in Asia and differentiated more successfully there than *Ensete*. *Musa ingens* and *Musa lasiocarpa* are regarded as relics of the early *Musa* line. SIMMONDS (1962) proposed a

scheme of banana evolution as shown in Figure 1.4.1. The evolution of edibility in the Eumusa section of cultivars began with wild *Musa acuminata* subspecies, which occur naturally in an area stretching from South Asia to Australia, but are concentrated mainly in South-East Asia (Figure 1.4.2) (JONES, 1999).

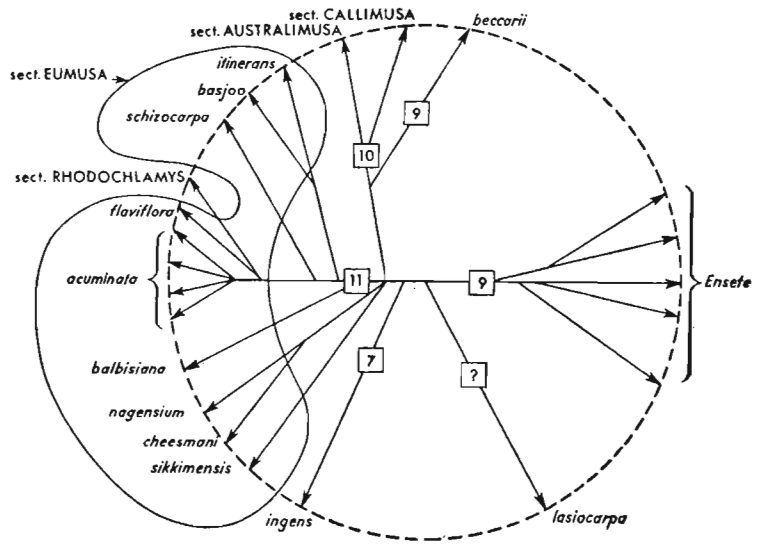

Figure 1.4.1: Evolution of wild bananas (SIMMONDS, 1962).

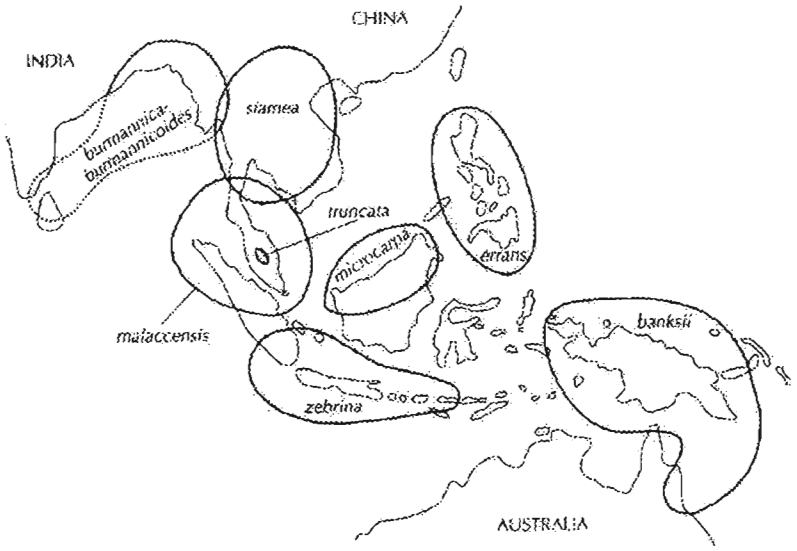

Figure 1.4.2: Distribution of Eumusa subspecies (JONES, 1999).

1.5 Cytogenetics, nomenclature and taxonomy of banana cultivars

1.5.1 Cytogenetics.

In the genus *Musa,* the main elements in the classification are the recognition of bi-specific origins of the group, coupled with knowledge of chromosome numbers (SIMMONDS and WEATHERUP, 1990b). All wild bananas that gave rise to edible bananas are diploid, with a basic chromosome number 2n=2x=22 in the section Eumusa and 2n=2x=20 in the section Australimusa. Most banana cultivars, and all plantains, have different levels of ploidy; diploid (2n=2x=22), triploid (2n=3x=33) tetraploid (2n=4x=44) and aneuploids (2n=3x=31) or (2n=3x=32) (SHEPHERD, 1999; MARTINA *et al.,* 2002). Usually, diploids have upright leaves and have more slender pseudostems than triploids. On the other hand, triploids have bigger fruits (Figure 1.5.1). Unfortunately, there are few natural tetraploids (2n=4x=44). However, DE LANGHE (1969) reported that they are vegetatively better-developed than triploids, and the fruit volume, as compared with that of the triploids, seems to have decreased slightly.

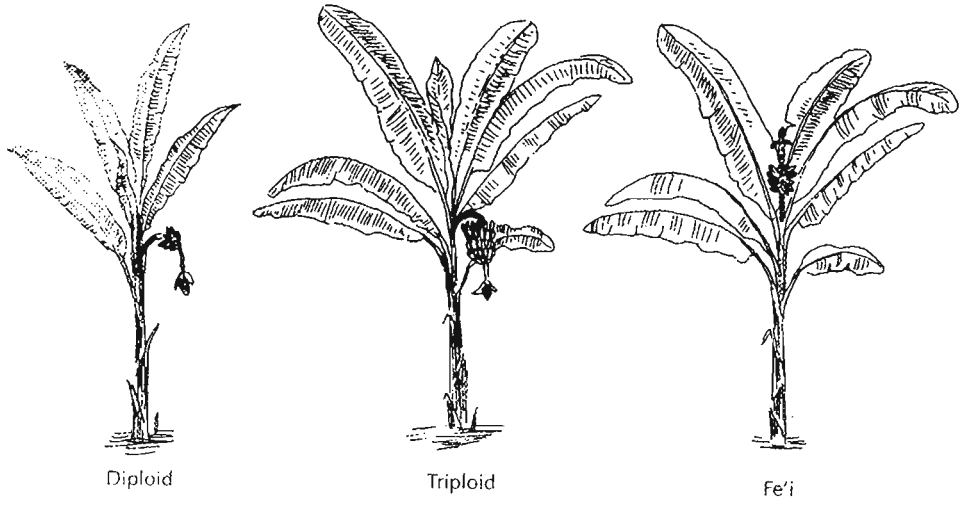

Figure 1.5.1: General appearance of diploid and triploid cultivars in the Eumusa section of edible banana and Fe'I cultivar in the Autralimusa section (BOURKE, 1976).

1.5.2 Nomenclature and taxonomy of banana cultivars.

The first and second names given by Carl Linnaeus for banana were *Musa paradisiaca* Linn and *Musa sapientum* Linn. Linnaeus separated the two species according to the persistence of male flowers. However, he did not know that both his descriptions were based on hybrids (SIMMONDS, 1966; SAMSON, 1980). Later names *Musa cavendishii* Lambert, *Musa sinensis* Sweet ex Sagot and *Musa nana* Lour designated the same clone 'Dwarf Cavendish' (VALMAYOR *et al.,* 2000).

To resolve the confusion in banana nomenclature, the notion of genome groups was suggested by SIMMONDS and SHEPHERD (1955) as the key to classification, where A and B represent respectively the genome from *Musa acuminata* Colla and *Musa balbisiana* Colla, which were involved in the evolution of the wild bananas to the majority of edible bananas (SIMMONDS, 1962; 1966; SAMSON, 1980; 1986).

The International Code of rules for the Botanical Nomenclature for Cultivated Plants state that plants which have originated through interspecific hybridization should have a scientific name which indicates this, where the hybrid nature of the species is indicated by a multiplication sign, for example *Musa x paradisiaca or Musa x sapientum* = AB, AAB, (SIMMONDS, 1966; De LANGHE, 1969). All banana taxonomists agree that no single scientific name can be given to all edible bananas and the hybrids cannot carry specific names due to their mixed composition and differences in ploidy (ROBINSON, 1996). Therefore 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. Table 1.5.1 presents the 15 diagnostic characters used to differentiate *Musa acuminata* clones from *Musa balbisiana* cultivars and their hybrids. The cultivars are classified by inspecting the expression of each character shown in Figure 1.5.2. The taxonomic scorecard suggested by SILILAYOI and CHOMCHALOW (1987) is a modified version of the original and was found very useful in segregation. These authors classified 137 accessions in the Thai banana gene bank on the same basis. They recognized some

imperfections in the original classification and modified it. A score of 1 is assigned for each character that adheres closely with wild *M. acuminata* and 5 for characters with extreme *M. balbisiana* expression. Intermediate expressions of the characters are assigned scores raging from 2, 3 or 4 depending on intensity. Pure *M. acuminata* (AA, AAA) varieties score between 15 and 25, while pure *M. balbisiana* cultivars range between 70 and 75 (BB, BBB) and the hybrids (AB, ABB, AAB, ABBB, AAAB) are expected to score between 26 to 69 points (SILILAYOI and CHOMCHALOW, 1987; ROBINSON, 1996; VALMAYOR *et al.,* 2002). The main difference between the classifications is the introduction of almost pure *M. balbisiana* clones, which did not appear in the list of the original classification.

Table1.5.1 Morphological differences between *Musa acuminata* Colla and *Musa balbisiana* Colla (SIMMONDS and SHEPHERD, 1955)

Character	*Musa acuminata* Colla	*Musa balbisiana* Colla
1. Pseudostem color	More or less heavily marked with brown or black blotches	Blotches slight or absent
2. Petiolar canal	Margin erect or spreading, with scarious wings below, not clasping pseudostem	Margin enclosed, not winged below, clasping pseudostem
3. Peduncle	Usually downy or hairy	Glabrous
4. Pedicels	Short	Long
5. Ovules	Two regular rows in each loculus	Four irregular rows in each loculus
6. Bract shoulder	Usually high (ratio< 0.28)	Usually low (ratio>0.30)
7. Bract curling	Bracts reflex and roll back after opening	Bracts lift but not roll
8. Bract shape	Lanceolate or narrowly ovate, tapering sharply from the shoulder	Broadly ovate, not tapering sharply
9. Bract apex	Acute	Obtuse
10. Bract color	Red, dull pulp or yellow outside; pink, dull pulp or yellow inside	Distinctive brownish-purple outside; bright crimson inside
11. Color fading	Inside bract color fades to yellow toward the base	Inside bract color continuous to base
12. Bract scars	Prominent	Scarcely prominent
13. Free tepal of male flower	Variably corrugated below tip	Rarely corrugated
14. Male flow color	Creamy white	Variably flushed with pink
15. Stigma color	Orange or rich yellow	Cream, pale yellow or pale pink

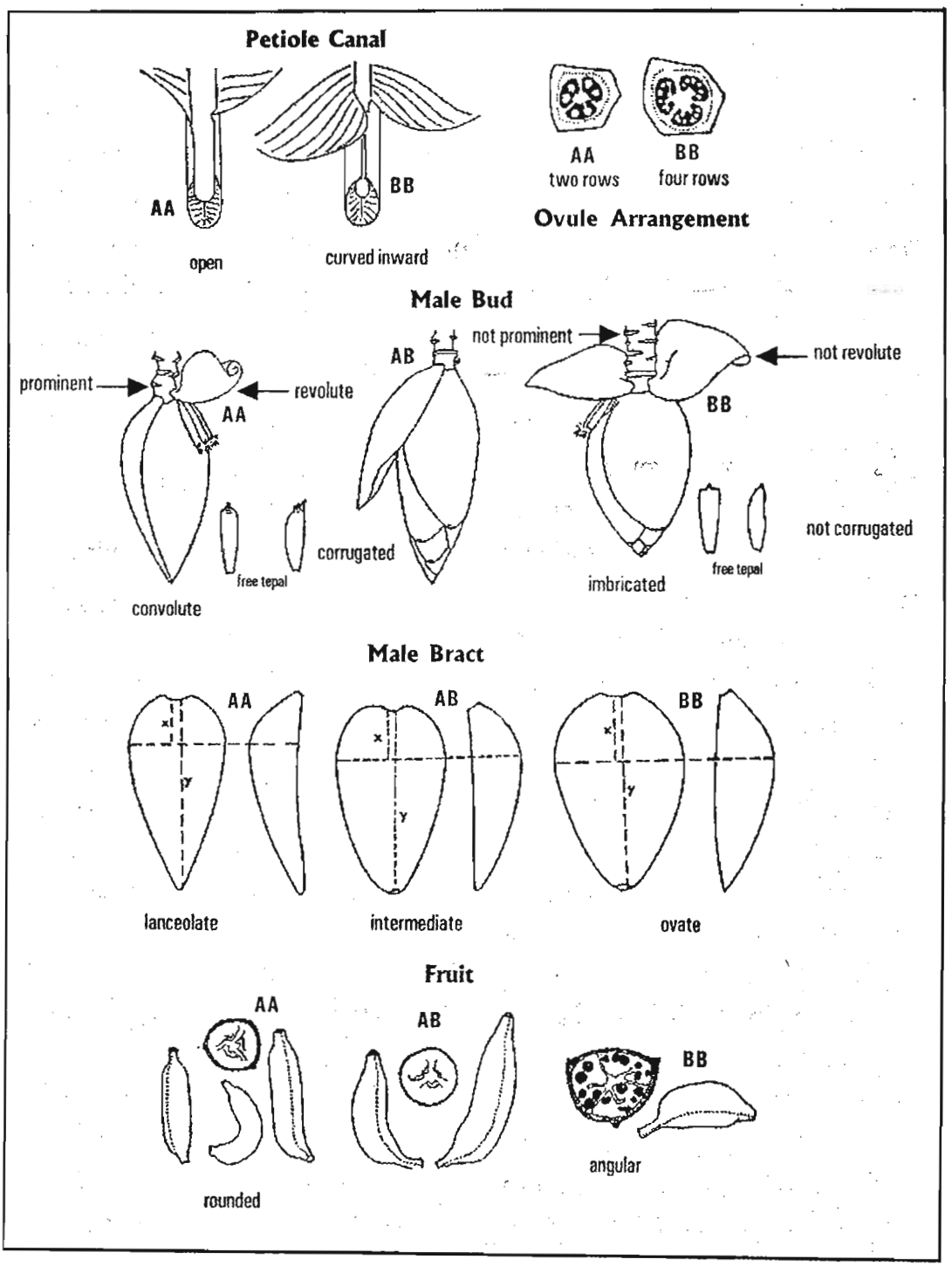

Figure 1.5.2: Characters important in determining cultivar group (VALMAYOR *et al.*, 2002).

1.6 Evolution of banana cultivars and their distribution

The first step and crucial phase of banana evolution was accomplished by the development of a highly parthenocarpic and reasonably female-sterile diploid (SIMMONDS, 1962; 1987; SHANMUGAVELU *et al.,* 1992). Parthenocarpy is the capacity of the fruit to grow and become full of edible parenchymatous pulp without pollination. Therefore the wild *acuminata* with the A genome gave rise to the edible cultivars. The second step was caused by chromosome restitution at meiosis of edible banana and gave rise to the A triploids. The third step was the dispersal of edible bananas by transport of vegetative planting material by humans to places where *Musa acuminata* is locally absent but where *Musa balbisiana* is native. This increased the crossing of the A and B genomes and gave rise to hybrids AB, AAB, ABB and AAAB. The last step of evolution was caused by somatic mutations (SIMMONDS, 1987, SHANMUGAVELU *et al.,* 1992). Figure.1.6.1 illustrates the evolution of the banana complex.

The spread of banana around the world from their center of origin (South-East Asia, India, Thailand, New Guinea Queensland and Australia) (SIMMONDS, 1987) remains the subject of speculation. Banana was considered a holy plant in the Koran, and had spread to Tunisia and Spain by the 12th and 13th Centuries (PRICE, 1995). It was suggested that banana moved from India through Palestine to Egypt in the 7th Century A. D.

It soon became popular in these areas and later spread to the East coast of Africa (SHANMUGAVELU *et al.,* 1992). However, movement of bananas from India through Palestine to Africa is unlikely because the climatic conditions along these routes are too dry for survival of this crop (PURSEGLOVE, 1972).

Modern views about the distribution of bananas to Africa consider the trade and contacts that historically existed between Asia and Africa. These contacts include the migration of Indo-Asian people to Madagascar between 0 – 500 A.D. and the Arab trade and

influence along the coast of East Africa after 600 A.D. (SIMMONDS, 1966; PURSEGLOVE, 1972). Early in the 16th Century, Portuguese mariners transported the plant from the West African coast to South America (MORTON, 1987; ROBINSON, 1996) (Figure 1.6.2).

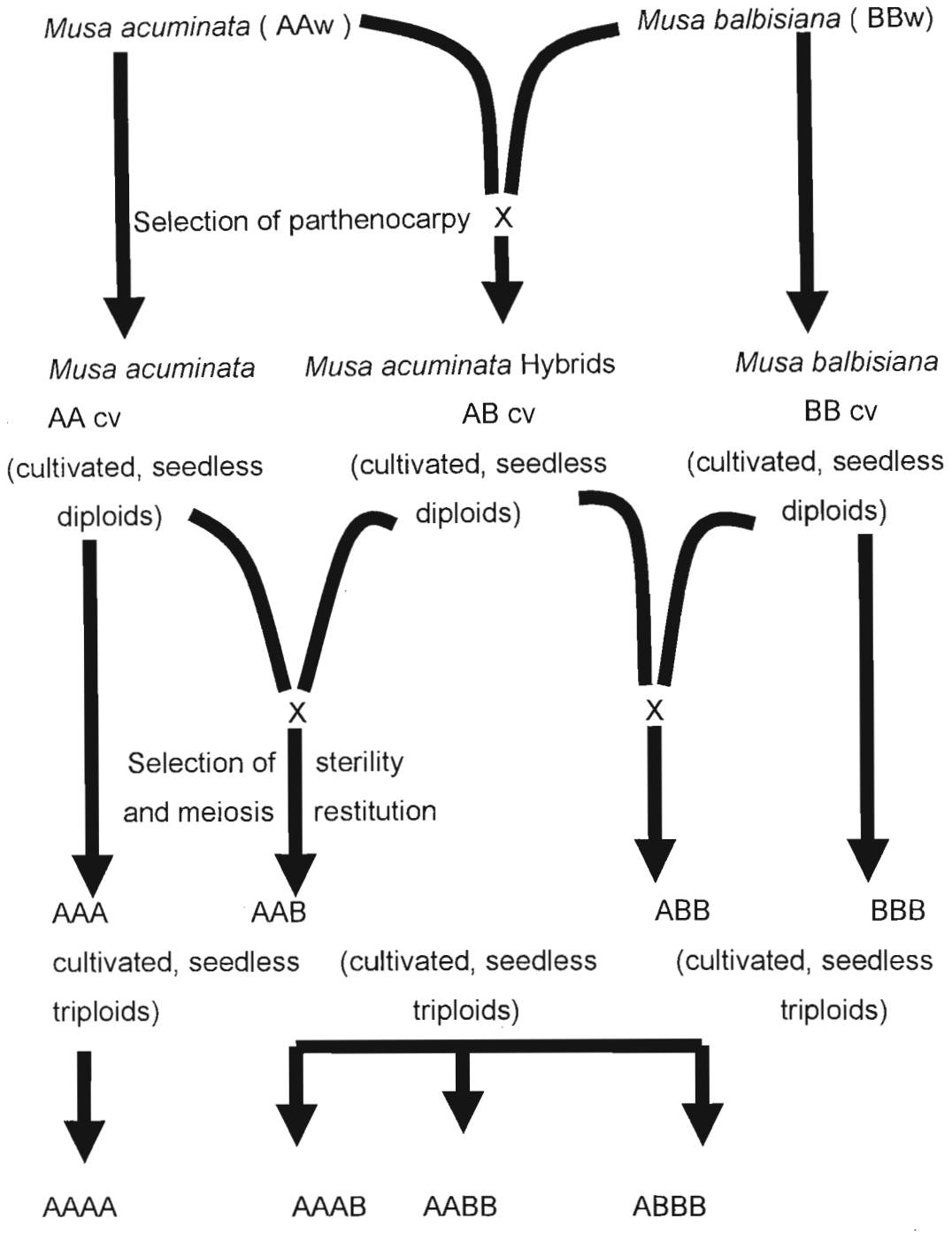

Figure 1.6.1: Evolution of edible banana (slightly modified from VALMAYOR et *al.*, 2002. Adapted originally from BREWBAKER and UMALI, 1956).

1.7 The introduction of bananas (*Musa* spp.) and their importance in Rwanda

The introduction of bananas from the coast to inland Africa is clarified by evidence obtained from languages. The general word signifying bananas in Uganda is Tooke. This word recurs with minor alteration in a broad corridor stretching through western Tanzania, Rwanda and Burundi and northern Malawi and down the Ruvuma valley near the coast in Tanzania (KARAMURA, 1998).

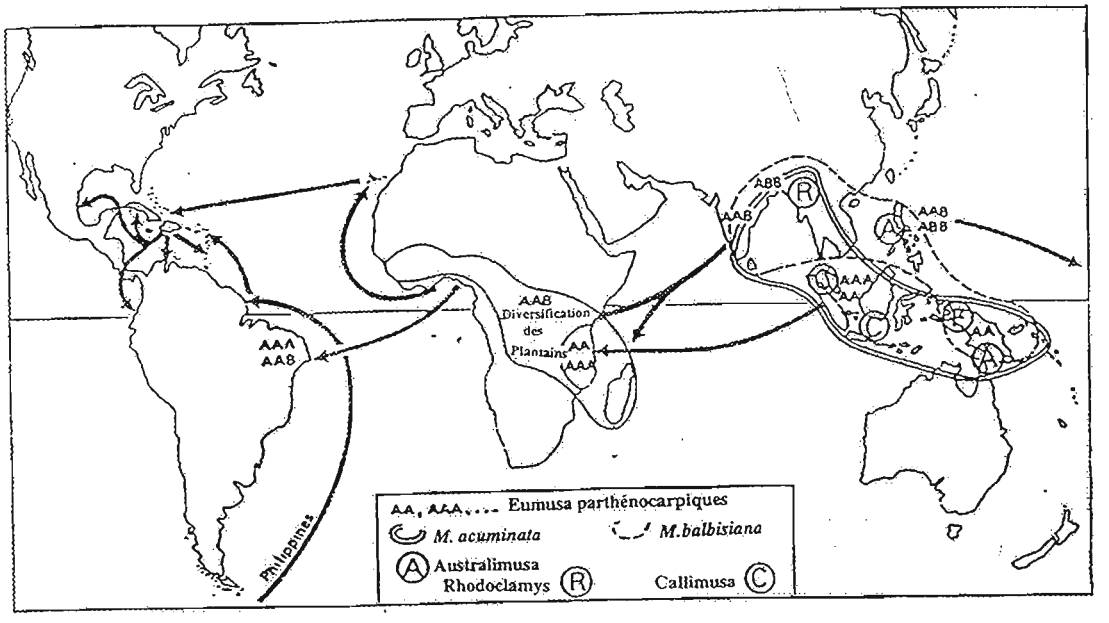

Figure 1.6.2: Origin and distribution of banana cultivars (CHAMPION, 1967).

The history of Rwanda shows that bananas were introduced in the time of governance of the 11th King of Rwanda, Mibambwe I (1411-1444), with the second invasion of the Abanyoro (KAGAME, 1972). During their occupation, the Abanyoro planted bananas in their settlements. They left Rwanda when their king-governor became sick and returned to his area of origin, a place corresponding to modern Mbarara in Uganda,. Bananas then spread in Rwanda from the Abanyoro settlements to the rest of the country.

Bananas are of great importance in Rwanda. The annual banana production is more than two million tones (39% of total raw food production). The crop is cultivated on more

than 180000 ha, that is 23% of the cultivated area in Rwanda (MPYISI *et al.*, 2000; MINECOFIN, 2001). Its production has expanded to almost all marginal areas under cultivation at altitudes of 2,200 meters (CHAHARLEY DE LA MASSELIERE, 1993). They are dominant features in the landscape of Rwanda (Figure. 1.7.1) and per annum consumption of bananas in Rwanda is one of the highest in the Great Lakes region, e.g., it was around 197 kg per capita per year in 2000 (Table 1.7.1). The cultivation of bananas has become woven into the socio-economic life of the communities (KANGASNIEMI, 1998).

Bananas are of exclusive social importance in the country, with banana wine being considered as a sign of sociability and an obligatory element of every ceremony traditionally existing over centuries (LASSOUDIÈRE, 1989; OKECH *et al.*, 2002.). Bananas are mostly grown as a subsistence crop.

Table 1.7.1 Consumption of bananas in (kg/capita/year)(FAO, 2001)

Country	Consumption kg/capita/year
Uganda	243
Rwanda	197
Burundi	89

Figure 1.7.1: Rwandan landscape dominated by banana plants (white arrow).

1.8 The end use of bananas in Rwanda

Banana utilization in Rwanda is currently limited to the fresh form, cooking or beverage production, with only rudimentary efforts to process fruits for storage in The Institute for Agricultural Sciences of Rwanda (ISAR). According to the survey done by the National Banana Program of ISAR and IITA in 2001, the bulk of banana fruit is utilized for beverage production and, to a lesser extent, bananas are utilized as cooked food (OKECH *et al.*, 2002).

1.8.1 Cooking and dessert bananas.

Traditionally, cooking bananas were cooked, with or without peels, in boiling water. When they are peeled, they can be cooked, mixed with beans, groundnuts or some other vegetables (Figure 1.8.1A). Dessert bananas are consumed raw at ripeness (Figure 1.8. 1B).

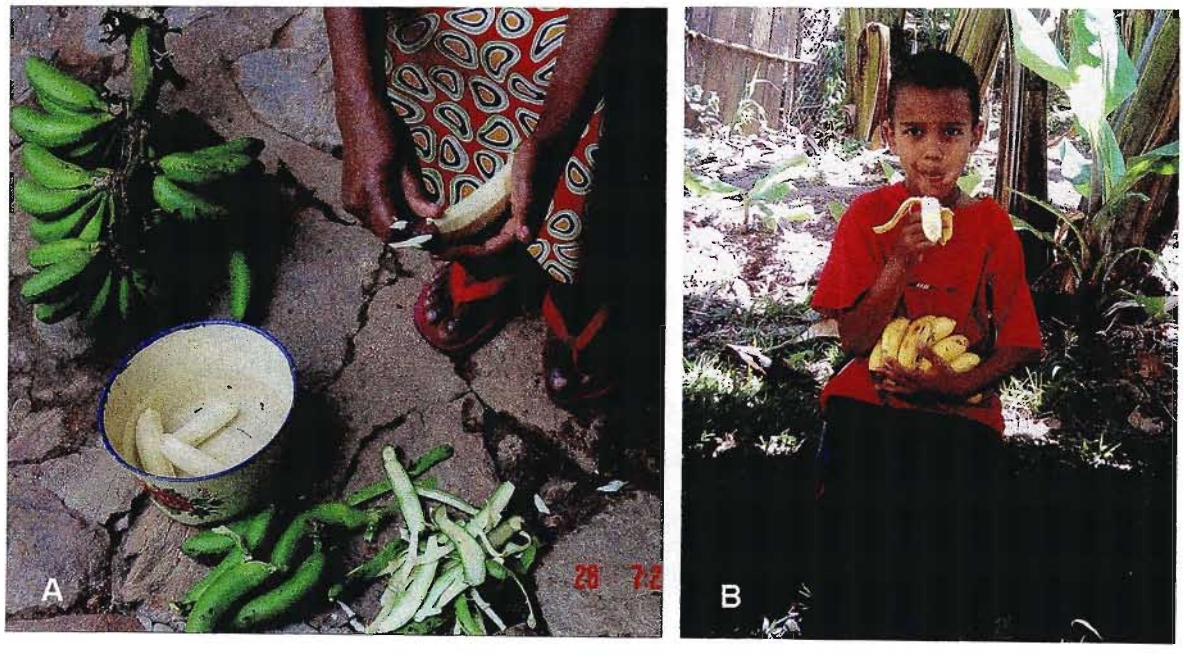

Figure 1.8.1: (A) Peeling of cooking banana belonging to the genomic grouping AAA-EA
(B) Eating dessert banana.

1.8.2 Beer bananas.

At ripeness, these bananas are processed into juice and further into the local beer. To accelerate the ripening process of banana fruits, the following procedure is applied in Rwanda: a conical pit of about 1 meter deep is constructed in the ground and dry banana leaves are burnt in the pit. The pit is then lined with fresh banana leaves (Figure 1.8.2A). Split bunches are then heaped in the pit, which are then covered with more banana leaves and then with soil. The process of ripening takes 5 days.

The juice extraction is done in canoes made from the 'Umuvumu' tree (*Ficus* sp.). The ripe bananas are peeled and put in the canoe (Figure 1.8.2B). The extraction starts with pressing the banana pulp, together with grass (*Vetiveria* sp.), by hand. The juice is then separated from the pulp (Figure 1.8.2C). The grass enhances the effectiveness of mashing and also entangles the pulp such that the juice is set free (Figure 1.8.2D). The juice is then filtered into containers (Figure 1.8.2E), pending consumption or further processing into beer.

Filtered juice is mixed with ground red sorghum or roasted ground red sorghum and put into a pot covered by banana leaves. The pot is placed in a warm place and allowed to ferment for 2 to 3 days. MUNYANGANIZI (1975) found that *Saccharomyces cerevisiae*, the synonym of *Sacchararomyces ellipsoideus,* which induces fermentation in grape juice to produce wine, is the principal agent for the transformation of banana juice to alcohol. He concluded that the sorghum serves as a source of additional nutrients for yeasts inducing fermentation.

Currently, banana fruit processing is modernized and there are many producers of banana wine for internal or export markets. An example is the Compagnie de la Valorisation Industrielle de la Banane au Rwanda (COVIBAR), which produces a highly distilled banana liquor and other banana beverages (Figure 1.8.2F).

Figure 1.8.2: (A) Fresh banana leaves lining the warm pits. (B) Peeling ripe bananas. (C) Pressing banana pulp together with the grass (*Vetiveria* sp.). (D) Juice is set free. (E) Filtration of banana juice. (F) Banana liquor with 41% alcohol, made in Rwanda.

1.9 Banana diversity in Rwanda

The East African highlands represent the secondary center of banana diversity, with the Highland banana cultivars being endemic to this region (SIMMONDS, 1966). East African Highland bananas form the most dominant group in Rwanda. Within this group in the National Banana Collection at Rubona station, cooking clones are more diverse when compared to beer clones. This diversity might be dependent on phenotypic characteristics or the general use of synonyms given by the people. Besides Highland bananas, bananas of the genomic groups AA, AAA, AAB, ABB are found in Rwanda. However, these groups are considered to be of recent introduction to Rwanda (ISAR, 1966; SEBASIGARE, 1987).

GOTANEGRE (1983) cited 67 known Highland banana cultivars in Rwanda. Some of them are widely distributed within the country but are not known to researchers or exist under different names in neighboring countries. Other cultivars are endemic to restricted areas within the country and are not known elsewhere. However, some banana cultivars, especially within the highland banana group, are already in decline well before they have been studied and characterized. Some of these cultivars may serve as important sources for future breeding of cooking bananas with high yield and taste quality.

1.10 Banana research in Rwanda

Interest in research on bananas started in 1953 at Rubona, one of the research stations of the former Institut National pour l'Etude Agronomique au Congo (INEAC). The first banana germplasm collection contained twelve accessions of the subgroup Mutika-Lujugira, and one Ducasse hybrid of the ABB group (ISAR, 1966). In 1961, this Banana germplasm collection was destroyed and replanted with new cultivars, with the objective of screening and promoting only dessert bananas suitable for the overseas export market. After Rwanda gained independence in 1962, and the transformation of INEAC into new institution, the research station Rubona became the Institut des Sciences

Agronomiques du Rwanda (ISAR) and the function of the banana germplasm collection at Rubona shifted towards the conservation of endemic and exotic banana and plantain germplasms. The number of accessions has increased drastically following survey and collecting missions conducted in major banana production areas in 1984 (ISAR, 1989).

In 1992, banana became a priority for research in the ISAR and the National Banana Program now concentrates on the issue of a decline in banana production. With the recommendation of LASSOUDIERE (1989) and the support of Cooperation Technique Française, a collaborative program was developed in 1992 to determine major production constraints, to characterize banana-based cropping systems and to prioritize research needs and directions. Germplasm characterization was one of the prioritized research needs. Unfortunately, with the 1994 war and genocide in Rwanda, that program was not sustained. In 1998, with the support of USAID through an ATDT project, ISAR – IITA and later ISAR – CIAT supported five research programs, including a banana program. This was the opportunity for the banana program to rehabilitate old banana germplasm collections, and conduct a second survey on farmer perceptions and production constraints, to increase the number of accessions in the field gene bank and to initiate further studies to classify and characterize all previously collected banana germplasm (ISAR, 2002).

1.11 Classification and nomenclature of banana in Rwanda

Bananas, being very important in Central and East Africa, have been diversified through somatic mutations and acquired names in various local languages in Uganda, Burundi, Tanzania and Rwanda (SEBASIGARE, 1990; KARAMURA, 1998). In the case of Rwanda, although only one language is spoken, many synonyms are commonly used in the different agro-climatic regions, while homonyms are also found.

Bananas in Rwanda are classified by their end use: cooking, beer and dessert. Cooking banana can be eaten raw but are usually eaten after cooking. They are known locally as 'Inyamunyu' and in the National banana collection they are represented by more clones

than the beer and dessert types. Beer bananas are known locally as 'Inkakama' as they have bitter astringent fruit which makes them unpalatable, therefore they are used exclusively for making beer and juice. SEBASIGARE (1990) gave some biochemical characteristics, concerning the size and viscosity of brownish droplets of pulp of freshly peeled green bananas, that can be used to differentiate between the cooking and beer varieties, but concluded that there are no morpho-taxonomical criteria by which to distinguish the cooking type from the beer one.

To resolve the problem, a series of morphological descriptors are now available which should allow some estimation of the degree of genetic variation between individual clones within germplasm collections (JARRET and LITZ, 1986). However, positive identification even of the most widely cultivated clones are difficult due to the multiple vernacular names for each clone and the lack of morphological and genetic variation between them (JARRET and LITZ, 1986).

The use of isozyme analysis and DNA-markers has thus received considerable attention for characterization and identification of germplasm (JARRET and LITZ 1986; KARP *et al.,* 1996).

1.12 Molecular markers in taxonomy studies

1.12.1 Biochemical markers.

MARKET and MOLLER (1959) first introduced the term "biochemical markers", often referred to as isozyme markers. They first coined the term "isozymes" to describe the different molecular forms of enzymes in a species that share a common catalytic activity.

Isozyme analysis results from translation events and relies on the detection of polymorphisms among isolated enzymes that differ in their electrophoretic mobility. A major disadvantage of this analysis is that the genes encoding isozymes represent a small, biased sample of the genome: only nucleotide substitutions that change the net

charge and the electrophoretic mobility of the enzyme molecule are detected. Many allelic variants remain undetected because of redundancy in the genetic code and similar migration of the enzymes they encode for in a gel (JASIENIUK and MAXWELL, 2001).

Isozyme studies require that the proteins be non-denatured in order for the staining methods to detect enzymatic activity. It should also be noted that in most cases, the polymorphism of isozyme markers is rather poor within a cultivated species, and the level of variability can be rather low in some organisms and thus mask the presence of population heterogeneity (CHAWLA, 2002).

1.12.2 Molecular marker systems.

DNA markers

The discovery of restriction endonucleases and the polymerase chain reaction (PCR) resulted in the development of molecular markers that allowed population biologists to detect variation in DNA sequences (JASIENIUK and MAXWELL, 2001). Today, DNA molecular markers are categorized into two basic techniques; non-polymerase chain reaction (PCR)-based techniques, and PCR-based techniques (CHAWLA, 2002).

Non-PCR-based techniques

Restriction Fragment Length Polymorphisms DNA (RFLPs)

Restriction Fragment Length Polymorphisms (RFLP) analysis involves several steps. Genomic DNA is extracted from the tissue and digested with restriction enzymes, followed by a hybridization step. The restriction fragments can be separated according to their length by agarose gel electrophoresis (WESTMAN and KRESOVICH, 1997). The fragments are then transferred to a membrane by Southern Blotting (SOUTHERN, 1975) and individual restriction fragments detected by nucleic acid hybridization with a

radioactively-labeled DNA probe. When the sizes of fragments hybridizing to the probe differ in the individuals being examined, a polymorphism exists (McCOUCH and TANKSLEY, 1991). Scoring of RFLPs is done by direct observation of an autoradiogram (CHAWLA, 2002), and the similarity of the patterns generated can be used to determine the phylogenetic relationships between varieties within a species, interspecific relationships, genomic evolution and many more (BONIERBALE *et al.,* 1988; BROWN, 1995).

The technique, however, has a relatively high cost and is of a technically demanding nature. Large amounts of DNA are required. Furthermore, a good supply of probes is needed and if heterogonous probes are unavailable, cDNA or genomic DNA probes must be developed. The blotting and hybridization steps are time consuming, expensive and difficult to automate (KARP *et al.,* 1996: DAMASCO *et al.,* 1998).

Techniques based on PCR

The polymerase chain reaction method, commonly called PCR, was developed by MULLIS and FALOONA (1987). This method was initially applied by researchers in human genetics to the analysis of human genetic variation (SAIKI *et al.,* 1988). The technique is based on an *in-vitro* method for the enzymatic synthesis of specific DNA sequences using two oligonucleotide primers that can hybridize to the opposite strands and flank the DNA region of interest. The PCR reaction typically requires cycling among three temperatures, the first to denature the template DNA strands, the second to anneal the primers, and the third to extend the new strands at a temperature which is optimal for the DNA polymerase (CHAWLA, 2002).

The discovery that the polymerase chain reaction can be used with an arbitrary selected primer to amplify a specific set of distributed loci in any genome laid the foundation for high output of genetic markers that can be used for a variety of purposes (WELSH and McCLELLAND, 1990; WILLIAM *et al.,* 1990). Since arbitrarily primed PCR is easily done with small amounts of DNA and without the requirement for clone banks or other forms

of molecular characterization of the species in question, many minor crop species, lacking sufficient research investment, became amenable to genetic studies. In addition, arbitrarily-primed PCR does not normally require radioactive-labeled nucleotides.

Arbitrarily-primed PCR polymorphisms are based on mismatches in primer binding sites or insertion/deletion events, and therefore usually result in the presence or absence of an amplified product from a single locus (WILLIAMS *et al.,* 1990; WELSH and McCLELLAND, 1990). This means that the arbitrarily-primed PCR markers are usually dominant and heterozygotes cannot be detected (KARP *et al.,* 1996; JASIENIUK and MAXWELL, 2001).

1. Random Amplified Polymorphic DNA (RAPD) markers

Random Amplified Polymorphic DNA (RAPD) analysis is a PCR-based genetic marker technique which was developed by WILLIAMS *et al.* (1990). RAPD markers are fragments of genomic DNA amplified through PCR using an oligonucleotide primer of random sequence. A number of closely related techniques based on the principle were developed almost at the same time. These techniques are:

RAPD: Random Amplified Polymorphic DNA (RAPDs)

DAF: DNA Amplifying Fingerprinting

AP-PCR: Arbitrarily-Primed Polymerase Chain Reaction

All these techniques refer to DNA amplification using single random primers and share the same principle with some slight differences in experimental detail (CHAWLA, 2002). Different RAPD patterns arise when the genomic regions vary for the presence or absence of complementary primer annealing sites. Without primer binding, sequence amplification cannot occur, resulting in the absence of a band in the analysis. The primers are typically 10bp long (WILLIAMS *et al.,* 1990) and no specific knowledge of a particular DNA sequence is required to choose or produce a primer. The RAPD process typically reveals several polymorphic genetic segments per primer within populations; other segments may appear as monomorphic bands within or across populations

(HADRYS *et al.,* 1992). RAPD analysis has several advantages over isozymes and RFLPs (KARP *et al.,* 1996; JASIENIUK and MAXWELL, 2001):

- Genetic variation at many loci from different regions of the genome can be examined quickly;
- Very small amounts of DNA are required;
- No prior template DNA sequence information is required; and it is
- Less costly and less time consuming.

2. DNA Amplification Fingerprinting (DAF)

A single, short, arbitrary primer of 5 - 8 nucleotides is used to amplify genomic DNA using PCR (CAETANO-ANOLLES *et al.,* 1991, CHAWLA, 2002). The DAF method differs from RAPDs not only in the length of primers used, higher primer concentration and two temperature cycles, but also in the gel matrix used for the visualization technique and staining (KARP *et al.,* 1996; CHAWLA, 2002).

3. Arbitrarily-primed Polymerase Chain Reaction (AP-PCR)

This is a special case of RAPDs wherein discreet amplification patterns are generated by employing single primers of 10 – 50 bases in length in PCR-amplification of genomic DNA. AP-PCR also differs from RAPDs in the gel matrix used for the visualization technique and staining (CHAWLA, 2002).

To cover the three techniques (RAPD, DAF, AP-PCR), CAETANO-ANOLLES *et al.,* 1992) suggested the term Multiple Arbitrary Amplicon Profiling (MAAP). Each method generates DNA profiles of varying complexity, primary defined by the sequence of the arbitrary primer used to direct amplification. RAPD describes the polymorphisms and DAF and AP-PCR describe the strategy used. Therefore, the term MAAP encompasses each variation of the overall strategy i.e describes the underlying characteristics,

multiple, arbitrary nature of target sites and the amplification of a range of characteristic DNA products.

4. Amplified Fragment Length Polymorphism (ALFP)

Amplified Fragment Length Polymorphism (AFLPs) methods combine RFLPs and PCR techniques, as they are specific, PCR-amplified, fragments of a restriction digest. They require a genomic restriction digestion, ligation of adapters to the restricted ends and the use of primers that contain the adapter sequence, the enzyme target sequence and selective nucleotides. This technique has the extra advantage of combining the speed of the PCR with the precision of restriction fragment length polymorphisms (VOS *et al.*, 1995).

The amplified products are radioactively or fluorescently-labeled and separated on a sequencing gel. In comparison with RAPD techniques, AFLPs are:

- Highly expensive and require more DNA per reaction; and
- Expensive to generate as silver staining, fluorescent dyes or radioactivity are used to detect bands.

5. Simple Sequences Repeats (Microsatellite)

The term "microsatellite" was coined by LITT and LUTTY (1989). Also known as Simple Sequences Repeats (SSRs) or Short Tandem Repeat (STR), microsatellites are present in the genome of all eukaryotes (CHAWLA, 2002). These terms are commonly used when the basic repeated unit is between 2 and 10 bases pairs in the length. The term "minisatellite" is used when the basic repeat is longer.

Polymorphism created by such elements is called Variable Number of Tandem Repeat (VNTR) polymorphism (JASIENIUK and MAXWELL, 2001). Microsatellite markers are currently the preferred technique for molecular characterization of different plant

species, because of their high information content; they are locus specific and co-dominant markers which allows distinction to be made between heterozygous and homozygous individuals. They are inherited in a Mendelian fashion which permits to carry out paternity analyses. However, this technique is very expensive due the need to identify polymorphic primer sites and to synthesize primers (PEAKALL, 1997; PARKER *et al.,* 1998; GUPTA and VARSHNEY, 2000; JASIENIUK and MAXWELL, 2001; WÜNSCH and HORMAZA, 2002).

1.12.3 Molecular markers in identification and characterization of banana genetic diversity.

Many studies have revealed how different molecular markers can be valuable for analyzing genetic diversity and determining relationships within and among populations (HARVEY and BOTHA, 1996; BEEBE *et al.,* 2000; CHEN and YAMAGUCHI, 2002). They are successful in generating groupings of germplasm that appear to be agronomically and biologically-meaningful, regardless of the technique employed (LASHRMES *et al.,* 1996; LAZARO and AGUINAGALDE, 1998; RÜTER *et al.,* 1999; YEE *et al.,* 1999; PARKER *et al.,* 2002; HUANG *et al.,* 2003; PEREIRA – LORENZO *et al.,* 2003).

In *Musa* taxonomic studies have been conducted using a wide array of techniques. SIMMONDS and SHEPHERD (1955) classified cultivated bananas using morphological traits, while SHEPHERD (1959) used cytogenetic characters. RIVERA (1983) used molecular markers and classified Saba cv as triploid *balbisiana*, while JARRET and LITZ (1986) confirmed the conventional classification scheme using isozyme methods. HORRY (1989) assayed eight isozyme loci from diverse geographic origins and their results suggested two independent centers of domestication for *Musa acuminata*, one in Southeast Asia and other in Papua New Guinea.

Restriction Fragment Length Polymorphism markers, which are normally codominant, have been used to confirm *Musa* classification and to amend the genome formula and

subspecies/subgroup classification of some varieties (JARRET, *et al.,* 1992; GAWEL *et al.,*1992; BHAT *et al.,*1994; CARREEL,1994; CARREEL *et al.,*1994).

BHAT *et al.* (1994) concluded that RFLPs may be used to group *Musa* clones, however, he suggested that the most accurate classification must be obtained using molecular methods in conjunction with morphological and cytological examinations.

The Random Amplified Polymorphic DNA technique has been used for identifying polymorphisms in a range of cultivars and species of *Musa* representing a series of genome configurations, and these authors concluded that RAPDs can be used for *Musa* germplasm characterization and identification of varieties (HOWELL *et al.,* 1994; BHAT and JARRET, 1995; THU *et al.,* 2002; ONGUSO *et al.,* 2004; UMA *et al.,* 2004).

ENGELBORGH *et al.* (1998) first reported the use of the Amplified Fragment Length Polymorphism (AFLP) technique on bananas and concluded that the technique can be used for identification of duplicates and somaclonal variants, as well as for the assessment of genetic relationships. This method has been used for classifying *Musa* species (WONG *et al.,* 200I; WONG *et al.,* 2002 ; UDE *et al.,* 2002a) and was successfully applied in the assessment of genetic diversity of banana cultivars (LOH *et al.,* 2000 ; UDE *et al.,* 2002b; UDE *et al.,* 2003; BHAT *et al.,* 2004).

CROUCH *et al.* (1997) demonstrated the usefulness of microsatellites as genetic markers in the genus *Musa* in an assessment of the level of heterozygosity present in a putative homozygous diploid banana accession which was commonly used in *Musa* genetic analysis and breeding studies. The analysis demonstrated that the mainly wild banana, Calcutta 4, used in breeding programs as a source of resistance to black sigatoka, is heterozygous. This may call for a reassessment of the use of Calcutta 4 as an homozygous tester genotype.

CRESTE *et al.* (2003) successfully used microsatellites and detected erroneously-classified banana cultivars and identified duplication in the Prata subgroup.

There are many studies on the characterization and identification of wild banana, Cavendish and Plantain because of their place in breeding and their importance for local and world markets (VISSER, 1998). However, very few studies using molecular markers have been done on East African Highland bananas.

1.12.4 Aims of this study.

Despite East African Highland bananas being very important in Central and East Africa, they remain poorly studied. Research has focused on dessert bananas, while East Highland bananas were neglected. Little work has been done using numerical taxonomy and has resulted in the subgroup being subdivided into five clone sets. Efforts to include East African Highland bananas in breeding schemes with the aim of improving agronomic performances and resistance to diseases have also been started (ORTIZ *et al.*, 1995). However, this subgroup has been diversified through somatic mutation and acquired many different names in various local languages within Uganda, Burundi, Tanzania and Rwanda. Therefore there is a strong need for the characterization of banana germplasm from this main center of diversity (Rwanda, Burundi, Uganda, Tanzania and Eastern Congo). To our knowledge, evaluation of different banana clones and collection of banana germplasm where undertaken in Rwanda during the 1980s (ISAR, 1983, 1989, 1992; SEBASIGARI, 1987). However, no banana germplasm in Rwanda has yet been characterized and classified. The aim of this study was to characterize the banana germplasm collection of the Institut des Sciences Agronomiques du Rwanda at the Rubona Station, with the following specific objectives:

- To characterize the banana and plantain varieties in National Banana Germplasm Collection at Rubona – Rwanda;
- To investigate the ploidy level of varieties in the banana germplasm collection at Rubona – Rwanda; and
- To assess genetic diversity of Rwandan Highland banana varieties using RAPD markers.

Chapter 2

Characterization of the banana germplasm collection from Rubona – Rwanda

2.1 Introduction

Banana germplasm in Rwanda contains different genotypes. The most important banana genotype belongs to the Subgroup Lujugira – Mutika, commonly known as the East African highland banana (AAA-EA). The efficient use of this crop, both by researchers and farmers, has been delayed due to taxonomic problems. Local germplasm has been collected but, to date, it has been poorly studied (SEBASIGARI, 1990). Earlier and during the 1994 genocide, some varieties disappeared or were given new names. This caused confusion in the nomenclature; therefore, urgent characterization of the banana germplasm in Rwanda is needed.

ORTIZ (1997) showed that the quantitative traits of fruit have high heritability, high repeatability and low coefficient variation. However, KARAMURA and PICKERSGILL (1999) used many characters including qualitative and quantitative traits for characterization of East African highland bananas and further subdivided them into five clone sets according to the following characteristics: Mbidde clone set is characterized by having bitter and astringent pulp, Nakitembe clone set by having a male inflorescence rachis with persistent neuter flowers and male bud imbricate, Nakabululu clone set by having a bunch orientation subhorizotal with fruit size length under 15 cm, Musakala clone set by having a bunch orientation oblique to pendulous with the length of fruit over 15 cm, and Nfuuka clone set by having a bunch shape mainly rectangular with compact fruits inflated, rounded or rectangular with intermediate shaped apices.

This study investigated for the first time the banana germplasm of Rwanda. Use was made of quantitative traits of the finger/fruit because of their high heritability.

2.2 Materials and Methods

The National Banana Germplasm Collection at Rubona is a field collection (Figure 2.1) maintained at 1723 m at 2° 29' 14.6 S latitude and 29° 46' 20.6 E longitude. Average annual rainfall is 1120 mm. Minimum annual temperature is 14°C, while the maximum temperature is 25°C. The soil is considered as ultisol with pH 5.5 and is mulched twice a year with *Tripsacum* species during the dry seasons (May-August) and (December-January). The accessions were planted in 8-plant plots in single rows for taxonomic and agronomic characterization. We used conventional planting material, such as explants derived from vigorous, healthy rhizomes from plants just prior to flowering. The explants were pared to remove all visible traces of nematode and banana weevil infestation and were immersed in hot water (52 – 55°C) for 20 mins. The distance between plants in the same row and between the rows was 3 m.

The germplasm received normal management, except that no chemical or mineral fertilizers were applied. During desuckering, surplus suckers were removed from the plant using a hoe, before they could become too large and unmanageable. The harvesting was done when the fingers were full of starch. This was determined by a breaking of peel on some clones, or the start of finger abscission on others (Figure 2.2). Post-harvest measurements were taken on the medium finger of medium hands of the bunch. Length of interior and exterior sides of the fruits (cm) were taken using a tape measure immediately after harvesting, weight of fruits (g), circumference of fruits (cm), green life (days) and post-green life (days) of fruits were done on five plants of each accession according to the recommendations for banana (IPGRI-INIBAP/CIRAD, 1996; DADZI *et al.*, 1997). The end of post green life of fruit for dessert banana, beer banana and plantains, was taken when the banana peel became brown and soft. The width of the fruit was calculated by circumference/3.14 and the actual length by formula: L= (le + li)/2, where 'le' is exterior length and 'li' is interior length of the fruit (Appendix 1).

Figure 2.1: National Banana Germplasm Collection Rubona-Rwanda

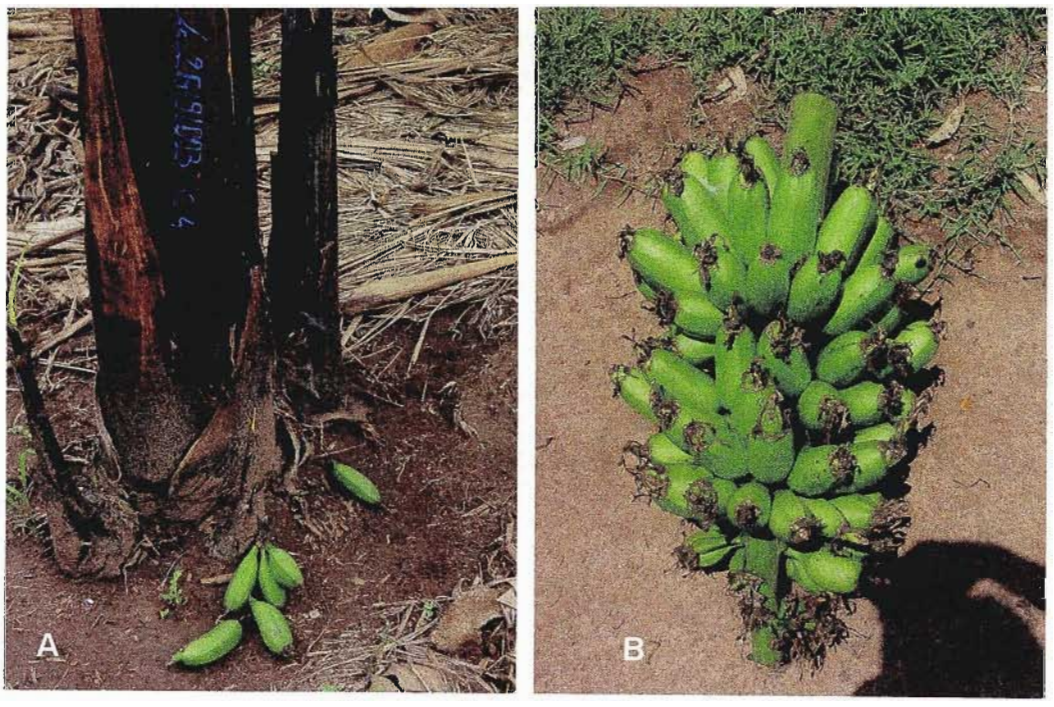

Figure 2.2: (A) Abscised fruits of 'Intuntu' a brewing variety ready for harvest.
(B) 'Inyabupfunsi' a cooking variety harvested.

2.3 Statistical analysis

All data were subjected to principal component analysis (PCA) (GenStat Release 7.1), based on group of means of quantitative traits of fruits. The eigenvectors were derived from the correlation matrix. Entries were plotted, according to their scores of the first and the second principal component, using Microsoft's Excel software.

2.4 Results and Discussion

In this study, a total of 90 accessions of bananas were characterized. Of these 62 accessions were from Subgroup Lujugira – Mutika, 8 from 2 plantain hybrids, 5 from Subgroup Cavendish, 2 from Subgroup Ibota, 2 from Subgroup Prata, and 1 from Subgroup Gros Michel, the genomic groups were 8 from ABB and 2 from AB (Table 2.1). When the variables were integrated by the principal component (PC) 92.9% of the variability observed was explained by the first three PCs (Table 2.2) and accounted for 48.8%, 27.1% and 17% of the variation in the data set respectively.

The most important variables contributing to the first component were the length, weight, width and length/width ratio of fruit, which were negatively correlated to the first component. In the second component, the contrast was between the green life and post-green life, while in the third, the contrast was amongst width and length/width ratio (Table 2.2). The plot of the first principal component scores against the second revealed that, all highland cooking banana clones were dispersed in the first and second quadrants. Beer clones belonging to the 'Mbidde' clone set were dispersed in the third and fourth quadrants, except for accessions 'Ingumba' (46) and Inyabukumwe (55), which were dispersed in the second quadrant. This may be due to their high level of green life (Figure 2.3).

Table 2.1 Banana cultivar accessions analyzed in the germplasm collection at Rubona Station – Rwanda

Cultivar name	Genomic group	Subgroup	Origin	Local use	Key
Americani	AAA	Cavendish	Ivory Coast	Dessert	86
Bakungu	AAA	Lujugira – Mutika	Rwanda	Cooking	42
Bakurura	AAA	Lujugira – Mutika	Rwanda	Cooking	4
Barabeshya	AAA	Lujugira – Mutika	Rwanda	Cooking	5
BS529	AAAB	H*	IRAZ	Cooking	77
Cyayaya	AAB	Plantain	Rwanda	Cooking	76
DIBIS	AAAB	H*	IRAZ	Cooking	78
Gisubi kagongo	ABB	Pisang Awak	?	Beer	62
Grande naine	AAA	Cavendish	Ivory Coast	Dessert	89
Gros Michel	AAA	Gros Michel	?	Dessert	85
Guindi	ABB	-	?	Beer	59
Ibotabota	AAA	Ibota	?	Beer	60
Ice cream	ABB	Bluggoe	?	Dessert	73
Icyerwa nini	AAA	Lujugira – Mutika	Rwanda	Cooking	7
Icyerwa ntoya	AAA	Lujugira – Mutika	Rwanda	Cooking	8
Igihobe	AAB	Plantain	Rwanda	Cooking	75
Igihuna	AAA	Lujugira - Mutika	Rwanda	Beer	67
Igihuni	AAA	Lujugira – Mutika	Rwanda	Cooking	28
Impigi	AAA	Lujugira – Mutika	Rwanda	Cooking	41
Impyisi	AAA	Lujugira – Mutika	Rwanda	Beer	51
Inconnu	AAA	Lujugira – Mutika	Rwanda	Cooking	36
Ingagara	AAA	Lujugira – Mutika	Rwanda	Cooking	30
Ingaju	AAA	Lujugira – Mutika	Rwanda	Cooking	16
Ingarama	AAA	Lujugira – Mutika	Rwanda	Cooking	3

Table 2.1 Banana cultivar accessions analyzed in the germplasm collection at Rubona Station – Rwanda (Continued)

Cultivar name	Genomic group	Subgroup	Origin	Local use	Key
Ingenge	AAA	Lujugira – Mutika	Rwanda	Cooking	26
Ingoromoka	AAA	Lujugira – Mutika	Rwanda	Beer	66
Ingote	AAA	Lujugira – Mutika	Rwanda	Beer	45
Ingumba	AAA	Lujugira – Mutika	Rwanda	Cooking	4
Ingumba	AAA	Lujugira – Mutika	Rwanda	Beer	46
Injagi	AAA	Lujugira – Mutika	Rwanda	Cooking	18
Injogo	AAA	Lujugira – Mutika	Rwanda	Cooking	1
Injumbura	ABB	Pisang Awak	IRAZ	Beer	65
Inkati	AAA	Lujugira – Mutika	Rwanda	Beer	50
Insana	AAA	Lujugira – Mutika	Rwanda	Cooking	12
Inshakabuhake	AAA	Lujugira – Mutika	Rwanda	Beer	57
Insiri	AAA	Lujugira – Mutika	Rwanda	Cooking	29
Insiri	AAA	Lujugira – Mutika	Rwanda	Beer	53
Intama	AAA	Lujugira – Mutika	Rwanda	Cooking	11
Intariho	AAA	Lujugira – Mutika	Rwanda	Cooking	9
Intembe	AAA	Lujugira – Mutika	Rwanda	Beer	69
Intobe	AAA	Lujugira – Mutika	Rwanda	Cooking	31
Intokatoke	AAA	Lujugira – Mutika	Rwanda	Beer	48
Intuntu	AAA	Lujugira – Mutika	Rwanda	Cooking	13
Intuntu	AAA	Lujugira – Mutika	Rwanda	Beer	47
Intutsi	AAA	Lujugira – Mutika	Rwanda	Cooking	34
Inyabukumwe	AAA	Lujugira – Mutika	Rwanda	Beer	55
Inyabupfunsi	AAA	Lujugira – Mutika	Rwanda	Cooking	37
Inyabutembe	AAA	Lujugira – Mutika	Rwanda	Cooking	15

Table 2.1 Banana cultivar accessions analyzed in the germplasm collection at Rubona Station – Rwanda (Continued)

Cultivar name	Genomic group	Subgroup	Origin	Local use	Key
Inyamico	AAA	Lujugira – Mutika	Rwanda	Cooking	17
Inyamunyo	AAA	Lujugira – Mutika	Rwanda	Cooking	19
Inyoya	AAA	Lujugira – Mutika	Rwanda	Cooking	40
Inziga	AAA	Lujugira – Mutika	Rwanda	Beer	71
Inzirabahima	AAA	Lujugira – Mutika	Rwanda	Cooking	22
Isha	AAA	Lujugira – Mutika	Rwanda	Beer	49
Ishika	AAA	Lujugira – Mutika	Rwanda	Beer	58
Kamaramasenge	AB	-	?	Dessert	83
Kayinja	AB	-	?	Beer	64
Kayuku	AAA	Lujugira – Mutika	Rwanda	Cooking	38
Kibuzi	AAA	Lujugira – Mutika	Rwanda	Cooking	35
Kintu	AAA	Lujugira – Mutika	Rwanda	Cooking	14
Kirayenda	AAA	Lujugira – Mutika	Rwanda	Cooking	27
Kivuvu	ABB	Bluggoe	?	Beer	63
Lacatan	AAA	Cavendish	?	Dessert	87
Mbirabire	AAA	Lujugira – Mutika	Rwanda	Cooking	33
Mbwaziruma	AAA	Lujugira – Mutika	Rwanda	Cooking	21
Mujuba	AAA	Lujugira – Mutika	Rwanda	Cooking	6
Mushayija-naranda	AAA	Lujugira – Mutika	Rwanda	Cooking	44
Muzibwe	AAA	Lujugira – Mutika	Rwanda	Beer	54
Naironi	AAA	Lujugira – Mutika	Rwanda	Cooking	20
Nsira	AAA	Lujugira – Mutika	Rwanda	Cooking	23
Nyabwihogora	AAA	Lujugira – Mutika	Rwanda	Cooking	39
Nyagashaba	AAB	Plantain	Rwanda	Cooking	79
Nyakababi	AAA	Lujugira – Mutika	Rwanda	Cooking	10

Table 2.1 Banana cultivar accessions analyzed in the germplasm collection at Rubona Station – Rwanda (Continued)

Cultivar name	Genomic group	Subgroup	Origin	Local use	Key
Nyakitembe	AAA	Lujugira – Mutika	Rwanda	Cooking	25
Nyakitengwa	AAA	Lujugira – Mutika	Rwanda	Cooking	24
Nyamabere	AAA	Lujugira - Mutika	Rwanda	Beer	56
Pelipita 2	ABB	-	?	Beer	72
Petite naine	AAA	Cavendish	?	Dessert	90
Pommes	AAB	Prata	?	Dessert	84
Poyo	AAA	Cavendish	Ivory Coast	Dessert	88
Prata	AAB	Prata	?	Beer	61
Radjang	ABB	Pisang Awak	?	Beer	68
Rugigana	AAA	Lujugira – Mutika	Rwanda	Beer	52
Rugondo	AAA	Lujugira – Mutika	Rwanda	Cooking	32
Rusatsi	AAA	Lujugira – Mutika	Rwanda	Cooking	43
Saba	ABB	Bluggoe	?	Beer	74
Tsambunu	AAB	Plantain	Rwanda	Cooking	82
Umushaba 1	AAB	Plantain	Rwanda	Cooking	80
Umushaba 2	AAB	Plantain	Rwanda	Cooking	81
Yangambi km 5	AAA	Ibota	?	Beer	70

- Unknown subgroup

H* Hybrid

? Unknown origin

IRAZ: Institut de Recherches Agronomiques et Zootechniques

Table 2. 2 Eigenvector contribution of quantitative traits of fruit to first, second and third principal components estimated in a collection of 90 accessions of bananas (*Musa* spp.) at the National Banana Collection Rubona - Rwanda

Trait	PC 1	PC 2	PC 3
Average length of fruit (cm)	-0.57097	-0.02500	0.19474
Post green life of fruit (days)	0.06716	-0.69946	0.04284
Fruit weight (g)	-0.55545	-0.11733	-0.21567
Green life of fruit (days)	-0.04817	0.70344	0.06655
Width of the fruit (cm)	-0.44867	0.02411	-0.62118
Length/Width ratio for fruit	-0.39666	0.03084	0.72349
Latent roots	2.931	1.621	1.021
Percentage of variation	48.8	27.1	17

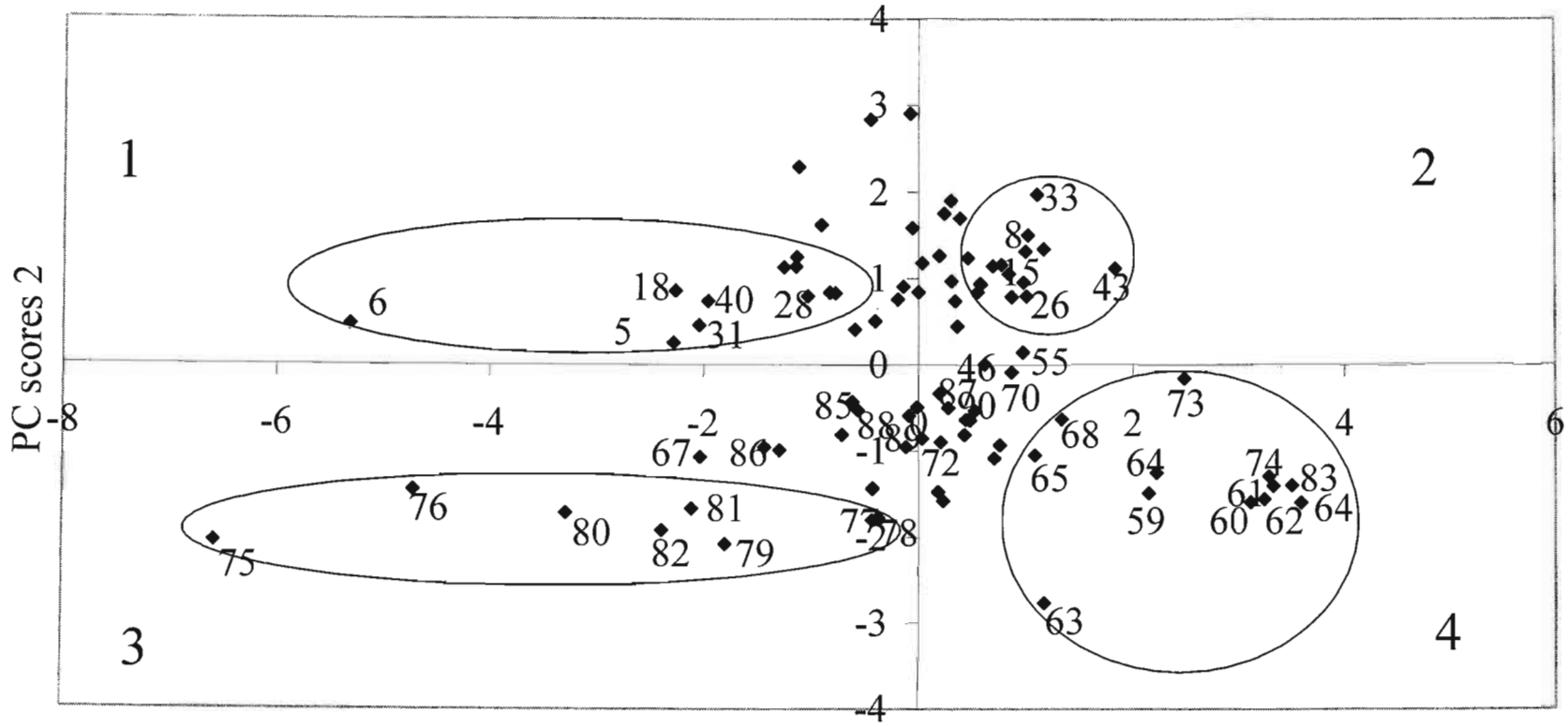

Figure 2.3 : Positions of scores of 90 accessions of banana entries for PC 1 and PC 2

Cycle in quadrant: (1) Musakalala clone set, (2) Nakabululu clone set,
(3) Subgroup Plantain, and (4) Mixed genomic group (AAA, AB, ABB, AAB)

The clones 'Barabeshya' (5), 'Mujuba' (6), 'Igihuni' (28), 'Intobe' (31), 'Inyoya' (40), had long fruits with high length/width ratios which were dispersed in quadrant 1. While the clones 'Inyabutembe' (15), 'Ingenge' (26), 'Mbirabire' (33), 'Rusatsi' (43) were dispersed in the second quadrant with other clones having small fruit size (Figure2.3).

According to the classification of Subgroup Lujugira – Mutika, established by KARAMURA and PICKERSGILL (1999), those clones with a long fruit length and a high length/width ratio belong to the clone set Musakala where the longest length was 24 cm, while fruits with short length belong to clone set Nakabululu. Nevertheless, some clones require supplementary characteristics, for example, the form of the male bud, for classifying their position in the clone set. This is specifically so for those that fit into the Nakabululu clone set, as they can have long fruit.

For the Nakitembe clone set, there is no need to use quantitative characteristics for identification, as they can be easily recognized by their male inflorescence rachis, which has persistent neuter flowers and imbricate male buds (KARAMURA and PICKERSGILL, 1999). The clones that are not placed in Nakubululu and Musakala should be included in the Nfuuka clone set.

Interestingly, all plantains; 'Igihobe' (75), 'Cyayaya' (76), 'Umushaba 1' (80), 'Tsambunu' (82), 'Umushaba 2' (81), 'Nyagashaba' (79), 'BS529' (77) and 'DIBIS' (78) were dispersed in the third quadrant due to their fruit length. According to the classification of plantain used by TEZENAS DU MONTCEL and DEVOS (1978), accession 75 is horn plantain, which is separated from others that have a long fruit size, followed by accession 76 (the false horn), while accessions 79, 80, 81 and 82 were considered as French plantain (Table 2.3). The hybrid plantains, 77 and 78, were grouped together (Figure 2.2).

Except for triploids, AAA genomic Subgroups such as Gros Michel, Cavendish, some of the Lugira–Mutika and Ibota were dispersed in the third and fourth quadrants. The accessions that belong to the AB, ABB and AAB groups were localized in the fourth

quadrant (Figure 2.3). These accessions are 'Guindi' (ABB) 59, 'Prata' (AAB) 61, 'Gisubi kagongo' (ABB) 62, 'Kivuvu' (ABB) 63, 'Kayinja' (AB) 64, 'Injumbura' (ABB) 65, 'Radjang' (ABB) 68, 'Ice cream' (ABB) 73, 'Saba' (ABB) 74, 'Kamaramasenge' (AB) 83 and 'Pomme' (AAB) 84. There are some exceptions such as accession 60 'Ibotabota' (AAA), which was closer to the ABB genomic group, while accession 70 'Yangambi Km 5', which is in the same subgroup with 'Ibotabota', is closer to the AAA genomic group. Another exception is accession 72 'Pelipita -2' which belongs to the ABB group which is closer to the AAA group (Figure 2.3). These exceptions showed us that quantitative traits such as fruit size cannot be used in differentiation of genomic groups for banana germplasm. It is important to note that even the use of fruit size in the Subgroup Lujugira – Mutika will need some supplemental qualitative characters. For example, the clone: 'Igihuni'(28) was placed into the Nakitembe clone set due to the presence of persistent neuter flowers and male bud imbricate (Table 2.4). It is also important to note that three clones from Subgroup Lujugira – Mutika: 'Ingumba' (2), 'Intuntu' (13) and 'Insiri'(29), have the same name but belong to two different clone sets, due to a change of qualitative characters as a result of a mutation of cooking varieties to beer banana varieties or *vice versa.*

Table 2.3. Classification of Plantain found in banana germplasm of Rwanda

Clones	Bunch types	Subgroup	Genomic group
Cyayaya(76)	False horn	Plantain	AAB
Igihobe(75)	Horn		
Nyagashaba(79)	French		
Tsambunu(82)	French		
Umushaba 2(81)	French		
Umushaba1(80)	French		

Table 2.4 Classification of some Highland bananas from the banana germplasm of Rwanda

Clones	Clone sets	Subgroup	Genomic group
Barabeshya	Musakala	Lujugira -Mutika	AAA
Icyerwa ntoya	Nakabululu		
Igihuna	Mbidde		
Igihuni	Nakitembe		
Impyisi	Mbidde		
Ingagara	Nakitembe		
Ingenge	Nakabululu		
Ingoromoka	Mbidde		
Ingote	Mbidde		
Ingumba	Nfuuka		
Ingumba	Mbidde		
Injagi	Musakala		
Injogo	Nfuuka		
Inkati	Mbidde		
Insana	Musakala		
Inshakabuhake	Mbidde		
Insiri	Mbidde		
Intama	Nakabululu		
Intembe	Mbidde		
Intokatoke	Mbidde		
Intuntu	Musakala		
Intuntu	Mbidde		
Intutsi	Nakitembe		
Inyabukumwe	Mbidde		
Inyabupfunsi	Nfuuka		
Inyabutembe	Nakabululu		
Inyoya	Nakitembe		

Table 2.4 Classification of some Highland bananas from the banana germplasm of Rwanda (Continued)

Clones	Clone sets	Subgroup	Genomic group
Inziga	Mbidde	Lujugira - Mutika	AAA
Ishika	Mbidde		
Kibuzi	Nakitembe		
Kintu	Nakabululu		
Kirayenda	Nakitembe		
Mbirabire	Nfuuka		
Mbwaziruma	Nakitembe		
Mujuba	Musakala		
Muzibwe	Mbidde		
Nyakitembe	Nakitembe		
Nyamabere	Mbidde		
Rugigana	Mbidde		
Rusatsi	Nfuuka		

2.4 Conclusion

The quantitative traits of fruit/finger used for characterization of banana germplasm using PCA clarified the separation of the subgroup plantain from other genomic groups. Measurement of fruit size can help in differentiation of clones in Subgroup Plantain and Subgroup Lujugira-Mutika as well, under similar environmental conditions. This study confirmed the findings of ORTIZ (1997) on the high heritability and low variation in fruit size and suggests that the classification system of Highland bananas used by KARAMURA and PICKERSGILL (1999) may be applied to classify banana germplasm in Rwanda. However, the curators of banana germplasm in East and Central Africa will need to have supplementary phenotype characteristics for the classification of some clones of Subgroup Mutika-Lujugira, as it has a high degree of morphological diversity.

Chapter 3

Distribution of banana cultivars in four major growing regions of Rwanda

3.1 Introduction

Banana cultivars in Rwanda exist under local names within limited habitats. However, due to high land pressures, diseases and plant nutritional problems, certain banana cultivars are disappearing and some new varieties are introduced or mutations occur within existing cultivars. Unfortunately there is no baseline information available for comparison with the current distribution of cultivars. Therefore the objectives of this study were, firstly, to establish the distribution of banana cultivars and their relative importance in four of the major growing regions and secondly to identify synonyms and new material for expansion of the National Banana Germplasm Collection.

3.2 Materials and Methods

The survey of banana germplasm was conducted during June 2001 in 12 districts from different provinces of Rwanda, representing four major growing regions of banana with high production potential (principally good rainfall and soil). These regions cover 41% of banana production in Rwanda (MPYISI *et al.,* 2000). The same sites were also used for a diagnostic survey of banana production constraints (OKECH *et al.*, 2002), with authorisation obtained from the Mayors of the different districts surveyed (Appendix 2). The four regions covered the provinces of Cyangugu, Kibungo, Kigali Rural and Kivu lake border, including some parts of Gisenyi and Kibuye (Figure 3.1). The districts within each region were selected on the basis of importance of banana to the local farming communities. The checklist (Appendix 3) used for the survey was from the diagnostic survey studies on banana conducted in Rwanda during June 2001.

3.2.1 Farm selection.

Sixty farms were surveyed (five farms per district). The farms were selected from a sampling frame provided by the extension service. The farms were selected randomly from a grid representing corners of central, north, south, east, and west sites. The distance between two farms ranged from 3 to 7 km. The selected farms consisted of at least 120 mats (in our case a mat consisted of a group of 3 or more plants) for adequate sample size (Figure 3.2). An area of 20 x 20 m with 40 to 50 banana mats, depending on the density, was selected to identify the cultivars.

3.2.2 Germplasm assessment.

The banana cultivars were identified by farmers and verified by the Germplasm Curators' research staff of the National Banana Germplasm Collection at ISAR Rubona, Rwanda. The number of mats of each cultivar in a farm was determined with the assistance of the farmers (counting each cultivar in the whole field). Information on cultivar discrimination criteria i.e. how one cultivar differs from another, as well as synonyms, were collected from farmers through informal discussions. Interviews were conducted in the local language (Kinyarwanda). The National Banana Germplasm Collection at Rubona station served as a reference for comparison of the banana cultivars found in different regions.

3.2.3 Statistical analysis.

Data recorded from the four regions surveyed were subjected to the analysis of frequency distribution of cultivars and genotypes, using Microsoft's Excel software.

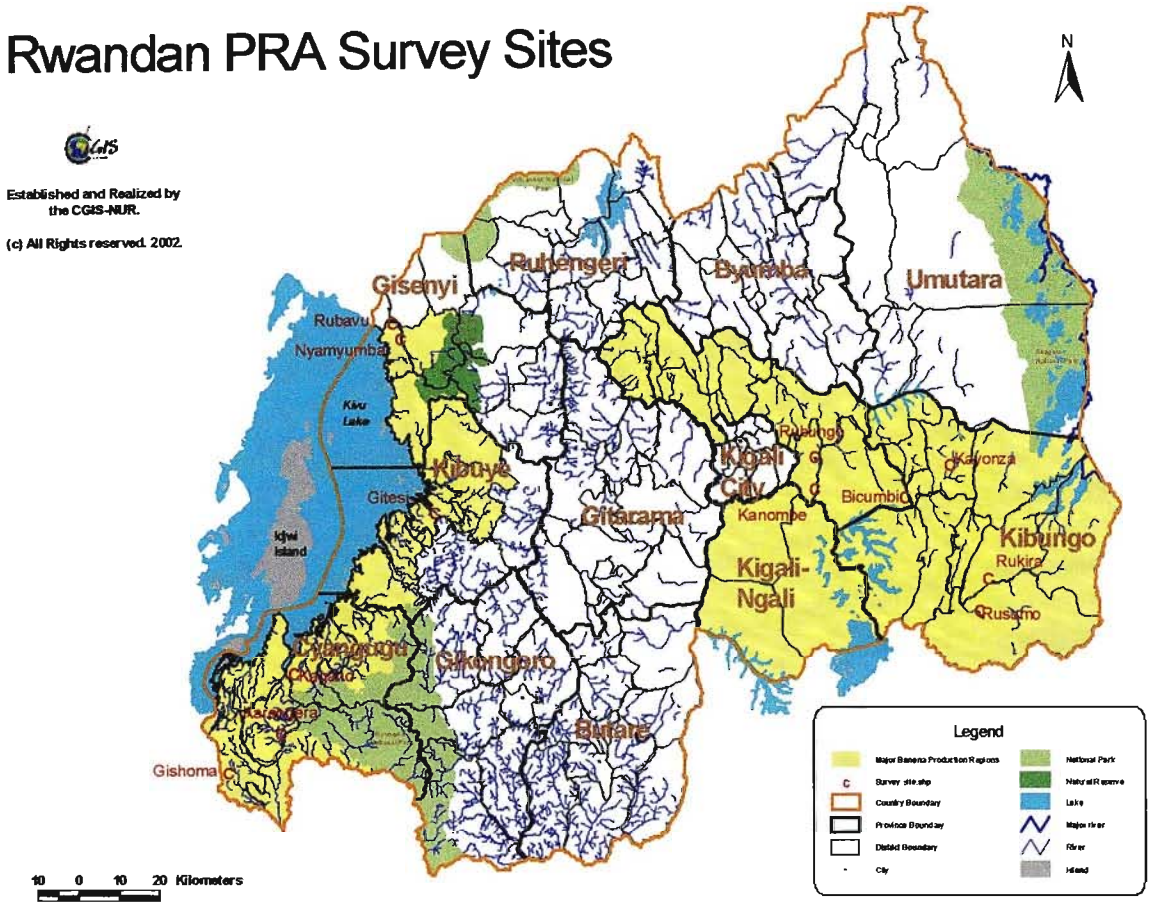

Figure 3.1: Map showing banana growing regions and districts surveyed.

Figure 3.2: Mat of banana in National Banana Germplasm Collection at Rubona – Rwanda (yellow arrow).

3.3 Results and Discussion

In total, 21708 mats of banana were counted, of which 6459, 5668, 5700 and 3881 were from Cyangugu, Kibungo, Kigali Rural, and Kibuye - Gisenyi respectively. The study showed that the major banana genotypes grown in Rwanda are AAA-EA (Subgroup Lujugira - Mutika), AAA (Subgroup Gros Michel), ABB (Subgroups Pisang Awak and Bluggoe), AAB (Subgroup Ney Poovan) and AAA (Subgroup Cavendish) (Figure 3.3).

The study showed that Subgroup Lujugira - Mutika constitutes the most important group of bananas in four major growing regions of Rwanda, with the frequency distribution

between 60 to 90% cultivation in the regions of Cyangugu, Kigali Rural, Kibungo and Kibuye – Gisenyi. This was followed by the Subgroups Pisang Awak and Bluggoe, with frequency distributions of 23.8 and 11.8% respectively. In Cyangugu and Kigali Rural, the Subgroup Ney Poovan, containing the 'Kamaramasenge' cultivar, showed the highest frequency distribution of 10%. In Kibungo, the other Subgroups, such as Ibotabota, Plantains, Gros Michel and Cavendish, accounted for less than 10% of the frequency distribution in the different regions surveyed (Figure 3.3). Interestingly, during the survey at Cyangugu, Kigali Rural and Kibuye - Gisenyi regions, we found some cultivars with unknown Subgroups (Figure 3.3).

Thirteen cultivars, such as 'Gros michel', 'Kamaramasenge', 'Intuntu', 'Inzirabahima', 'Ingenge', 'Gisubi', 'Kayinja', 'Gisukali', 'Madamu', 'Mbwaziruma', 'Mitoki', 'Poyo' and 'Yangambi', appeared across all of the growing regions for banana, whilst other cultivars were found only in certain regions (Table 3.1).

The factors influencing cultivar distribution may vary with the sites. Usually, cultivar preferences are given on the basis of bunch size, food taste, maturation time, tolerance to the soil, nutrient deficiency, drought, wind lodging and diseases. The other factor for cultivar selection includes income-generating activities. Banana brewing, for instance, occurs at a high frequency in the regions of Kibuye - Gisenyi and Cyangugu, and less so in Kigali Rural and Kibungo. On the other hand, the dessert bananas occur at a high frequency in Kibungo and Kigali Rural because the cultivar 'Kamaramasenge' is exported under the name of apple banana due to its flavour. Finally, the maximum distribution of cooking bananas is found in the Kigali Rural and Kibungo regions (Figure 3. 4), due to the demand in the Kigali city for starchy foods (GAIDASHOVA *et al.*, 2005).

In this survey, names of 56 cultivars, with 34 synonyms, were recorded for the four major growing regions of Rwanda (Table 3.1 – 3.2). The synonymous nomenclature of cultivars appears to result from different phenotypic expressions under varying ecological conditions. Probably some cultivars are named according to their origin, for instance, the cultivar 'Congo' may have originated from the Congo.

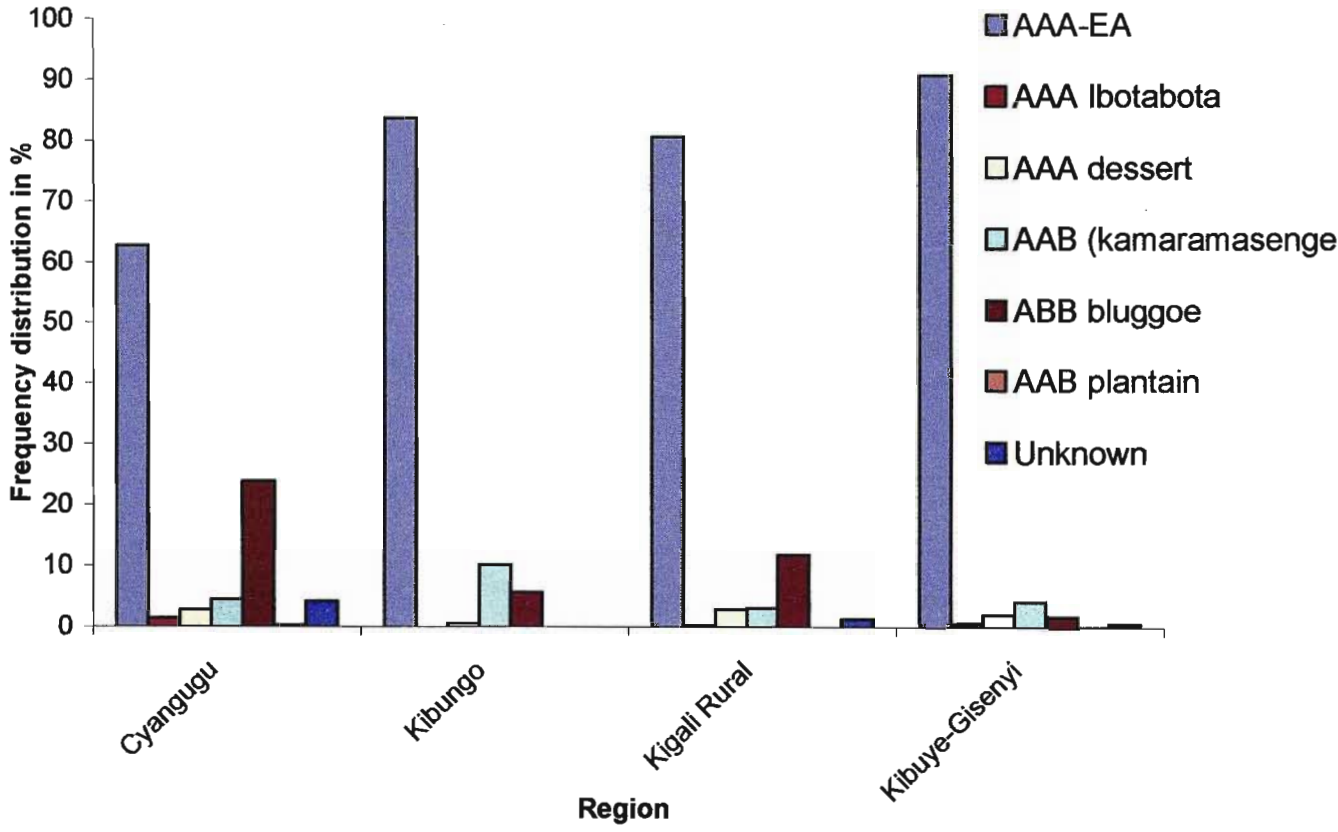

Figure 3.3: Frequency distribution of banana genomic groups in four regions of Rwanda

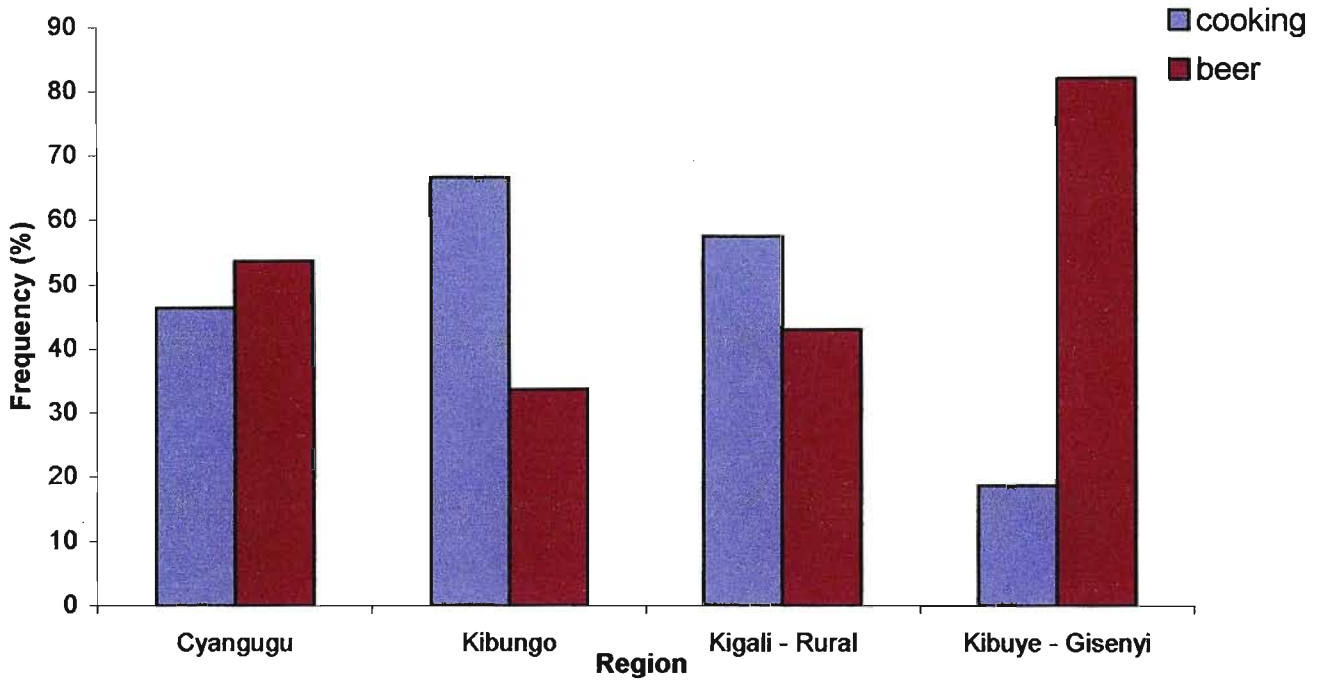

Figure 3.4: Frequency distribution of Subgroup Mutika – Lujugira in four regions of Rwanda

Table 3.1 Varieties found in four banana growing regions of Rwanda

Variety	Genomic group	Local use	Region
Bakurura	AAA	Cooking	2
Barabeshya	AAA	Cooking	1
Bugoyi	AAA	Cooking	4
Gisubi	AB	Brewing	1,3,4
Gisukari	X	Dessert	1,2,4
Grande naine	AAA	Dessert	3,4
Gros Michel	AAA	Dessert	1,2,3,4
Icyerwa	AAA	Brewing	2
Igihuna	AAA	Brewing	1,3
Incakara	AAA	Cooking	3,4
Ingagara	AAA	Cooking	2,3
Ingaju	AAA	Brewing	2,3
Ingaju	AAA	Cooking	2,3
Ingenge	AAA	Brewing	3
Ingenge	AAA	Cooking	1,2,3,4
Ingoromoka	AAA	Brewing	2
Ingote	AAA	Brewing	1,3
Ingumba	AAA	Brewing	2,3
Injagi	AAA	Cooking	2,3

Table 3.1 Varieties found in four banana growing regions of Rwanda (Continued)

Cultivar name	Genomic group	Local use	Region
Inkonkobora	AAA	Cooking	2
Insiri	AAA	Brewing	3
Intama	AAA	Cooking	2
Intembe	AAA	Brewing	2, 3
Intobe	AAA	Cooking	2, 3
Intokatoke	AAA	Brewing	2, 3
Intokatoke	AAA	Cooking	1, 3
Intokekazi	AAA	Cooking	1
Intuntu	AAA	Brewing	1, 2, 3, 4
Intutsi	AAA	Cooking	2, 3
Inyabukumwe	AAA	Cooking	1
Inyabupfunsi	AAA	Cooking	1
Inyabutembe	AAA	Brewing	2
Inyabutembe	AAA	Cooking	2, 3
Inyamunyu	AAA	Cooking	1, 4
Inyamico	AAA	Cooking	2
Inyoya	AAA	Cooking	2
Inzirabahima	AAA	Cooking	1, 2, 3, 4
Isha	AAA	Brewing	3

Table 3.1 Varieties found in four banana growing regions of Rwanda (Continued)

Cultivar name	Genomic group	Local use	Region
Ishika	AAA	Brewing	4
Kayinja	ABB	Brewing	2, 3, 4
Kamaramasenge	AAB	Dessert	1, 2, 3, 4
Kibuzi	AAA	Cooking	3
Kintu	AAA	Cooking	2
Madamu	X	Dessert	1, 3, 4
Mbwaziruma	AAA	Cooking	2, 3, 4
Mitoki	AAA	Cooking	1, 3, 4
Mujuba	AAA	Cooking	2, 4
Mushaija Naranda	AAA	Cooking	2
Mutsimawuburo	AAA	Cooking	2, 3
Nakitengwa	AAA	Cooking	3
Pome	AAB	Dessert	4
Poyo	AAA	Dessert	1, 3, 4
Rwabuganga	AAA	Brewing	2
Umushaba	AAB	Cooking	1, 4
Umuzibo	AAA	Brewing	1, 4
Yangambi Km 5	AAA	Brewing	1, 3, 4

X: Unknown subgroup

Region 1: Cyangugu, Region 2: Kibungo, Region 3: Kigali Rural

Region 4: Kibuye - Gisenyi

Table 3.2 Cultivar name and synonyms found in four banana growing regions of Rwanda

Cultivar	Subgroup	Synonym
Inyabukumwe	Lujugira - Mutika	Inyamakure
Muzibwe	Idem	Imizibo , Muzibo, Umuzibo, Imizibwe
Intuntu	Idem	Mazizi, Inkara, Igishumbu, Ishumbu, Inkashi, Inzizi, Ingashi
Inyabupfunsi	Idem	Inyamafuri, Ishoke
Inyamunyu	Idem	Gisenyi
Inzirabahima	Idem	Maraya, Inyonza, Bagabobaradya, Inshana, Banabazare
Mutsimawuburo	Idem	Inzirabushera
Injagi	Idem	Nyabigoro
Intutsi	Idem	Insenene
Ingote	Idem	Urugote
Ingoromoka cook	Idem	Incakara
Yangambi Km 5	Ibotabota	Indaya, Kagongo, Bavumbanyinshi
Gros Michel	Gros Michel	Sindika
Grande Naine	Cavendish	Ingurube, Ikingurube
Gisukari	?	Igihushamuhoro
Pommes	Bluggoe	Insambari
Madamu	?	Kampala

? Unknown subgroup

3.4 Conclusion

The results from the survey showed that East African Highland bananas (AAA-EA) are the most important genotype group in the four major banana growing regions of Rwanda that were studied. In this survey, several new Highland banana cultivars were recorded. These were; 'Intokatoke', 'Igihuna', 'Ingenge', 'Ingaju', 'Icyerwa', 'Mitoki', 'Madamu', 'Inkokobora', 'Intokekazi', 'Bugoyi', 'Ishoki'. Amongst these cultivars, some were classified as cooking and others as brewing bananas. However, in the National Banana Germplasm Collection, the uses of these cultivars are recorded differently therefore increasing the need for agro-morphological characterization.

The use of one language would probably not reduce the presence of synonyms, due to the instability of some phenotypical characters and the taste of unripe fruit of Highland bananas. For exotic bananas such as 'Yangambi km5', 'Gros Michel', 'Grande Naine' and 'Pommes', there is a tendency to give new names in the local language 'Kinyarwanda', such as 'Indaya', 'Kagongo', and 'Bavumbanyinshi' for 'Yangambi Km 5', 'Sindika' for Gros Michel, 'Ingurube', 'Ikingurube' for Grande Naine and 'Insambira' for Pommes instead of retaining the original names.

Chapter 4

Ploidy investigation of bananas (*Musa* spp.) from the National Banana Germplasm collection at Rubona - Rwanda

4.1 Introduction

Knowledge of the ploidy of a variety is important to breeders when attempting to manipulate a multi-ploidy crop such as banana (PILLAY *et al.,* 2003a). Crossing of a triploid banana with a diploid variety generates diploid, triploid, tetraploid, aneuploid and hyperploid progeny (VUYLSTEKE *et al.,* 1993). In the genus *Musa*, accurate determination of the ploidy by chromosome counting is laborious. A variety of phenotypic traits, including stomata size, stomata density and pollen size, are used as alternative approaches to estimate ploidy (HAMILL *et al.,* 1992; DOLEZEL, 2004).

These techniques do not always provide consistent data, mainly due to strong genotypic influences (VANDENHOUT *et al.,* 1995, VAN DUREN *et al.,* 1996). DOLEZEL *et al.,* (1997) demonstrated that rapid and reliable ploidy screening in *Musa* could be performed using DNA flow cytometry.

Flow cytometry has frequently been used in ploidy analysis (AWOLEYE, 1994, DOLEZEL *et al.,* 1997, JOHNSON *et al.* 1998, EGESI *et al.,* 2002, EMSHWILLER 2002, STACY *et al.,* 2002, BEATSON *et al.,* 2003, WALKER *et al.,* 2005) as it has an advantage over the traditional chromosome counting technique in that it can be used to screen many plants in a short time and can be applied to any plant tissue (ROUX *et al.,* 2003, DOLEZEL and BATROS, 2005).

SGORBATI *et al.,* (1986) used fixed tissues of plants for analysis of nuclear DNA content and the cell cycle and found reliable results when compared with flow cytometry of fresh isolated nuclei. As far as can be ascertained from the literature, ploidy determination using frozen banana plant material has not previously been attempted.

The objectives of this study were to examine the feasibility of using banana leaves stored at – 70°C for ploidy determination to investigate the ploidy level of banana germplasm from Rwanda.

4.2 Material and Methods

4.2.1 Plant material.

The fresh banana leaf material from National Banana Collection at Rubona was swirled in 95% ethanol for 1 min followed by decontamination in commercial bleach (JIK, 3.5% [m/v] NaOCl) for 5 min. Samples were then re-immersed in fresh 95% ethanol for 30 sec and stored in plastic bags in a refrigerator at 4 °C. The following day, the leaves were transported in a vacuum flask to the Research Centre for Plant Growth and Development (RCPGD) at the University of KwaZulu-Natal, South Africa where they were stored at – 70 °C until used.

4.2.2 Flow cytometric analysis.

The flow cytometric analysis started with the preparation of the samples using a modified Galbraith's (GALBRAITH *et al.,* 1983) buffer (45 mM $MgCl_2$; 36 mM of trisodium-citrate; 22 mM MOPS; 0.1% (v/v) of Triton X-100, pH 7.14). Approximately 40 mg of frozen leaves were chopped with a sharp razor blade in a plastic Petri dish containing 2 ml buffer and 10 mM dithiotreitol (DTT) incubated for 30 min on ice. The suspension was filtered through a 40 µm nylon filter and 500 µl was placed into a plastic tube. After that, the nuclear DNA of the samples was stained by adding 500 µl of 0.02 mg ml^{-1} propidium iodide.

The sample was mixed gently and incubated briefly on ice before mixing again. Prior to standardisation of flow cytometry using a known banana diploid from the *Musa* Germplasm Transit Centre, we used flow check fluorespheres (Beckman Coulter) and controlled the half-peak coefficient of variance (HPCV) to less than 2%. Relative fluorescence intensity of stained nuclei was analyzed using a Beckman Coulter Epics XL-MCL Flow Cytometer with a 488- nm laser. The ploidy analyzer was calibrated so

that the G1 peak of stained nuclei from *Musa acuminata* subspecies *Maleccensis*, diploid, (fresh material) was set at channel 200 and the flow was given a stop time of 300 sec, allowing a total of 5000 – 10000 nuclei to be analyzed per sample.

4.3 Results and Discussion

A total of 91 accessions were analysed (Table 4.1). Of these, ITC 0249 and ITC 0084, with known ploidy level, were fresh samples, received from INIBAP. The other 89 were from the National Banana Germplasm collection at Rubona station – Rwanda, of which, 18 accessions; 'Kamaramasenge', 'Pommes', 'Gisubi kagongo' (Figure 4.1), 'Poyo', 'Kivuvu', 'Dibis', ' Gisubi kayinja', 'Petite Naine', Guindi, 'Pelipita 2', 'Saba', 'Americani', 'Prata', 'Ice cream', 'Lacatan', 'Grande Naine', 'Naasuna' and 'Yangambi km5', were exotic. The histograms of the flow cytometric analysis of mixed nuclei from fresh material of diploid *Musa acuminata* subspecies *Burmannicoides* (ITC 0249) and triploid 'Mbwazirume' (ITC 0084) are shown in Figure 4.2A. The dominant peak corresponding to the G1 nuclei reflects the ploidy of each plant. The peak of the diploid ITC 0249 was approximately on channel 200, while the peak for the triploid 'Mbwazirume' (ITC0084) was at channel 300. There was no peak at channels 400 and 600 which would represent the G2 phase for the diploid and triploid phases respectively. This suggests that there was no further cell division related to the leaf position of samples.

Figure 4.1: Some banana cultivars classified as diploid and triploid at National Banana Germplasm Collection at Rubona – Rwanda. (A) Pommes. (B) Kamaramasenge. (C) Gisubi kagongo.

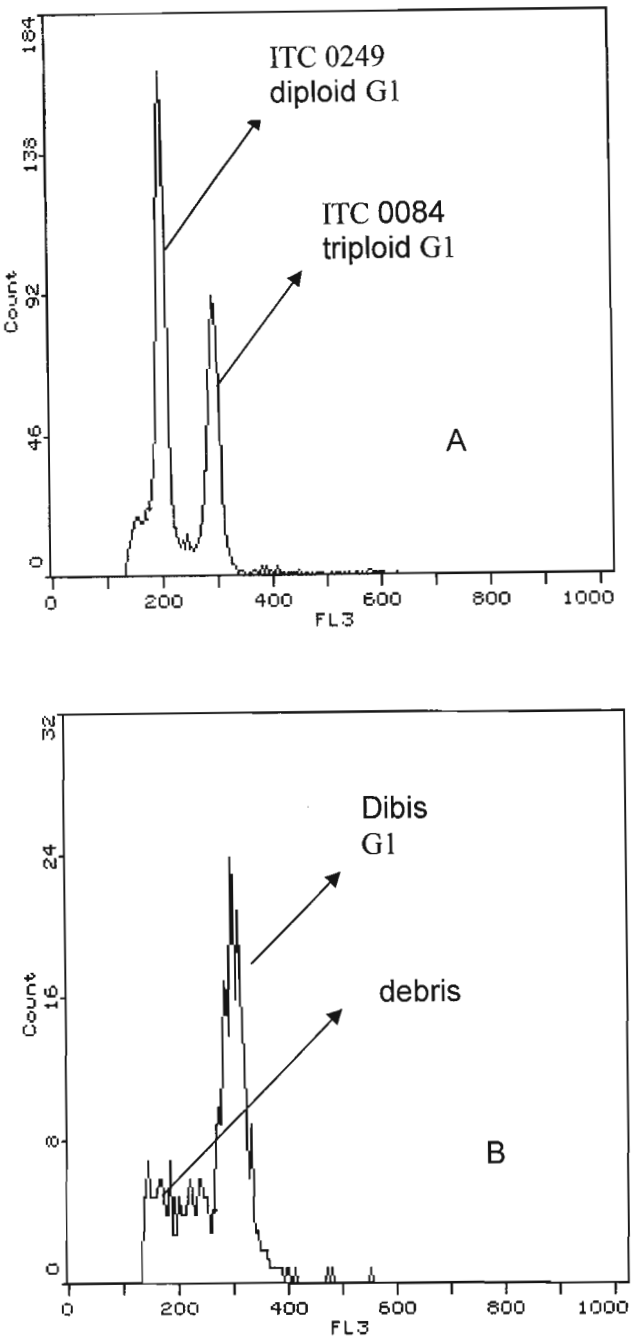

Figure 4.2: A) Histogram of relative nuclear DNA content from fresh leaves of diploid *Musa acuminata* subspecies *Burmannicoides* (ITC0249) and triploid 'Mbwazirume' (ITC0084). B) Histogram of relative nuclear DNA content from frozen material of the triploid hybrid 'Dibis'.

The histograms of the frozen leaves are shown in Figures 4.2A-B. Most histograms revealed a coefficient of variation (CV) of less than 5% (Table 4.1). The histograms of the fresh material showed less debris than those for the frozen samples and the

nuclei count was higher (Figures 4.2 A-B). The low nuclear count, the presence of debris and the presence of a high CV are presumably due to mechanical damage to the cells caused by storage at – 70 °C and the brief storage in liquid nitrogen before the tissues were chopped. However, this debris did not affect the determination of ploidy levels of the bananas.

Table 4.1: Ploidy level of 91 accessions of bananas used for ploidy analysis by flow cytometry (FC) and coefficient of variation (CV) of their histograms

Name	Genomic group	Origin/donor	Expected ploidy	FC-determined ploidy	CV %
1. Nyamabere	AAA	Rwanda	3x	3x	1.92
2. ITC 0084	AAA	Burundi	3x	3x	3.66
3. Isha	AAA	Rwanda	3x	3x	4.98
4. Gisubi Kagongo	?	X	3x	2x	1.11
5. Dibis	?	Burundi	4x	3x	0.72
6. Umushaba II	AAB	Rwanda	3x	3x	0.77
7. Ingenge	AAA	Rwanda	3x	3x	3.75
8. Inzirabahima	AAA	Rwanda	3x	3x	0.72
9. Mbirabire	AAA	Rwanda	3x	3x	3.13
10. Nyabwihogora	AAA	Rwanda	3x	3x	3.58
11. Inyabupfunsi	AAA	Rwanda	3x	3x	0.35
12. Inyoya	AAA	Rwanda	3x	3x	4.66
13. Rugigana	AAA	Rwanda	3x	3x	3.95
14. Mushaija Naranda	AAA	Rwanda	3x	3x	1.75
15. Kibuzi	AAA	Rwanda	3x	3x	0.34
16. Tsambunu	AAB	Rwanda	3x	3x	1.35
17. Impigi	AAA	Rwanda	3x	3x	1.04
18. Intama	AAA	Rwanda	3x	3x	1.67
19. Insiri cook	AAA	Rwanda	3x	3x	2.13

Table 4.1: Ploidy level of 91 accessions of bananas used for ploidy analysis by flow cytometry (FC) and coefficient of variation (CV) of their histograms (continued)

Name	Genomic group	Origin/donor	Expected ploidy	FC-determined ploidy	CV %
20. Injogo	AAA	Rwanda	3x	3x	0.55
21. Icyerwa ntoya	AAA	Rwanda	3x	3x	6.48
22. Intutsi	AAA	Rwanda	3x	3x	0.86
23. Poyo	AAA	Ivory Coast	3x	3x	4.71
24. Intariho	AAA	Rwanda	3x	3x	2.38
25. Ingumba cook	AAA	Rwanda	3x	3x	1.04
26. Insana	AAA	Rwanda	3x	3x	4.00
27. Rugondo	AAA	Rwanda	3x	3x	2.30
28. Kuvuvu	ABB	X	3x	3x	3.34
29. Inyamunyu	AAA	Rwanda	3x	3x	1.44
30. Intokatoke brewing	AAA	Rwanda	3x	3x	0.33
31. ITC 0249	AAw	INIBAP	2x	2x	4.27
32. Muzibwe brewing	AAA	Rwanda	3x	3x	2.32
33. Ingumba brewing	AAA	Rwanda	3x	3x	0.85
34. Nsira cook	AAA	Rwanda	3x	3x	0.37
35. Nyakababi	AAA	Rwanda	3x	3x	0.27
36. Intobe	AAA	Rwanda	3x	3x	1.67
37. Naironi	AAA	Rwanda	3x	3x	1.47
38. Bakungu cook	AAA	Rwanda	3x	3x	0.35
39. Inconnu cook	AAA	Rwanda	3x	3x	3.72
40. Gisukari	?	Rwanda	3x	3x	0.32
41. Yangambi km5	AAA	X	3x	3x	1.53
42. Ingoromoka	AAA	Rwanda	3x	3x	3.8
43. Umushaba I	AAB	Rwanda	3x	3x	4.45
44. Insiri brewing	AAA	Rwanda	3x	3x	0.30

Table 4.1: Ploidy level of 91 accessions of bananas used for ploidy analysis by flow cytometry (FC) and coefficient of variation (CV) of their histograms (continued)

Name	Genomic group	Origin/donor	Expected ploidy	FC-determined ploidy	CV %
45. Ingagara	AAA	Rwanda	3x	3x	2.60
46. Mutsimawuburo	AAA	Rwanda	3x	3x	2.76
47. Inkati brew	AAA	Rwanda	3x	3x	3.01
48. Inyabutembe	AAA	Rwanda	3x	3x	5.90
49. Inshakabuhake	AAA	Rwanda	3x	3x	0.19
50. Bakurura cook	AAA	Rwanda	3x	3x	4.11
51. Kintu	AAA	Rwanda	3x	3x	0.58
52. Petite Naine	AAA	X	3x	3x	2.40
53. Intuntu cook	AAA	Rwanda	3x	3x	0.68
54. Impysi	AAA	Rwanda	3x	3x	3.52
55. Guindi	ABB	X	3x	3x	1.51
56. Kirayenda	AAA	Rwanda	3x	3x	0.76
57. Intuntu brewing	AAA	Rwanda	3x	3x	1.06
58. Ingaju	AAA	Rwanda	3x	3x	3.50
59. Barabeshya	AAA	Rwanda	3x	3x	1.24
60. Ishika brew	AAA	Rwanda	3x	3x	0.49
61. Kamaramasenge	AB	X	2x	3x	2.31
62. Pommes	AB	X	2x	3x	3.44
63. Gisubi kayinja	AB	X	2x	3x	0.43
64. Mbwaziruma	AAA	Rwanda	3x	3x	5.10
65. Nyakitengwa	AAA	Rwanda	3x	3x	3.87
66. Ingote	AAA	Rwanda	3x	3x	4.15
67. Indenge	AAA	Rwanda	3x	3x	2.33
68. Rwabuganga brew	AAA	Rwanda	3x	3x	0.66
69. Igihuni cook	AAA	Rwanda	3x	3x	2.15
70. Nyakitembe	AAA	Rwanda	3x	3x	4.51

Table 4.1: Ploidy level of 91 accessions of bananas used for ploidy analysis by flow cytometry (FC) and coefficient of variation (CV) of their histograms (continued)

Name	Genomic group	Origin/donor	Expected ploidy	FC-determined ploidy	CV %
71. Cyayaya	AAB	Rwanda	3x	3x	4.48
72. Mujuba cook	AAA	Rwanda	3x	3x	2.03
73. Muzuzu	AAA	Rwanda	3x	3x	3.65
74. Inyamico	AAA	Rwanda	3x	3x	1.14
75. Igihuna	AAA	Rwanda	3x	3x	3.99
76. Ingarama	AAA	Rwanda	3x	3x	4.13
77. Inyabukumwe	AAA	Rwanda	3x	3x	0.43
78. Icyerwa Nini	AAA	Rwanda	3x	3x	4.26
79. Inzinga	AAA	Rwanda	3x	3x	1.58
80. Intembe	AAA	Rwanda	3x	3x	2.73
81. Naasuna	AAA	Uganda	3x	3x	2.58
82. Kayuku cook	AAA	Rwanda	3x	3x	3.59
83. Igihobe	AAB	Rwanda	3x	3x	4.61
84. Saba	ABB	X	3x	3x	2.53
85. Americani	AAA	Ivory Coast	3x	3x	3.42
86. Prata	AAB	X	3x	3x	3.11
87. Ice cream	ABB	X	3x	3x	2.80
88. Pelipita 2	ABB	X	3x	3x	2.33
89. Lacatan	AAA	X	3x	3x	4.32
90. Rusatsi	AAA	Rwanda	3x	3x	2.33
91. Grande Naine	AAA	Ivory Coast	3x	3x	2.35

? : Unknown genomic group

X: Unknown origin/donor

w: Wild

INIBAP: International Network for Improvement of Banana and Plantain

Our study indicated that the accession 'Dibis', previously reported as a tetraploid hybrid received from Burundi, is in fact a triploid, whilst 'Pomme', 'Kamaramasenge', 'Gisubi kayinja', and 'Gisubi kagongo' reported as diploid, diploid, diploid and triploid, respectively, turned out to be triploid, triploid, triploid and diploid, respectively (Table 4.2). Our results concerning the ploidy level were identical to those reported recently by DOLEZELOVÀ *et al.*, (2005) for fresh material of 'Kamaramasenge'.

Table 4.2: The accessions where the determined ploidy differed from the expected

Name	Genomic group	Origin/donor	Expected ploidy	FC-determined ploidy	CV, %
Gisubi Kagongo	?	X	3x	2x	1.1
Dibis	?	Burundi	4x	3x	0.7
Kamaramasenge	AB	X	2x	3x	2.3
Pommes	AB	X	2x	3x	3.4
Gisubi kayinja	AB	X	2x	3x	0.4

? : Unknown genomic group

X: Unknown origin/donor

4.4 Conclusion

All 65 East African Highland bananas analysed were triploid (Table 4.1). These Highland bananas are phenotypically triploid with the A genome and belong to the subgroup Lujugira-Mutika (SIMMONDS, 1966). Our results confirmed their ploidy level using high-resolution methods. This study showed the feasibility of using banana leaves stored at - 70 °C for analysis of ploidy using flow cytometry. These results should be valuable for the National Banana Germplasm collection, and specifically for banana breeders, as some Highland banana cultivars can be used as female parents for a crossing program in the improvement of East African Highland bananas (SsEBULIBA *et al.*, 2005).

Chapter 5

Assessment of genetic diversity of Highland bananas from the National Banana Germplasm Collection at Rubona – Rwanda

5.1 Introduction

Analysis of genetic relationships in crop species is an important component of crop improvement programs, as it serves to provide information about genetic diversity, and is a platform for stratified sampling of breeding populations (MOHAMMADI and PRASANNA, 2003). Investigation of genetic diversity of germplasm collections can facilitate reliable classification of accessions and identification of subsets of core accessions with possible utility for specific breeding purposes (MOHAMMADI and PRASANNA, 2003).

Genetic improvement of Highland bananas is to some degree limited by poor knowledge of genetic diversity within the subgroup. To make the collection useful for breeders and farmers, morphological and molecular characterization of this germplasm is needed. Differentiation of cultivars through morphological characteristics is inefficient and inaccurate. This problem is further compounded by the perennial nature of the crop. The disadvantages of phenotype-based assays can be overcome by direct identification of genotypes with DNA-based diagnostic assays using Random Amplified Polymorphic DNA (RAPD) markers. Molecular markers have previously been employed in the characterization and evaluation of genetic diversity in *Musa* species (CARREEL *et al.*, 2002). However, only a few molecular analyses have been conducted on Highland bananas (PILLAY *et al.*, 2001).

The objective of this study was to evaluate the genetic diversity of Highland bananas from the National Banana Collection at Rubona - Rwanda using RAPD markers.

5.2 Materials and Methods

5.2.1 Plant material.

Banana leaves were collected from the National Banana Germplasm collection as detailed in Chapter 4. The seeds of *Ensete ventricosum* were collected in the National Botanical Garden Pietermaritzburg KwaZulu – Natal South Africa and grown in a glasshouse, while i*n vitro* plantlets of *Musa Balbisiana* Colla were obtained from the Musa Germplasm Transit Center, International Network for Improvement of Banana and Plantain (INIBAP), Catholic University of Leuven, Belgium. A total of 49 accessions from the National Banana Germplasm Collection at Rubona-Rwanda were used in this study. These are listed in Table 5.1.

5.2.2 DNA extraction.

The genomic DNA was extracted from banana leaves collected using a slightly modified protocol described by RICHARDS (1997). Approximately, 0.5 g of frozen banana leaves were ground to fine powders in liquid nitrogen in a sterile mortar and pestle and placed in a 1.5 ml microfuge tube. Extraction solution (500 µl) containing 2% (w/v) cetyltrimethylammonium bromide (CTAB), 100 mM Tris-Cl, 0.2 mM EDTA, 1.4 M NaCl at pH 8, 2% (w/v) polyvinylpolypyrolidone (PVPP) and 1% (v/v) 2-Mercaptoethanol (2-ME) was added. The samples were mixed well and then incubated for 1 h at 65°C with occasionally mixing. An equal volume of 24:1 chloroform/isoamyl alcohol was added to the homogenate and the sample mixed well. This was followed by centrifugation at 10,000 rpm (7500 x g) for 5 min. The supernatant was removed to a fresh, sterile microfuge tube and 1/10 volume 10% (w/v) CTAB, 0.7 M NaCl added. After thorough mixing by inversion, an equal volume of 24:1 chloroform:isoamyl alcohol was added and the solution was again mixed. Following centrifugation for 5 min at 10,000 rpm (7500 x g), the aqueous layer was recovered. Then 1 ml of solution containing 1% (w/v) CTAB, 50 mM Tris.HCl, 10 mM EDTA at pH 8.0, pre-warmed to 65°C, was added. In most

cases the precipitate was visible at this stage, but in cases where it was not, an additional incubation of 30 to 60 min was applied. The mixture was centrifuged for 5 min at 2700 rpm (500 x g) and the pellet recovered by removing the supernatant. The pellet was dissolved in high salt TE buffer (10 mM Tris.HCl, 0.1 mM EDTA and 1 M NaCl, pH 8.0) The genomic DNA was re-precipitated by adding 0.6 volumes of ice-cold isopropanol, followed by gentle mixing and centrifugation for 15 min at 10,000 rpm (7,500 x g). The supernatant was decanted and the pellet washed three times with 70% (v/v) ethanol to remove residual salts, followed by one wash with absolute ethanol to remove the water for ease of drying of the pellet. The dried pellet was then suspended in a minimal volume of buffer (10 mM Tris-HCl at pH 8.0) and stored at -20°C.

5.2.3 DNA quantification.

The DNA concentration was calculated by measuring absorption at 260, 280 and 320 nm, using a UV-Visible spectrophotometer (Varian Cary 50). Ratio, percentage purity and concentration were calculated using the following formulae:

Ratio = A_{260}/A_{280}

Purity (%) = Ratio/1.8

Concentration in ($\mu g/\mu l^{-1}$) = (Corrected A_{260} x ΔE X dilution)/1000 where corrected A_{260} = $A_{260} - A_{320}$ and ΔE = 50 (extinction coefficient for genomic DNA).

The concentration was then confirmed by agarose electrophoresis, in which the DNA was visualized using ethidium bromide staining under UV light.

Table 5.1: Cultivar names and genomic group of bananas from the National Banana Germplasm Collection at Rubona-Rwanda used in the RAPD analysis

Key	Cultivar name	Genomic group	Local use
1	Impysi	AAA	Brewing
2	Inkati	AAA	Brewing
3	Inshakabuhake	AAA	Brewing
4	Indenge	AAA	Brewing
5	Inyabutembe	AAA	Cooking
6	Umushaba 1	AAB	Cooking
7	Kintu	AAA	Cooking
8	Kirayenda	AAA	Cooking
9	Bakurura	AAA	Cooking
10	Insiri	AAA	Brewing
11	Inyabukumwe	AAA	Brewing
12	Ingarama	AAA	Cooking
13	Nyakitembe	AAA	Cooking
14	Igihuna	AAA	Brewing
15	Igihuni	AAA	Cooking
16	Ingoromoka	AAA	Brewing
17	Ingagara	AAA	Cooking
18	Rwabuganga	AAA	Brewing
19	Barabeshya	AAA	Cooking
20	Naasuna	AAA	Cooking
21	Nyakitengwa	AAA	Cooking
22	Icyerwa Nini	AAA	Cooking
23	Intembe	AAA	Brewing
24	Inziga	AAA	Brewing
25	Ishika	AAA	Brewing
26	Kayuku	AAA	Brewing

Table 5.1: Cultivar names and genomic group of bananas from the National Banana Germplasm Collection at Rubona-Rwanda used in the RAPD analysis (continued)

Key	Cultivar name	Genomic group	Local use
27	Mutsimawuburo	AAA	Cooking
28	Intuntu brewing	AAA	Brewing
29	Ingote	AAA	Brewing
30	Intokatoke	AAA	Brewing
31	Muzibwe	AAA	Brewing
32	Isha	AAA	Brewing
33	Yangambi km 5	AAA	Brewing
34	Nyamabere	AAA	Brewing
35	Ingumba	AAA	Brewing
36	Intutsi	AAA	Cooking
37	Impigi	AAA	Cooking
38	Inyamunyu	AAA	Cooking
39	Rugigana	AAA	Brewing
40	Intobe	AAA	Cooking
41	Inconnu	AAA	Cooking
42	Insiri	AAA	Cooking
43	Gisukali	AAA	Brewing
44	Mbirabire	AAA	Cooking
45	Nyakababi	AAA	Cooking
46	Inyoya	AAA	Cooking
47	Intariho	AAA	Cooking
48	Enset*	?	X
49	Balbisiana*	BB	X
50	Mbwaziruma	AAA	Cooking
51	Mushayija Naranda	AAA	Cooking

?: Unknown genomic group X: Unknown local use *Outgroup

5.2.4. Random Amplification of Polymorphic DNA Analysis.

The reactions were performed in a total volume of 25 µl. The reaction mixtures contained 30-60 ng template DNA, 1.25 units of *Taq* DNA polymerase (Roche Diagnostics, Germany), 0.2 µM primer (Operon Technologies, USA), 0.2 µM each of dATP, dCTP, dGTP, dTTP (Roche) and 2.5 µl PCR buffer (1.5 mM $MgCl_2$, 50 mM KCl and 10 mM Tris-HCl, pH 8.3; Roche). Sterile HPLC grade water was used to adjust the final volume and the reactions were overlaid with 60 µl of paraffin oil to prevent evaporation. Amplification was performed in a Hybaid Thermal Reactor (HYBAID, UK) using the following cycling parameters: 1 cycle of 95°C for 1 min, 36°C for 20 sec and 72°C for 2 min followed by 45 cycles of each 95°C for 10 sec, 36°C for 20 sec and 72°C for 2 min and a final elongation of 1 cycle of 72°C for 5 min and 35°C for 1 min. The amplification products and the negative controls, in which DNA was omitted, were electrophoresed in 1.5% agarose (HISPAN, Spain) gels containing 1 µg ml^{-1} ethidium bromide. The buffer used for preparing gels was 1x TAE buffer (40 mM Tris base, 2.0 mM EDTA, with pH adjusted to 8.0 using acetic acid) The wells were loaded with 12.5 µl reaction containing 1.25 µl of loading buffer (50 mM NaOH, 1 mM EDTA, 2.5% Glycerol, 0.25% Bromophenol Blue) and the gel was run in 1x TAE buffer for 2 - 3h at $5V.cm^{-1}$(100V). Fragment sizes were estimated by interpolation using DNA molecular weight marker XIV (Roche). Gel images were captured using a UVItec UVIdoc photodocumentation system (UVItec, UK).

5.2.5 Morphological characterization of banana cultivars used for screening of primers.

All bananas used for screening of primers were Highland bananas with dark bronze, black or brown blotches on the pseudostem. However, some additional characteristics such as the taste of the pulp, form of the bunch and the stem were also taken into consideration.

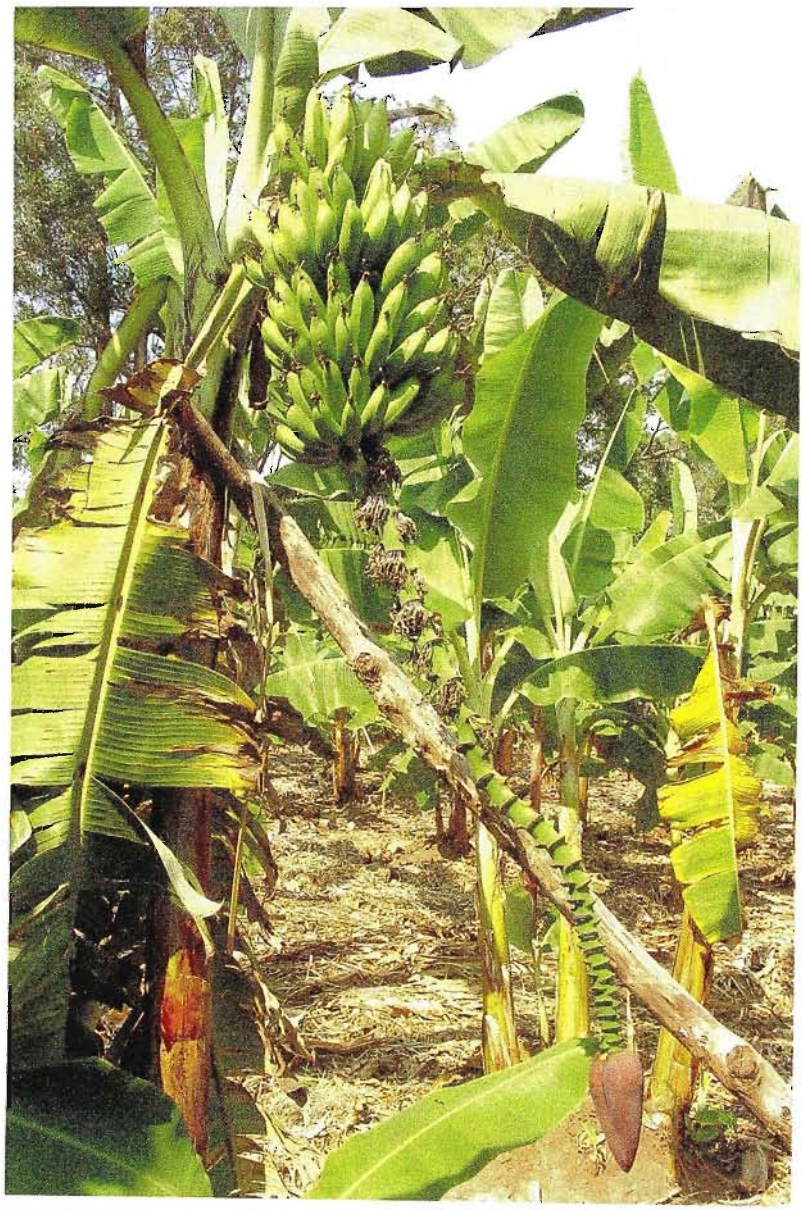

Figure 5 A: 'Rwabuganga' a beer banana clone belonging to the Mutika – Lujugira Subgroup.

Figure 5 B: 'Inzinga' a beer banana clone with a spiraloid hand, belonging to the Mutika – Lujugira Subgroup.

Figure 5 C: 'Mbwaziruma' a cooking banana clone with male inflorescence rachis with persistent neuter flowers and male bud imbricate, belonging to the Mutika – Lujugira Subgroup.

Figure 5 D: 'Mushayija Naranda' a cooking banana clone having the pseudostems emerging at an angle, belonging to the Mutika – Lujugira Subgroup.

5.2.6 Primer screening.

A total of 120 RAPD primers (Operon Technologies kits B – G) were used in three steps of primer screening. The initial step was done using 'Rwabuganga', one of the banana cultivars used for brewing. Those primers that showed good amplification were next screened against 'Mbwaziruma', used for cooking. For the final step of screening, useful primers identified from the first two rounds were applied against four cultivars which had the greatest morphological differences (Figure 5 A-D). Each sample was amplified at least twice to verify reproducibility. A total of 14 RAPD primers (Table 5.2) that showed good amplification for the cultivars were then selected for further analysis of the remainder of the accessions. The gel images of amplified product of 51 accessions were saved in TIFF format to be analyzed by the UVIDoc software (UVItec). Databases were created by analyzing the banding patterns in each gel image.

Table 5.2: Sequences of primers used in our study and results of amplification with 4 banana accessions used during screening

Primer	Sequence (5' to 3')	No. of generated fragments	No. of polymorphic fragments
OPA-18	AGGTGACCGT	5	4
OPB-08	GTCCACACGG	10	10
OPC-05	GATGACCGCC	5	4
OPC-07	GTCCCGACGA	16	16
OPC-13	AAGCCTCGTC	9	9
OPD-18	GAGAGCCAAC	11	11
OPD-20	ACCCGGTCAC	5	5
OPE-01	CCCAAGGTCC	14	13
OPE-02	GGTGCGGGAA	9	9
OPE-15	ACGCACAACC	5	2
OPE-18	GGACTGCAGA	5	3
OPF-01	ACGGATCCTG	17	17

Table 5.2: Sequences of primers used in our study and results of amplification with 4 banana accessions used during screening (continued)

Primer	Sequence (5' to 3')	No. of generated fragments	No. of polymorphic fragments
OPG-02	GGCACTGAGG	5	3
OPG-08	TCACGTCCAC	9	9
OPG-17	ACGACCGACA	7	5
Total		132	120

5.2.7 Statistical analysis.

Band positions for each banana accession and primers combination were scored as either present (1) or absent (0). We considered as homologous all bananas where bands of identical size were amplified with the same primer, since WILLIAMS *et al.*(1993), THORMANN *et al.*(1994) and REISERBERG (1996) showed a high proportion of homology among co-migrating RAPD fragments detected in close species (≥ 80%).

The scores of bands were then assembled into a binary data matrix in a database program (Microsoft Excel). Using the binary matrix, coefficients of similarity (Simple Matching Index of Similarity) as described by SNEATH and SOKAL (1973) were calculated using the Sequential Agglomerative Hierarchical Nested cluster analysis (SAHN) procedure of NTSYS – PC (Numerical Taxonomy and Multivariate Analysis) System version 2.11U (ROHLF, 1994).

The Simple Matching Index of similarity is calculated as:
Sij = (a + d) / (a + b + c + d) where, a = number of amplified products present in both accession 'i' and 'j',
B = number of amplified products present in both accession 'i' and not in 'j',
C = number of amplified products absent from accession 'i' but present in 'j',

d= number of amplified products absent from 'i' and 'j', but scored for other accessions.
The similarity matrix obtained indicates the similarity indices for each accession compared with other accessions.
To group similar accessions together, a dendrogram was constructed based on the data of the similarity matrix, using the unweighted pair group method with arithmetic average (UPGMA). The correspondence between the morphological and RAPD-based similarity coefficient matrices was tested based on correlation analysis and the MANTEL (1967) matrix correspondence test. The Mantel matrix correspondence test was carried out using the MXCOMP procedure in NTSYS – PC version 2.11U (ROHLF, 1994).

5.3 Results and Discussion

5.3.1 DNA extraction.

The extraction of DNA from banana leaf samples collected from National Banana Germplasm Collection at Rubona-Rwanda stored at + 4°C for 2 days using a slightly modified protocol described by RICHARDS (1997) gave DNA with more than 85% purity. For PCR, DNA samples were selected only if their purity was greater than 91% (HONEYCUTT *et al.,* 1992). The quality of the DNA had been confirmed by electrophoretic analysis. An example of the PCR amplification product profiles of 51 varieties is shown in Figure 5 E.

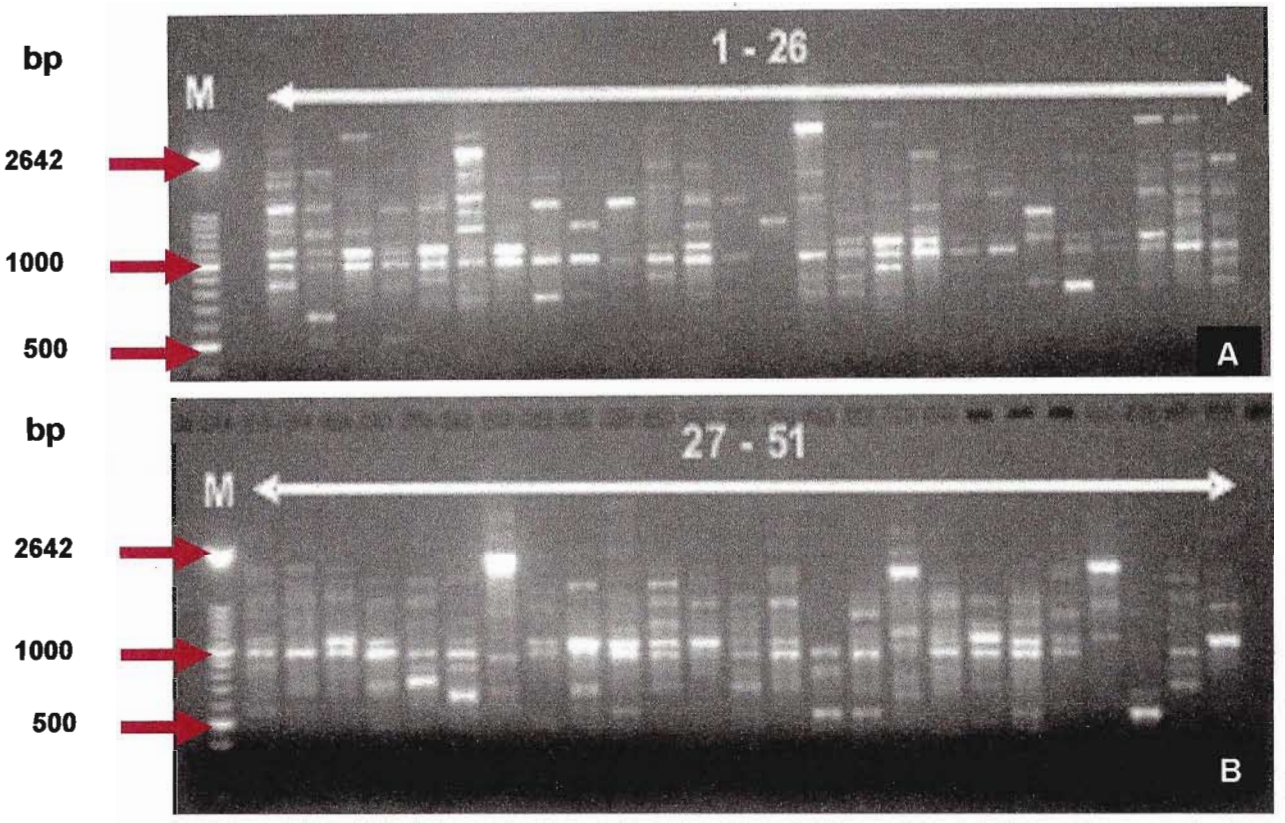

Figure 5 E: A – B RAPD marker profiles generated for 49 banana accessions from National Banana Germplasm Collection at Rubona – Rwanda and two accessions one from the ITC other from the National Botanical Garden Pietermaritzburg KwaZulu – Natal South Africa (lanes 48 and 49) using primer OPF-01. M = DNA molecular weight marker XIV (Roche).

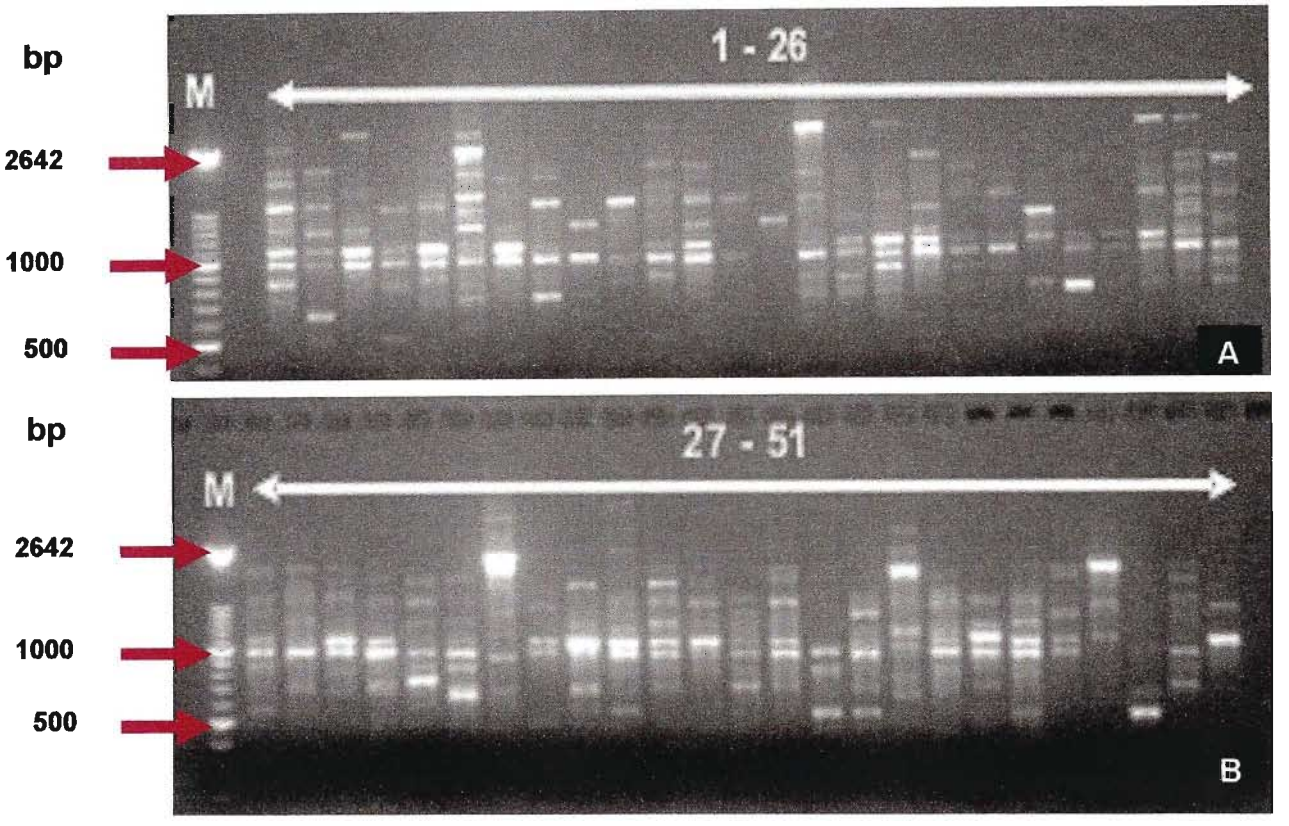

Figure 5 E: A – B RAPD marker profiles generated for 49 banana accessions from National Banana Germplasm Collection at Rubona – Rwanda and two accessions one from the ITC other from the National Botanical Garden Pietermaritzburg KwaZulu – Natal South Africa (lanes 48 and 49) using primer OPF-01. M = DNA molecular weight marker XIV (Roche).

5.3.3 Genetic similarities.

In this study, from the 120 primers screened, 15 were selected on the basis of providing good amplification and which also revealed a high level of variation between cultivars. (Figure 5A-D). According to BHAT and JARRET (1995), the use of a small number of primers is sufficient when morphological variation is high. Since it was found that there is higher variation in morphological characteristics, the 15 primers were used for further analysis of all accessions, comprising 49 samples of banana including 21 Highland bananas used for brewing beer, 1 plantain ('Umushaba 1') and one example from the Bluggoe subgroup ('Yangambi km 5'). The remaining bananas from Rwanda were considered as cooking Highland bananas and contained all types of clone sets found in Highland banana (Nakitembe, Nakabululu, Musakala, Nfuuka). Included in this analysis as outgroups were *Ensete ventricosum,* which was collected from the National Botanical Garden Pietermaritzburg KwaZulu - Natal South Africa and grown in a glasshouse in the University of KwaZulu - Natal Botanic Garden in Pietermaritzburg (South Africa), and *M. balbisiana* obtained from the Musa Germplasm Transit Center, International Network for Improvement of Banana and Plantain (INIBAP).

An average of 8.8 bands per primer, with 5 to 17 bands per primer, was obtained for each primer in the 4 banana cultivars used for primer screening (Table 5.2). The primers selected were then tested further on the 51 accessions previously described. The total number of scorable bands amplified from the 51 accessions was 222. However, lack of amplification in some cultivars was observed (Appendix 4). It may reflect deviation in the sequence flanking the primer binding loci or a complete inhibition of amplification. This lack of amplification is common in *Musa* (CROUCH *et al.,* 1998, 1999a, 1999b).

For the analysis of genetic similarities, a Simple Matching coefficient was used. This is not a simple pair-wise comparison between two genotypes, but also takes into account the information given by all genotypes. For example, the absence of an amplified product in two genotypes which is present in others is given equal weighting as the shared presence of a band in calculating similarity. This is in contrast to the Jacquard

index which scores shared presence while ignoring shared absence (JACQUARD, 1974). With RAPD markers, fragments are randomly amplified and no relationship can be made between the absence of the fragment and the presence of a new one. Therefore the index of Simple Matching is appropriate for analyzing RAPD markers as it includes joint absences of a fragment in the numerator and denominator.

To estimate the similarities amongst the banana germplasm from Rubona, the Simple Matching coefficient gave similarity values ranging from 0.46 to 0.85. (Table 5.3). UPGMA cluster analysis of the similarity matrix (Table 5.3) separated all genotypes of the banana from Rubona, as well as *Musa balbisiana* and *Ensete ventricosum*.

The dendrogram separated the 51 accessions into four major clusters. The first cluster contained 14 accessions. This cluster was further subdivided into several sub-clusters containing seven brewing bananas; 'Impyisi' 'Inkati' 'Inshakabuhake', 'Indege', 'Inyabukumwe' 'Insiri' and 'Igihuna' and seven cooking bananas; 'Inyabutembe', 'Kintu', 'Kirayenda', 'Ingarama', 'Bakurura', 'Nyakitembe', 'Igihuni'. The clones: 'Impysi', 'Inkati', 'Inshakabuhake' and 'Indege' were in the same sub-cluster while other beer bananas were randomly clustered with cooking bananas.

Interestingly 'Igihuna' and 'Igihuni' were very close in the dendrogram (Figure 5.G) with a similarity coefficient of 0.81 (Table 5.3). These two accessions share a large number of morphological characteristics, such as the presence of persistent neuter flowers and male bud imbricate on their male inflorescence rachis and the two clones were very close to 'Nakitembe', the example of the Nakitembe clone set. These results are consistent with classification based on morphological characters. However, the bitter taste of the pulp of Igihuna was the most pronounced reason to classify this clone in Mbidde clone set, while 'Igihuni' was classified to Nakitembe clone set (NSABIMANA and VAN STADEN, 2005). On the other hand, there are two clones having the same name (homonymous) 'Insiri', with a low similarity coefficient of 0.69 (Table 5.3). These two clones share the same morphological characteristics, such as the color of the pseudostem (a strong black color), medium size fruit, but were classified separately

according to the taste of the pulp; one in the Mdidde clone set and the other as a Nfuuka clone (NSABIMANA and VAN STADEN, 2005). For these clones, the amplification of the primers did not associate with the morphological characteristics. This result shows that RAPD markers can without difficulty distinguish between Highland banana cultivars.

The second cluster was made up of 23 accessions and many small sub-clusters were formed, starting from accession 'Mutsimawuburo' to accession 'Impigi'. The third cluster had small sub-clusters and contained 11 accessions, from 'Ingoromoka' to 'Kayuku', but did not show a high consensus tree for these accessions (Figure 5G). However, some accessions such as; 'Gisukali', 'Yangambi km 5' and Umushaba 1, appeared alone in the clusters (Figure 5G). These accessions do not belong to the subgroup Mutika – Lujugira. The accession 'Gisukali', having a red-brown pseudostem and brown fruit color, sometimes changing to green, is triploid AAA. The accession 'Yangambi km 5' was classified into Subgroup Ibotabota (DANIELLS *et al.,* 2001), while the accession 'Umushaba 1' goes to the subgroup Plantain (NSABIMANA and VAN STADEN, 2005).

The fourth cluster comprised only the outgroups, *M. balbisiana* and *Ensete ventricosum.* All of the outgroups belong to the family *Musaceae* respectively to the genus *Musa* and *Ensete.*

According to JENNY *et al.* (1999), variability within Subgroups is mainly dependent on the genotype and the frequency with which the clone is multiplied and planted. High levels of similarity are expected between cultivars from the same subgroup because they share a common origin. However, with only fifteen primers, we have a coefficient of similarity of between 0.46 and 0.85 in Highland bananas from Rwanda. This shows how the cultivars in the same subgroup are genetically diversified.

Diversity of bananas in Africa is considered to be the result of accumulated somatic mutations (SIMMONDS, 1966). The exact mechanisms of these mutations remain unknown. Involvement of retrotransposons in spontaneous mutations has been reported for rice (HIROCHIKA, 1997). Retrotransposons are abundant and comprise over 50% of

nuclear DNA content in many species. They are generally dispersed over plant chromosomes (ASHALATHA *et al.,* 2005). The retrotransposons insert themselves into the genome, and act as mutagenic agents and in that way provide a potential source of biodiversity (HESLOP-HARRISON, 2000). Retrotransposons have been identified in banana (PILLAY *et al.,* 2003b; ASHALATHA *et al.,* 2005). It is also known that retrotransposons are activated by stress and environmental factors (GRANDBASTIEN, 1998). KANGASNIEMI (1998) reported a high frequency of multiplication and planting of Highland bananas in Rwanda, which can also play a role in the diversification of bananas in this region.

Table 5.3: Similarity matrix of 51 accessions analyzed according to the Simple Matching algorithm of the NTSYS program

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51
1	1.00																																																	
2	0.79	1.00																																																	
3	0.78	0.76	1.00																																																
4	0.76	0.74	0.77	1.00																																														
5	0.76	0.75	0.71	0.73	1.00																																													
6	0.64	0.63	0.69	0.63	0.68	1.00																																													
7	0.73	0.77	0.73	0.74	0.80	0.69	1.00																																											
8	0.69	0.73	0.76	0.80	0.78	0.71	0.80	1.00																																										
9	0.66	0.66	0.72	0.74	0.74	0.68	0.75	0.83	1.00																																									
10	0.69	0.69	0.78	0.76	0.73	0.68	0.74	0.80	0.79	1.00																																								
11	0.74	0.74	0.74	0.77	0.76	0.69	0.80	0.85	0.76	0.78	1.00																																						
12	0.68	0.75	0.70	0.69	0.78	0.68	0.79	0.79	0.77	0.75	0.81	1.00																																				
13	0.66	0.69	0.72	0.73	0.71	0.64	0.74	0.78	0.75	0.78	0.79	0.78	1.00																																		
14	0.64	0.64	0.68	0.72	0.69	0.65	0.74	0.76	0.77	0.74	0.76	0.79	0.82	1.00																																	
15	0.63	0.65	0.64	0.66	0.67	0.67	0.70	0.73	0.70	0.72	0.73	0.77	0.75	0.81	1.00																																
16	0.64	0.62	0.65	0.66	0.63	0.66	0.69	0.69	0.69	0.70	0.68	0.71	0.70	0.80	0.76	1.00																															
17	0.64	0.58	0.66	0.68	0.63	0.63	0.59	0.68	0.69	0.69	0.66	0.66	0.69	0.71	0.74	0.77	1.00																														
18	0.61	0.59	0.66	0.62	0.63	0.64	0.64	0.65	0.65	0.66	0.66	0.66	0.64	0.73	0.73	0.79	0.79	1.00																													
19	0.58	0.50	0.58	0.59	0.57	0.59	0.56	0.60	0.58	0.64	0.58	0.59	0.60	0.62	0.64	0.66	0.74	0.74	1.00																												
20	0.61	0.59	0.68	0.65	0.65	0.64	0.61	0.67	0.70	0.68	0.65	0.61	0.68	0.68	0.69	0.69	0.75	0.77	0.75	1.00																									
21	0.59	0.57	0.63	0.63	0.60	0.64	0.62	0.68	0.68	0.65	0.64	0.61	0.59	0.67	0.67	0.69	0.72	0.77	0.71	0.76	1.00																								
22	0.54	0.52	0.59	0.59	0.58	0.59	0.55	0.59	0.62	0.59	0.59	0.55	0.59	0.64	0.61	0.60	0.67	0.69	0.76	0.72	0.75	1.00																							
23	0.64	0.56	0.64	0.67	0.63	0.63	0.59	0.69	0.73	0.69	0.66	0.64	0.67	0.70	0.66	0.68	0.74	0.74	0.69	0.80	0.79	0.78	1.00																						
24	0.59	0.54	0.59	0.60	0.61	0.61	0.58	0.60	0.66	0.60	0.61	0.59	0.59	0.66	0.67	0.64	0.70	0.75	0.70	0.79	0.70	0.76	0.79	1.00																					
25	0.51	0.48	0.52	0.52	0.49	0.47	0.47	0.48	0.49	0.50	0.51	0.49	0.51	0.56	0.53	0.51	0.55	0.63	0.65	0.64	0.59	0.70	0.65	0.69	1.00																				
26	0.61	0.59	0.60	0.64	0.60	0.59	0.60	0.60	0.64	0.63	0.67	0.62	0.64	0.69	0.66	0.66	0.65	0.70	0.63	0.74	0.69	0.64	0.74	0.72	0.70	1.00																			
27	0.72	0.72	0.71	0.67	0.69	0.60	0.69	0.69	0.70	0.71	0.68	0.65	0.68	0.69	0.66	0.65	0.61	0.63	0.57	0.66	0.60	0.56	0.66	0.62	0.50	0.65	1.00																		
28	0.69	0.69	0.69	0.71	0.69	0.61	0.66	0.69	0.71	0.69	0.66	0.64	0.67	0.67	0.62	0.63	0.63	0.61	0.52	0.62	0.59	0.55	0.65	0.59	0.50	0.66	0.81	1.00																	
29	0.74	0.73	0.72	0.66	0.74	0.63	0.74	0.70	0.71	0.69	0.68	0.68	0.67	0.66	0.63	0.63	0.61	0.64	0.57	0.63	0.60	0.54	0.63	0.59	0.47	0.63	0.83	0.79	1.00															
30	0.67	0.67	0.69	0.73	0.69	0.63	0.67	0.73	0.75	0.74	0.68	0.64	0.67	0.67	0.63	0.63	0.65	0.60	0.55	0.64	0.62	0.56	0.68	0.63	0.49	0.64	0.76	0.79	0.78	1.00														
31	0.60	0.61	0.64	0.67	0.63	0.59	0.64	0.69	0.69	0.66	0.63	0.58	0.61	0.62	0.58	0.63	0.59	0.57	0.48	0.62	0.62	0.56	0.65	0.55	0.57	0.58	0.74	0.71	0.73	0.75	1.00													
32	0.61	0.61	0.65	0.63	0.56	0.55	0.59	0.64	0.67	0.66	0.62	0.58	0.64	0.67	0.61	0.61	0.64	0.59	0.52	0.63	0.63	0.56	0.66	0.57	0.62	0.63	0.69	0.66	0.67	0.71	0.84	1.00												
33	0.64	0.62	0.68	0.64	0.64	0.62	0.62	0.66	0.70	0.66	0.66	0.64	0.66	0.69	0.65	0.66	0.68	0.66	0.55	0.65	0.63	0.58	0.63	0.60	0.50	0.69	0.74	0.72	0.69	0.72	0.65	0.69	1.00											
34	0.67	0.68	0.70	0.70	0.64	0.64	0.68	0.74	0.72	0.71	0.69	0.66	0.66	0.70	0.69	0.69	0.68	0.65	0.57	0.70	0.68	0.59	0.71	0.61	0.49	0.64	0.78	0.71	0.76	0.74	0.74	0.74	0.72	1.00										
35	0.67	0.67	0.69	0.65	0.65	0.64	0.65	0.69	0.66	0.67	0.68	0.67	0.66	0.64	0.61	0.61	0.64	0.61	0.58	0.65	0.64	0.59	0.67	0.60	0.46	0.58	0.72	0.68	0.72	0.73	0.69	0.66	0.72	0.79	1.00									
36	0.67	0.66	0.65	0.65	0.67	0.62	0.68	0.69	0.69	0.70	0.70	0.66	0.68	0.67	0.60	0.65	0.62	0.64	0.57	0.63	0.62	0.54	0.65	0.55	0.51	0.64	0.77	0.75	0.78	0.74	0.70	0.67	0.72	0.76	0.74	1.00								
37	0.60	0.59	0.60	0.61	0.58	0.55	0.65	0.62	0.62	0.60	0.59	0.61	0.58	0.60	0.56	0.63	0.62	0.61	0.56	0.57	0.56	0.49	0.58	0.51	0.55	0.57	0.67	0.68	0.71	0.67	0.63	0.64	0.64	0.75	0.72	0.69	0.64	0.65	0.67	0.72	1.00			
38	0.66	0.69	0.65	0.66	0.67	0.68	0.64	0.69	0.67	0.68	0.64	0.65	0.64	0.68	0.65	0.69	0.71	0.71	0.62	0.68	0.66	0.61	0.70	0.66	0.54	0.63	0.71	0.75	0.74	0.74	0.67	0.67	0.73	0.74	0.74	0.76	0.72	1.00						
39	0.64	0.66	0.69	0.62	0.61	0.60	0.62	0.68	0.67	0.68	0.61	0.59	0.60	0.59	0.56	0.62	0.64	0.63	0.59	0.65	0.65	0.57	0.68	0.59	0.49	0.59	0.64	0.68	0.70	0.70	0.65	0.64	0.67	0.73	0.78	0.75	0.65	0.76	1.00					
40	0.68	0.65	0.68	0.67	0.61	0.58	0.63	0.66	0.64	0.70	0.62	0.59	0.59	0.63	0.59	0.67	0.69	0.64	0.59	0.64	0.68	0.57	0.68	0.59	0.53	0.60	0.71	0.72	0.68	0.75	0.67	0.66	0.68	0.74	0.75	0.69	0.67	0.80	0.77	1.00				
41	0.66	0.68	0.73	0.65	0.64	0.66	0.67	0.70	0.69	0.73	0.64	0.63	0.66	0.68	0.66	0.67	0.68	0.64	0.60	0.66	0.64	0.61	0.69	0.60	0.49	0.61	0.73	0.71	0.74	0.71	0.66	0.68	0.69	0.76	0.76	0.74	0.68	0.78	0.79	0.76	1.00			
42	0.67	0.66	0.70	0.66	0.60	0.63	0.65	0.70	0.65	0.69	0.66	0.63	0.62	0.66	0.65	0.66	0.66	0.67	0.60	0.67	0.64	0.55	0.64	0.61	0.50	0.60	0.70	0.68	0.71	0.67	0.63	0.64	0.64	0.75	0.72	0.69	0.64	0.77	0.73	0.77	0.83	1.00		
43	0.61	0.61	0.65	0.66	0.59	0.60	0.60	0.63	0.65	0.66	0.61	0.58	0.61	0.62	0.59	0.59	0.62	0.59	0.51	0.60	0.59	0.53	0.64	0.58	0.47	0.59	0.67	0.69	0.68	0.74	0.61	0.64	0.65	0.71	0.69	0.68	0.58	0.68	0.69	0.70	0.70	0.71	1.00	
44	0.62	0.66	0.73	0.68	0.63	0.62	0.65	0.70	0.69	0.74	0.65	0.65	0.64	0.67	0.64	0.65	0.64	0.64	0.55	0.67	0.64	0.57	0.65	0.63	0.53	0.63	0.74	0.69	0.70	0.72	0.66	0.67	0.68	0.75	0.70	0.72	0.62	0.74	0.73	0.74	0.80	0.81	0.74	1.00
45	0.66	0.68	0.72	0.70	0.65	0.61	0.64	0.71	0.69	0.69	0.65	0.64	0.63	0.68	0.59	0.67	0.67	0.69	0.56	0.66	0.66	0.61	0.71	0.65	0.56	0.66	0.73	0.72	0.73	0.72	0.69	0.69	0.69	0.78	0.74	0.72	0.64	0.80	0.77	0.78	0.77	0.79	0.74	0.84
46	0.61	0.64	0.67	0.65	0.60	0.59	0.65	0.67	0.67	0.69	0.64	0.63	0.61	0.65	0.62	0.66	0.66	0.64	0.55	0.62	0.62	0.57	0.67	0.60	0.54	0.64	0.70	0.72	0.66	0.72	0.64	0.64	0.69	0.72	0.70	0.73	0.66	0.74	0.72	0.78	0.80	0.78	0.69	0.82
47	0.61	0.64	0.69	0.67	0.59	0.61	0.62	0.69	0.69	0.68	0.65	0.58	0.61	0.66	0.62	0.67	0.65	0.65	0.50	0.64	0.66	0.57	0.65	0.60	0.50	0.62	0.69	0.69	0.65	0.75	0.66	0.67	0.71	0.74	0.67	0.68	0.63	0.69	0.69	0.72	0.75	0.73	0.73	0.74
48	0.57	0.57	0.61	0.57	0.57	0.57	0.54	0.60	0.62	0.62	0.57	0.52	0.56	0.60	0.59	0.59	0.58	0.63	0.51	0.60	0.61	0.55	0.65	0.61	0.50	0.56	0.62	0.62	0.59	0.63	0.55	0.54	0.60	0.68	0.63	0.56	0.51	0.64	0.62	0.64	0.66	0.64	0.61	0.65
49	0.48	0.51	0.54	0.53	0.55	0.53	0.52	0.59	0.60	0.60	0.56	0.53	0.58	0.59	0.55	0.56	0.54	0.57	0.50	0.59	0.56	0.53	0.6	0.56	0.41	0.54	0.56	0.53	0.57	0.59	0.51	0.51	0.60	0.59	0.63	0.58	0.49	0.58	0.59	0.53	0.62	0.58	0.54	0.61
50	0.65	0.64	0.69	0.68	0.59	0.62	0.59	0.71	0.70	0.71	0.66	0.64	0.69	0.72	0.65	0.73	0.71	0.68	0.60	0.67	0.65	0.59	0.71	0.62	0.55	0.67	0.69	0.70	0.66	0.76	0.66	0.69	0.72	0.70	0.68	0.70	0.65	0.74	0.72	0.75	0.72	0.71	0.69	0.75
51	0.64	0.65	0.71	0.76	0.62	0.64	0.59	0.71	0.69	0.72	0.67	0.63	0.69	0.73	0.66	0.68	0.73	0.65	0.59	0.71	0.67	0.63	0.72	0.65	0.59	0.66	0.67	0.67	0.63	0.71	0.66	0.66	0.65	0.73	0.69	0.64	0.61	0.71	0.67	0.69	0.74	0.72	0.67	0.74
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51

1 – 51 correspond to the list of accessions in Table 5.1.

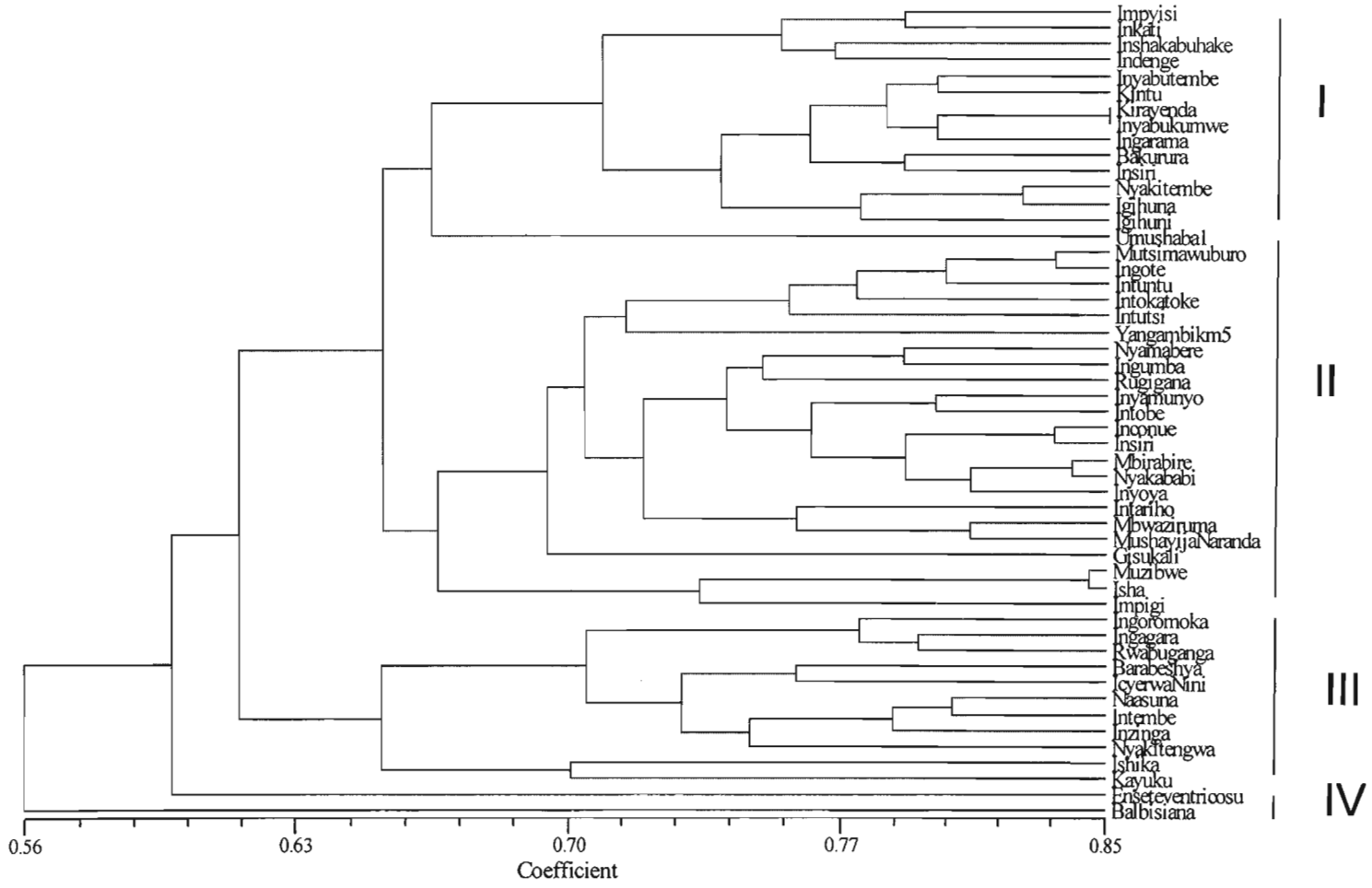

Figure 5 G: Dendrogram of 50 accessions of bananas and one *Ensete ventricusom* using RAPD markers with 15 primers.

I, II, III, IV: Clusters.

5.3.4 Correspondence between marker systems.

To understand the degree of correspondence between marker systems, if any, a pairwise comparison of similarity matrices, based on morphological characteristics of fruits used in Chapter 2 and RAPD markers, was carried out based on the Mantel correspondence test (MANTEL, 1967).

Fifty-one accessions were considered for the correlation study, of which only 45 have morphological characteristics of fruit data. This data was not available for the remaining 6 accessions. No significance and quite low correlation between the matrices was obtained ($r = 0.04$, $p = 0.78$) after carrying out 1000 random permutations.

A comparison between morphological and molecular markers has been investigated in several studies. Some significant correlations between the two markers were reported (BURSTIN and CHARCOSSET, 1979). However, in most cases the correlation existed either in autogamous crops or in inbred lines, where genotypes tend to be homozygous (ARCHAK *et al.,* 2003). In the case of bananas, a clonally propagated crop, the low correlation between morphological characteristics of fruit and RAPD markers may be attributed to the missing data and the few morphological traits used.

5.4 Conclusion

Molecular characterization using RAPD markers was conducted to assess the degree of diversity in the Banana Germplasm Collection at Rubona-Rwanda. Among 49 accessions appraised, 46 belong to the Subgroup Mutika – Lujugira, one accession to the Subgroup plantain, other accession to the subgroup Ibotabota and a third accession to an unknown Subgroup. The extent of variability among the clones was found to range from 0.46 to 0.85. They were grouped into 3 clusters with several subclusters and one cluster for the outgroups.

Using RAPD analysis, we could not discriminate completely between cooking and beer bananas. This may be because the primers screened were not associated with the gene(s) controlling bitterness of the fruit, the characteristic that is usually used as the discriminating factor between cooking and beer bananas. The genomic composition of Highland bananas was confirmed by RAPD markers as triploid with the A genome.

A comparison of morphological and RAPD markers shows a low correlation between the two. However, correlation between them could be improved if more morphological traits were analyzed or more primers screened. The results indicate that a few primers are sufficient for assessing variability in Highland bananas and that RAPD markers could readily distinguish between Highland banana cultivars.

5.5 General conclusion

Bananas are monocotyledons in the genus *Musa*, of the family *Musaceae*. Banana is the third most important starchy staple crop in the world after cassava and sweet potato. This crop is of great importance in Rwanda. In terms of consumption, Rwanda takes the second position in the world after Uganda with a consumption of 197 kg per capita per annum. Therefore, there is a need for the characterization of the East African Highland bananas to include them in breeding schemes with the aim of improving the agronomic performance and diseases resistance.

In this study, the characterization of bananas from the National Banana Germplasm Collection at Rubona - Rwanda was done, using morphological, cytological and molecular markers, while the survey was conducted to evaluate the distribution of banana cultivars in four major growing regions of Rwanda.

The results of this study showed that the National Banana Germplasm Collection at Rubona - Rwanda contained different genomic groups of bananas such as AB, AAA, ABB AAB and AAAB. The dominant genomic group was AAA which is subdivided in different Subgroups mainly Mutika – Lujugira, Gros Michel, Cavendish and Ibotabota .

Using Principal Component Analysis, the Subgroup Mutika – Lujugira was subdivided into clone sets such as Musakala, Nakabulu and Beer bananas. Few clones from the Mutika – Lujugira Subgroup were classified into Nfuuka and Nakitembe clone sets. The plantains belonging to the AAB genomic group were subdivided in false, horn and French according to their bunch type. The other cultivars belonging to the AB and ABB genomic groups were grouped together. However, the germplasm characterization need to be updated especially within the Subgroup Mutika – Lujugira as there are some beer bananas having the same morphological characteristics as cooking bananas. Therefore, it is recommended that, the Curator should always first to taste the pulp of the bananas as it is the major factor in identifying the East - African Highland banana. Secondly, to observe the persistence of neuter flower and bract as these are discriminating factors for

the Nakitembe clone set and finally to take measurements of fruit size at harvesting so that he can discern a difference between the Musakalala clone set and the Nakabululu clone set, as they have long finger and short finger size of fruit in the middle of the bunch respectively.

The survey on distribution of banana cultivars in Rwanda showed that Subgroup Mutika – Lujugira was the major subgroup of AAA in the four regions of Rwanda such as Cyangugu, Kibungo, Kigali Rural and Kibuye – Gisenyi with the frequency distribution ranging between 60% to 90% in Cyangugu and Kibuye – Gisenyi respectively. However, the use of one language in Rwanda did not reduce the occurrence of synonyms. The cultivars having the same name in four regions of Rwanda must be corrected and after quarantine period, they should be planted in the National Banana Germplasm Collection at Rubona – Rwanda, for further evaluation and characterization.

Using high-resolution methods "Flow cytometry analysis" for determination of ploidy levels of bananas from National Banana Germplasm Collection at Rubona - Rwanda, it was found that there was misclassification of some exotic banana. However, all cultivars belonging to the Subgroup Mutika – Lujugira were confirmed to be triploid. The present results established the feasibility of analysis of ploidy level of bananas using leaves of banana stored at - 70 °C.

Using Random Amplified Polymorphic DNA markers, the genomic group of Highland bananas analyzed from the National Banana Gremplasm Collection at Rubona – Rwanda was established using OPA-18 primer and OPG-17 primers. These primers showed band 441 and 443 bp respectively for the accession having only the B genome whilst they were absent for the accessions having only an A genome.

Using flow cytometry analysis, all Highland bananas analyzed were shown to be AAA, which is in accordance with the results of SIMMONDS (1966). The results of 15 primers combined for RAPD analysis showed a low coefficient similarity ranging between 46 to

85% and each accession was clearly separated demonstrating the usefulness of RAPDs in analysis of genetic diversity.

The results of this study are very important for Curators and Breeders from Eastern Central African Region where the Highland banana is localized. However, for better understanding of this crop future research can be focused on using Inter – Retrotransposon Amplified Polymorphism (IRAP) which is used to detect retrotransposon insertional polymorphism, and to develop Microsatellites for the Highland bananas to further separate cooking and beer bananas at the DNA level.

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Appendix 1

Quantitative trait of fruits used for Principal Component Analysis (PCA).

Key	Circ. (cm)	Extl. (cm)	Wfr.(g)	Pstgl.(days)	Gl.(days)	Intl.(cm)	Avl(cm)h	Wdfr.(cm)	L/Wdfr
1	13.6	20.32	196.1	0	13.8	14.9	17.61	4.33	4.06
2	15.32	17.56	196.2	0	10.5	13.14	15.35	4.87	3.15
3	12.62	15.72	139.1	0	11.4	12.08	13.9	4.02	3.45
4	13.68	17.9	180	0	11.6	13.24	15.57	4.35	3.58
5	14.28	22.64	240.6	0	10.3	16.94	19.79	4.54	4.36
6	17.02	28.02	402.5	0	12.2	20.08	24.05	5.42	4.44
7	14.14	16.12	160.9	0	11.6	11.62	13.87	4.5	3.08
8	13.72	15.06	136.9	0	14	11.22	13.14	4.37	3.01
9	15.32	16.18	189.8	0	14.6	12.5	14.34	4.88	2.93
10	13.38	20.12	196.1	0	13.4	15.22	17.67	4.26	4.15
11	14.9	15.78	151	0	11.8	11.68	13.73	4.74	2.9
12	14.92	18.96	194.4	0	10.2	13.12	16.04	4.75	3.38
13	12.88	16.14	134.9	0	12.4	11.74	13.94	4.1	3.4
14	14.1	14.8	136.2	0	11.8	11.7	13.25	4.49	2.95
15	15.02	14.82	152.8	0	12.6	11.32	13.07	4.78	2.73
16	14.32	16.18	160.7	0	11.2	12.44	14.31	4.56	3.17
17	13.4	19.22	188.8	0	15.2	15.04	17.13	4.26	4.02
18	14.68	22.14	242.9	0	12.6	16.92	19.53	4.67	4.18
19	13.1	16.98	153.9	0	10.2	12.6	14.79	4.17	3.57
20	13.88	17.72	171.8	0	11.8	12.62	15.17	4.42	3.43
21	13.74	17.26	148.4	0	19.8	13.8	15.53	4.37	3.55
22	13.56	16.82	141.9	0	15.2	13.34	15.08	4.32	3.49
23	13.72	18.22	146.1	0	19.6	14.88	16.55	4.37	3.79
24	13.84	15.94	162.7	0	13.2	12.24	14.09	4.4	3.2
25	14.74	17.04	177.3	0	13	12.24	14.64	4.69	3.12
26	14.1	15.28	140.6	0	11.2	10.92	13.1	4.49	2.92
27	14.26	18.68	201.5	0	12	14.32	16.5	4.54	3.63
28	13.54	19.34	194.2	0	12	15.6	17.47	4.31	4.05
29	13.14	14.24	131.3	0	13.4	11.94	13.07	4.18	3.13
30	13.22	20.62	164.3	0	17.8	15.52	18.07	4.21	4.29
31	15.24	20.44	262	0	11	16.48	18.46	4.85	3.81

Key	Circ. (cm)	Extl. (cm)	Wfr.(g)	Pstgl.(days)	Gl.(days)	Intl.(cm)	Avl(cm)h	Wdfr.(cm)	L/Wdfr
32	13.24	16.86	141.8	0	15	12.68	14.77	4.21	3.51
33	13.32	14.94	119.6	0	15.8	11.7	13.32	4.24	3.14
34	14.74	18.74	207.7	0	12	14.02	16.38	4.69	3.49
35	14.68	18.24	179.8	0	12	12.04	15.14	4.67	3.24
36	13.66	16.2	161.7	0	12.2	13	14.6	4.35	3.36
37	14.66	13.96	152.3	0	13.2	11.24	12.6	4.66	2.7
38	15.9	18.44	228.5	0	13.2	14.88	16.66	5.06	3.29
39	13.62	16.7	162.8	0	13.4	12.98	14.84	4.33	3.43
40	15.68	21.2	257.1	0	12	15.12	18.16	4.99	3.64
41	13.24	15.84	149	0	12.8	12.06	13.95	4.21	3.31
42	15.18	15.28	167.5	0	11.8	11.24	13.26	4.83	2.74
43	13.58	12.32	133.9	0	12.3	10.1	11.21	4.32	2.59
44	13.48	16.84	145	0	15.8	12.84	14.84	4.29	3.46
45	14.94	20.38	226.6	5.6	10.7	14.9	17.64	4.75	3.71
46	14.24	15.34	170.4	4.6	13	12.04	13.69	4.53	3.02
47	15.32	17.94	210.4	5.4	8.4	13.22	15.58	4.88	3.19
48	14.22	15.88	166.7	5.1	9.2	11.58	13.73	4.53	3.03
49	13.7	17.32	172.9	6	8.8	13.12	15.22	4.36	3.49
50	14.62	18.44	204.4	4.9	8	13.4	15.92	4.65	3.42
51	14.08	16.24	167.8	5.3	11.2	12.34	14.29	4.48	3.19
52	15.16	18.48	216.6	5.2	10.8	13.82	16.15	4.82	3.35
53	13.44	16.08	147.6	5.3	10	12.34	14.21	4.28	3.32
54	14.24	16.7	179.4	5.4	10.4	12.84	14.77	4.53	3.26
55	13.72	15.04	165.3	5.2	14.1	11.44	13.24	4.37	2.92
56	15.12	14.82	176.2	5.3	11.5	12.38	13.6	4.81	2.83
57	13.08	17.44	160.6	4.8	7.2	13.54	15.49	4.16	3.72
58	14.48	16.72	171.6	5	10.2	11.76	14.24	4.61	3.09
59	9.92	14.18	79	5.6	8	11.08	12.63	3.16	4
60	9.54	12.3	59.6	5.3	7	9.44	10.87	3.04	3.57
61	11.4	11.32	74.5	5.6	7.8	8.38	9.85	3.63	2.71
62	10.74	11.24	75.2	5.1	6.8	9.14	10.19	3.42	2.98
63	14.54	14	142.7	8.8	6.1	12.56	13.28	4.63	2.87
64	12.82	13.28	107.3	5.8	8.8	9.74	11.51	4.08	2.82
65	13.82	14.68	148.6	4.9	9	11.78	13.23	4.4	3.01
66	13.9	17.86	175.9	4.9	9.8	13.72	15.79	4.42	3.57

Key	Circ. (cm)	Extl. (cm)	Wfr.(g)	Pstgl.(days)	Gl.(days)	Intl.(cm)	Avl(cm)h	Wdfr.(cm)	L/Wdfr
67	14.02	21.94	250	5.4	10.6	16.7	19.32	4.46	4.33
68	13.24	14.64	129.6	5.2	10.9	11.54	13.09	4.21	3.11
69	14.52	16.58	174.9	5.4	11.3	11.62	14.1	4.62	3.05
70	13.18	16.08	137.8	5.4	13.4	12.04	14.06	4.19	3.35
71	12.42	18.68	152.8	5.4	12.2	13.64	16.16	3.95	4.09
72	15.42	15.64	213.7	5.8	11	12.54	14.09	4.91	2.87
73	12.26	12.32	82.3	5.2	12.4	10.32	11.32	3.9	2.9
74	11.46	10.62	67.7	5.8	8.4	9.48	10.05	3.65	2.75
75	17.34	30.04	477.7	5	8	22.9	26.47	5.52	4.79
76	19.84	23.98	439	6	10.4	17.74	20.86	6.31	3.3
77	14.04	18.72	200.9	5.6	7.4	13.44	16.08	4.46	3.6
78	14.84	18.84	197.5	5.6	7.2	13.38	16.11	4.72	3.41
79	13.96	22	230	5.3	6.4	16.62	19.31	4.44	4.35
80	17.74	22.2	398	5.4	8.5	15.04	18.62	5.65	3.29
81	15.14	22.3	230.5	4.6	7.2	17.4	19.85	4.82	4.12
82	15.3	22.94	280.4	4.7	6.6	16.14	19.54	4.87	4.01
83	10.98	10.68	64.7	5	7.2	8.72	9.7	3.49	2.78
84	10.94	11.1	68.3	4.4	5.8	7.82	9.46	3.48	2.72
85	12.8	20.4	173.3	4.2	11.4	14	17.2	4.07	4.23
86	13.68	21.5	218	5.2	10.6	15.34	18.42	4.35	4.23
87	12.26	18.14	153.8	5.4	12.8	13.08	15.61	3.9	4
88	13.52	19.52	191.5	5	11.8	13.78	16.65	4.3	3.87
89	12.28	18.72	165.9	4.9	11.4	13.48	16.1	3.91	4.12
90	12.82	17.7	145.2	5.8	12.4	13.4	15.55	4.1	3.79

Circ.- circumference (cm) Dirl.- direct length (cm) Extl.- exterior length of fruit (cm) Wfr.- weight of fruit(g)

Pstgl.- post green life of fruit (days) Gl.- green life of fruit (days) Intl. – interior length of fruit (cm) Avl – average length of fruit (cm)

Wdfr.- width of fruit (cm) L/Wdfr ratio

Appendix 2

Please note that this is a permission letter written in the local working language, Kinyarwanda by the General Director of ISAR, sent to District Mayors to undertake the survey.

INSTITUT DES SCIENCES AGRONOMIQUES DU RWANDA
I S A R
B.P 138 BUTARE
DIRECTION GENERALE

Rubona, le 11./02./2000
N°...111./00/D.G/K.X

Kuli Bwana Bourgmestre wa Komini Mabanza.

Bwana Bourgmestre,

Abashakashatsi b'Ikigo Gishinzwe Ubushakashatsi mu Buhinzi n'Ubworozi mu Rwanda (ISAR), bariho barakora enquête irebana n'ibibazo umusaruro w'ibitoke uhura nabyo.

Komini yanyu iri muzatoranyijwe. Turabasaba rero ngo mubafashe gukora uwo murimo muri Komini yanyu.

Mugire akazi keza.

Umuyobozi mukuru wa ISAR,
Dr. MUHINDA MUGUNGA

Appendix 3

Survey - Juin 2001.

Prefecture/ commune/ secteur/
cellule:_____
Nom du fermier : _____
Date d'observation :_____
Age de bananeraie :_____
Altitude : _____

Germoplasme:

Type de banane	Genotype	Nom de cultivar	Nombre de pieds par cultivar dans le champ
A cuire			
Total à cuire :			
A dessert			
Total à dessert :			
A bière			
Total à bière :			
Total pieds dans le champ :			

Appendix 4

Binary data matrix for RAPD data.

Impyisi
10111011010100111010100110010100001100011110111000101001110
11000010000001100101000010110000100000100001001001010010000000000
11011111011110000000000001100000000001100000000101000100000010 0
10010011100111100011000001000000001
Inkati
10111111010100111010100110000110001100011010100101001000101
01010000000001000101000001010000010001101001001100000011000010000
11011111010110000000000101001000000000110001100010100010000000000
10101010010110010101000001000000001
Inshakabuhake
01000101010100100000000100010010000100010110100101000000101
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