December 2012 IJPSS Volume 2, Issue 12 ISSN: 2249-5894

POTENTIAL OF GENETICS FOR BANANA PRODUCTION IMPROVEMENT

Wisdom J. Changadeya*

Aggrey J. D. Ambali**

Abstract

Global annual banana and plantain production estimated at 100 million tonnes is generally insufficient given an ever-increasing population especially for regions where bananas and plantains are a staple food. Manipulation of the genetics of bananas through various methods such as conventional and mutation breeding, cytogenetics, genomics, genetic transformation and application of tissue culture techniques and morphological, biochemical and molecular markers hold promise to improve production.

Keywords: Bananas and plantains, morphological and molecular markers, breeding, cytogenetics, genetic transformation.

* Department of Biological Sciences, University of Malawi, Chancellor College, Zomba, Malawi.

** NEPAD African Biosciences Initiative, Policy Alignment and Programme Development Directorate, NEPAD Agency, Republic of South Africa.

Introduction

Bananas and plantains (*Musa* spp.) are the fourth most important crops in the developing world after rice, maize and wheat in terms of gross value of production and important staples and income generating fruits for millions of poor people in the tropical and subtropical countries (Ssebuliba *et al*., 2005; FAOSTAT, 2007). They are divided into two main categories: dessert and cooking bananas, the former constituting 43% of the world production and are usually eaten fresh when ripe, since they are sugary and easily digestible. Cooking bananas, which account for 57% of the world production, are usually starchier when ripe and boiled, fried or roasted (Jones, 2000). Cavendish bananas, which are dessert bananas constitute the most commercially important component of world banana production, accounting for 47% of the global banana production (Arias *et al.,* 2003).

Frison and Sharrock, (1999) estimated the world annual production at 103 million tonnes, of which about a third is produced in each of the African, Asia-Pacific and Latin American and Caribbean regions. Recent estimates put world banana and plantain production at about 100 million tonnes (FAOSTAT, 2008). About 87% of the world banana/plantain production is by small-scale farmers for home consumption or for sale in local and regional markets (Frison and Sharrock, 1999). Africa produces nearly 31 million tonnes of bananas yearly, which is mostly consumed without entering the international market system. About 1% of the annual 20 million tonnes of banana produced by the eastern and southern African regions is exported, while the western and central African regions export 4 % of their 11 million tons annual production. Production levels especially for the sub-saharn Africa are below potential levels due to numerous challenges facing the banana sector.

Problem statement

Banana production in general is beset with a number of challenges, which include variability of genetic material, low productivity, drought, poor husbandry practices and susceptibility to diseases and pest infestation. Bananas throughout the world suffer from relatively small number of pests and diseases which are highly devastating to yield and production (Swennen *et al.,* 1989). Among the most serious pests and diseases are banana weevil (*Cosmopolites sordidus*), a

complex of nematodes (*Pratylenchus goodeyi*, *Helicotylenchus multicinctus* and *Radopholus similis*), black streak/black Sigatoka (*Mycosphaerella figiensis*), yellow Sigatoka (*Mycosphaerella musicola*), Fusarium wilt (*Fusarium oxysporum*) also called Panama disease, banana bunchy top virus and banana streak virus. Of these black streak/Sigatoka is considered to be the most serious biotic constraint to banana and plantain production in Africa (Swennen *et al.,* 1989). The quality and quantity of the fruit is generally low because of the above pests and diseases that affected banana production considerably in Africa.

The abiotic and biotic challenges dwindling banana production can be addressed through various means, which include genetic improvement. There is enormous potential for increasing yields in bananas and plantains through genetic improvement (de Vries *et al*., 1967). This potential remains to be fully exploited in the sub Saharan Africa and other parts of the world despite considerable advances in the understanding of the genetic basis of specific traits in *Musa* (Ortiz, 1997), which form the basis for future genetic improvement programmes. However, there are various genetic improvement programmes employed in various countries. This review details progress made in different banana genetic improvement programmes.

Evolutionary origins of bananas and plantains

Cultivated bananas are believed to have resulted from interspecific hybridisation of two wild seminiferous diploid species, *Musa acuminata* (AA) and *M. balbisiana* (BB) that were donor of the A and B genomes, respectively (Pillay *et al.,* 2003; Pollefeys *et al.,* 2004). The natural intra or interspecific hybridisation of the two species was possible because the wild species are fertile (Carreel *et al*., 2002). The hybridisation gave rise to three additional genotypes, AB, AAB, and ABB (Carreel *et al*., 2002). The evolutionary pathway, which led to the emergence of cultivated varieties, involved two critical events. Initially vegetative parthenocarpy and female sterility appeared in *M. acuminata*, allowing the production of pulp without seeds as evidenced by the occurrence of parthenocarpic and seedless diploid *M. acuminata* (Simmonds, 1962). Parthenocarpy is believed to be based on mutations in A genome because it has not been identified in B genome yet hybrids of A and B show the character (Heslop-Harrison and Schwarzacher, 2007). Secondly, crosses within *M. acuminata (*intraspecific hybridisation) or

between *M. acuminata* and *M. balbisiana* (interspecific hybridisation*)*, coupled with female restitution and haploid fertilization, which implies formation of diploid (2n) gametes, gave rise to homogenomic hybrids which are essentially AAA dessert and highland bananas and heterogenomic hybrids comprising the AAB plantains and the ABB cooking bananas (Pillay *et al.,* 2004; Raboin *et al*., 2005).

Historical origins and centres of diversity of bananas and plantains

The exact origins of banana and its domestication remain in doubt. However, the earliest evidence of banana in the archaeobotanical record, provided by phytoliths (literally "plant stones") is from Papua New Guinea (PNG), which is an island country of south East Asia (Vrydaghs and De Langhe, 2003). Wilson (1988) reported the occurrence of *Musa* phytoliths at Kuk site, in the upper valley of the Wahgi on Mount Hagen in Papua New Guinea.
Modern bananas and plantains appear to have originated in the south-east Asian and western Pacific regions where their inedible, seed-bearing, diploid ancestors can still be found in the natural forest vegetation (Simmonds, 1966). In the wild, they are distributed in forest habitats of Asian-Pacific region ranging from Australia, the Pacific to northern India. The region also comprises other countries like, Bangladesh, Nepal, Bhutan, Myanmar, Indonesia, Malaysia, Solomon Islands, Papua New Guinea, Fiji and Samoa and is renowned for its richness in agro-biodiversity consisting of primitive forms, landraces and farmers' varieties (FAO, 2002).

Wild parents of bananas are found in the Asian-Pacific region which is a primary centre of diversity of bananas and the greatest diversity of banana cultivars, are known to grow in this region, each adapted to a specific ecoregion and selected by farmers for special eating or cooking qualities (Pillay, *et al*., 2004). There are other two important secondary centres of diversity outside of South East Asia. One exists in humid lowlands of West and Central Africa, where the greatest variability of plantains in the world occurs hence considered a secondary centre of plantain diversification. The other is in East Africa Highlands around the Great Lakes Zone where a distinct group of highland cooking and beer bananas are grown. This region is considered a secondary centre of diversity for the East African Highland banana cultivars (*Musa sp*. AAA) (Pillay, *et al*., 2004). Diversity among cultivated bananas has developed through

natural mutations and /or hybridisation involving wild diploids, *M. acuminata* (AA) and *M. balbisiana* (BB). Genetic variability is valuable in bananas since sterility of most bananas makes generation of variation through hybridisation almost impossible. Therefore, efforts are being made to conserve it in different countries around the world. A large proportion of cultivated banana cultivars diversity is also maintained *in vitro* in the International *Musa* Germplasm Collection by the International Network for the Improvement of Banana and Plantain (INIBAP) at the *Katholieke Universiteit Leuven* in Belgium (Nsabimana and Staden, 2006)

Classification of bananas

Banana is a herb of the family *Musaceae,* which are large, often tree-like perennial herbs comprising two genera, *Musa* and *Ensete* (Gill, 1988). Genus *Ensete* is composed of monocarpic herbs, none of which bear edible fruits but can be used as fibre and vegetable while *Musa* provides both fruit and fibre. The genus *Musa* L. that comprises bananas and plantains is generally divided into four sections, which are *Eumusa*, *Rhodochlamys*, *Callimusa* and *Australimusa*. Another section *Ingentimusa*, was created to accommodate the species *M. ingens* (2n=14) found in the highlands of Papua New Guinea (Argent, 1976). Table 1 shows banana classification based on recent classification of flowering plants (Bremer *et al*., 1998). The section *Eumusa* is the largest and geographically widespread and has given rise to the majority of cultivated bananas and plantains. All cultivated banana in Africa belong to *Eumusa* section (Laisnez, 2005) while the section *Australimusa* contains the Fe'i bananas and includes *M. textiles*, which is of commercial importance for its fibre. The *Rhodochlamys* and *Callimusa* sections comprises individuals of ornamental interest (Pillay *et al*., 2004). The genus *Musa* has five sections based on differences in chromosome numbers and morphological characters. However, validation of the classification system is questionable due to hybridisation incidents across sections. Amplified Fragment Length Polymorphism (AFLP) analysis has shown no distinct genetic differences between section *Rhodochlamys* and *Eumusa* suggesting merging of the two while sections *Callimusa* and *Australimusa* were also not genetically distinct and would be merged into a single section (Wong *et al*., 2002). Despite these findings, classification system is still confused with other authors recognizing only four sections excluding *Ingentimusa* (Dolezel and Bartos, 2005; Bartos *et al*., 2005).

Table 1 Classification of bananas.

Classification					
Kingdom	Plantae				
Super phylum	Tracheata				
Phylum	Angiospermophyta				
Class	Monocotyledoneae				
Superorder	Zingiberanae				
Order	Scitamineae/ Zingiberales				
Family	Musaceae				
Genus	**chromosome number**	**Section**	**Distribution**	**Species number**	**Uses**
Ensete	9		W. Africa to New Guinea	7-8	Fibre, Vegetable
Musa	10	Australimusa	Australia to Philippines	5-6	Fibre, Fruit
	10	Callimusa	Indo-China and Indonesia	5-6	Ornamental
	11	Eumusa	South India to Japan and Samoa	9-10	Fruit, Fibre, Vegetable
	11	Rhodochlamys	India to Indo-China	5-6	Ornamental
	14	Ingentimusa	Papua New Guinea	1	

Source: Laisnez, 2005

Genomes in *Musa*

Cultivated bananas are known to exhibit four genomes, A, B, S and T, the A, B and S genomes are characteristic of species in the section *Eumusa*. The A and B genomes were derived from

wild diploid species *M. acuminata* and *M. balbisiana* respectively (Cheesman, 1948). The S genome is known to be present only in the diploid *M. schizocarpa* (2n=2x=22), while the T genome is characteristic of the section *Australimusa* (2n= 2x= 20). Cultivars with the S and T genomes have been identified in Papua New Guinea (Shepherd and Ferreira, 1984) and are found in a few cultivars unlike A and B (Sharrock, 1989). The classification of edible bananas into genomic groups is based on a system developed by Simmonds and Shepherd, (1955). Under this system, 15 morphological features characteristic of *M. acuminata* and *M. balbisiana* were isolated and designated arbitrary numerical values ranging from 1 to 5, which were used to score the features. Using the visual assessment of the features to assign scores and knowledge of chromosome numbers of the plants, banana plants were assigned to various genome groups. The major genomic groups include diploids (2n=2x=22) (AA, BB, AB), triploids (2n= 3x=33) (AAA, AAB, ABB, BBB) and tetraploids (2n=4x=44). (AAAA, AAAB, AABB, ABBB). Although the genomic groups are based on morphological data, modern genetic techniques have largely supported this grouping (De Langhe, 2000). Genome composition has played an important role in the classification of bananas. Clones that share similar characteristics are considered to have arisen from a single base clone by mutation to form subgroups. Bananas that constitute each genomic group can however be very different. For instance, the AAA group contains the sweet dessert bananas as well as the cooking bananas of the East African highlands (Pillay *et al*., 2004).

Genomic groups with S genome include AS, AAS and ABBS, while those with the T genome are AAT, AAAT and ABBT (D'hont, *et al*., 2000). Carreel (1995) reported that molecular markers revealed that *M. schizocarpa* and one or more species from the section *Australimusa* played a role in the origin of some cultivars in Papua New Guinea. Genomic *in situ* hybridisation has helped to validate the involvement of the S and T genomes in some of these cultivars (D'hont, *et al*., 2000).

Most cultivated bananas are triploids. They vary in genome composition with the most common types being AAA (dessert), AAB (plantain) and ABB (cooking banana). Banana improvement programmes employ interspecific hybridisation for gene introgression, therefore knowledge of the exact ploidy level and genome composition of a plant is important for breeding purposes

(Pillay *et al.,* 2003). Crossing a triploid banana with diploid accessions generates diploid, triploid, tetraploid, aneuploid and hyperploid progeny (Vuylsteke *et al*., 1993a). The selection of a hybrid of a desired ploidy level requires precise determination of ploidy of its parents. The ploidy level of a plant is generally determined either by a physical count of its chromosomes or by DNA flow cytometry (Pillay *et al*., 2003).

***Musa* improvement programmes**

Despite the importance of bananas and plantains in international trade and food security especially in tropical regions, little has been done to genetically improve them in comparison to other major food crops. This is because major advances in *Musa* productivity have traditionally relied on improvements in crop husbandry practices (Crouch *et al*., 1999). *Musa* breeders face many problems specific to this crop, which retard genetic improvement. Cross breeding, that involves gene recombination, is difficult in *Musa* due to extremely complicated genetic systems such as different genomic constitutions, heterozygosity, polyploidy and sterility of edible varieties (Bhat *et al.,* 1995). The main hindrances to genetic improvement of *Musa* spp are; (i) most bananas and plantains are triploids, therefore virtually or completely sterile because plant triploids generally do not produce viable gametes during meiosis due to chromosome pairing problem, and (ii) bananas have long life cycle of almost two years from seed to seed (Crouch *et al*., 1998; Heslop-Harrison and Schwarzacher, 2007). Seed set, seed quality and embryo germination has also been found to be cultivar dependent hence only a few cultivars are available as females for crossings yet success in banana breeding relies on the identification of female fertile landraces that produce high rate of quality seeds (Ssebuliba *et al*., 2006).

However, breeders have generated promising tetraploid hybrids through interspecific and intraspecific hybridisation of triploid landraces with diploid accessions from wild, cultivated or improved gene pools (Ortiz and Vuylsteke, 1995). Creation of the tetraploids hybrids takes advantaged of rare residual female fertility present in some triploids (Ortiz and Vuylsteke, 1995). Further, mutation breeding has been employed in *Musa* breeding programmes. Hautea *et al.* (2004) reported that induced mutation either by chemicals or irradiation, coupled with *in vitro* propagation technique such as shoot-tip culture has been established as a tool for genetic

improvement by generating variations in banana cultivars. The use of mutation agents such as nuclear irradiation to induce variability in crops championed by the International Atomic Energy Agency (IAEA) has over the years resulted in numerous crop varieties in wheat, barley, oats, rice, beans, onions apples, grapes and soybeans (Micke, 1991).

The recent advances in biotechnology offer possibilities of generating planting materials that can tolerate or withstand both abiotic and biotic challenges facing bananas. Genetic transformation has become an important tool for crop improvement. Relative success in genetic engineering of bananas and plantains has been achieved recently, enabling the transfer of foreign genes into the plant cells. Lately, transgenic approaches have been reported to provide solutions to Black Sigatoka and nematodes control through production of disease resistant plants (Tripathi, 2003).

Conventional banana breeding

Important progress has been made in the genetic improvement of *Musa* through several conventional breeding programmes across the world, which have generated promising tetraploid hybrids, with combined good agronomic performance and disease resistance (Escalant *et al.*, 2002). These new varieties are now becoming available from breeding programmes. The important Centres for banana and plantain improvement are in; Hondurus, at the Fundacion Hondurena de Investigacion Agricola (FHIA); in Onne, Nigeria, at the International Institute for Tropical Agriculture (IITA); in Brazil, at the Empresa Brasileira de Pesquisa Agropecuaria (EMBRAPA); in Montpellier, France at the Centre International de Recherches Agricole pour le Développment (CIRAD-FLHOR); in Cameroon, at the Centre de Recherches sur la Banane et Plantain (CARBAP); at the Agricultural Research Centre in South Africa; and at the Banana Board of Jamaica (Escalant *et al*, 2002, Pollefeys *et al.*, 2004).

The tetraploid hybrids stem from a hybridisation technique first developed by FHIA and now adopted by other programmes. It is based on interspecific and intraspecific hybridisation of triploid landraces with diploid accessions from wild, cultivated or improved gene pools (Vuylsteke *et al.,* 1993b; Vuylsteke *et al.*, 1995). Improved fertile diploids possessing useful resistance traits introduced from wild sources in an improved genetic background are used to

pollinate popular triploids varieties to produce tetraploid hybrids. The resultant tetraploid hybrids express traits of both parents. Using this system FHIA has produced a number of improved tetraploid hybrids some of which are widely distributed and are adopted by farmers in some countries (Escalant *et al*, 2002). Various improved varieties incorporating disease resistance have been produced by all major breeding programmes and have been sent to the International Network for the Improvement of Banana and Plantain (INIBAP) for testing (Table 2) (Escalant *et al*., 2002).

Table 2 Improved banana varieties from conventional breeding programmes.

Breeding Programme	Group	Accession
FHIA	Sweet varieties	FHIA-02, FHIA-17, FHIA-23, SH-3640
FHIA	Sweet-acid	FHIA-01, FHIA-18
FHIA	Cooking	FHIA-03, FHIA25
FHIA	Plantain	FHIA-21
IITA	Cooking	BITA-2, BITA-3
IITA	Plantain-type	PITA-16
CARBAP	Plantain-type	CRBP-39
CIRAD	Dessert	IRFA-911, IRFA-904
EMBRAPA	Sweet-acid	PA-03-22, PA-12.03, PV-03-44, PV-42-320, PV-42-53, PV-42-81

Source: Escalant *et al.*, 2002

In 1987, IITA screened *Musa* accessions for resistance to black sigatoka/ black streak and found 30 sources of resistance, mainly from fertile diploid types (Swennen and Vuylsteke, 1991). Resistant tetraploid hybrids were developed following breeding techniques developed by Rowe and Rosales (1993). Researchers crossed male-fertile, resistant diploid accessions with female fertile, triploid cultivated varieties to obtain resistant tetraploids. Additional diploid accessions produced through this process have since been crossed with tetraploid hybrids to obtain male-sterile, secondary triploids (Ortiz and Vuylsteke, 1994). IITA has developed five hybrid cultivars (PITA-2, PITA-3, PITA-8, PITA-14 and PITA 17) with resistance to black sigatoka. Another

IITA line PITA-14 has been shown to be black sigatoka resistant and virus tolerant (Ortiz and Vuylsteke, 1998).

The main constraints of this breeding strategy are; (i) very low fertility of the desired female triploid parents, and (ii) residual fertility in the tetraploid progeny leads to seed bearing fruits under certain circumstances, which is undesirable in the banana industry. To overcome the latter constraint, tetraploids undergo additional crosses to produce secondary sterile triploids (Escalant *et al.,* 2002). Another challenge facing the tetraploid hybrids is that progeny of tissue-culture tetraploid hybrids have been diagnosed with high percentage infection of Banana Streak Virus (BSV) and have been quarantined in some parts of the world (Frison and Sharrock 1998). Despite these challenges, tetraploid banana hybrids have potential for increased production because of their favourable traits, which will eventually lure commercially, minded farmers resulting in increased adoption rate.

Mutation breeding of bananas

Spontaneous somatic mutations are known to have played a major role in speciation and domestication of bananas and plantains. As an evolutionary force, mutations potentially alters frequencies of alleles within a population, which is vital to upsetting genetic identity of a population (Harlt and Clark, 1989). In the case of bananas, there is a school of thought that present banana diversity is due to evolutionary selection in prehistorical time from spontaneous mutations, naturally occurring mutations (Buddenhagen, 1986). Unfortunately, the rate of occurrence of spontaneous mutations is too low to provide sufficient genetic variability to meet practical breeding needs. Consequently, mutagenic agents such as radiations and certain chemicals such as sodium azide, diethyl sulphate and ethel methanesulphonate have been used to induce mutations at higher frequency and generate genetic variability from which desirable mutants may be selected. Induced mutation techniques are especially important for banana and plantains where there is limited sexual reproduction that can generate sufficient genetic variation, the basis for selection and breeding (Bhagwat and Duncan, 1998a; Roux, 2004). Induced mutation coupled with *in vitro* propagation techniques such as shoot-tip culture has been established as a tool in mutation breeding for generation of variation in a number of vegetatively

propagated crops (Micke, 1991; Donini and Sinnino, 1998). Induced mutants are usually differentiated from each other and from the original cultivar by phenotypic analysis based on agromorphological and physiological traits (Hautea *et al*., 2004). *In vitro* mutagenesis raised hopes in the 1980s and 1990s but not many useful mutants were obtained through this method due to high degree of chimerism (mixoploid) that occurs during mutagenesis (Roux *et al.,* 2004). Chimerism refers to a condition where cells or tissues exhibit two different genotypes, which is a result of cells, or tissues having different chromosome numbers within the same individual, also called mixoploidy. Mixoploidy often result in abnormally developed plants. Use of somatic embryogenic cell suspensions for mutagenesis has proved to be an effective way for eliminating chimeras since embryos are presumed to regenerate from single cells (Van Harten, 1998).

The Taiwan Banana Research Institute (TBRI) is one of the institutions conducting banana mutation breeding. Mutant variants of Cavendish bananas with resistance to race 4 of Fusarium wilt have been identified at TBRI. Four such accessions (GCTCV-247, GCTCV-106, GCTV-215 and GCTCV-119) were forwarded to INIBAP (Escalant *et al*., 2002). Using gamma rays, Roux (2004) reported different banana mutants with improved morphological characteristics of bunch size and cylindrical shape (mutant line name: Klue Hom Thong KU1) in Thailand, and plant height (dwarfness) (mutant line names: SH3436-L9 and 6.44) in Cuba. Mutants with increased tolerance to *Fusarium oxisporium* (mutant line names: Mutiara and Novaria) in Malaysia and to toxin of *Mycosphaerella fijiensis* (mutant line names: GN35-I to GN35-VIII) by IAEA were reported. Apart from gamma rays, ion beam technique has been used to produce a wide range of mutants (Reyes-Borja *et al*, 2007). Fukuda *et al*. (2004) indicated that ion beams frequently produced large DNA alteration such as inversion, translocation, and large deletion rather than point mutation, resulting in producing desirable characteristics in mutant banana. These mutation breeding success stories point to a critical role that this technology has in banana genetic improvement endeavours and emphasizes the need to harness and regulate seemingly dangerous technologies to the benefit of mankind.

Banana tissue culture

In banana production use of pest and disease infected suckers in new fields, has been identified as the major reason for the spread of pests and diseases (Jones and Diekmann, 2000). Fungal and bacterial wilt pathogens can occur symptom-less in the roots and rhizomes of suckers taken from infected fields, while nematodes, banana weevils and Sigatoka diseases can also be transmitted through infected material (Viljoen *et al*., 2004). The introduction of tissue culture 20 years ago as first generation biotechnology tool led to the production of genetically uniform and disease free planting material (Viljoen *et al*., 2004). Plant tissue culture is the science of growing plant cells, tissues, or organs isolated from the mother plant, on artificial media. Both organized and unorganized growth is possible *in vitro* (George, 1993). Organised growth of banana tissue *in vitro* is limited to embryo culture and shoot tip culture. Embryo culture, which is the culture of embryos on nutrient media, is an important aid for classical breeding in banana since the germination frequency of seed is extremely low. Embryo rescue, which is an *in vitro* technique used to culture hybrid embryos which otherwise abort *in vivo*, can increase this frequency 10 times (Vuylsteke and Swennen, 1992). Major applications of shoot tip culture are mass clonal propagation and germplasm conservation. In the former already existing shoot tips are stimulated to multiply rapidly while in the latter multiplication rate is slowed down for conservation purposes. This technique is employed to conserve the international *Musa* germplasm collection at INIBAP Transit Centre at K.U. Leuven (Strosse *et al*, 2004). *In vitro* banana collection maintenance is labour intensive even under reduced growth conditions and loss of accessions due to contamination and human error is possible. Moreover, *in vitro* material is subject to somaclonal variation, which contravenes the purpose of conservation. Cryopreservation or freeze-preservation at ultra-low temperatures (-196°C) is the preferred method since under these conditions; biochemical and most physical processes are docile, so plant materials can be stored for unlimited periods (Panis *et al*., 2004). Tissue culture techniques have therefore a vital role to play in banana production by providing disease and pest free planting materials as well as conserving banana germplasm which harbour gene diversity on which future genetic improvement programmes rely.

Tissue culture of banana via multiple shoot tip culture based on addition of cytokinins to standard media has become a wide spread method of propagating selected, clean clones for cultivation in fields and plantations (Vuylsteke *et al*, 1997). In Kenya and Tanzania selected

farmers have adopted cultivation of tissue-cultured banana plants on both large and small scale. Tissue cultured banana plants exhibit significant increased vigour and yield and early maturity. Although tissue culture plants are generally free from fungi, bacteria, insects and nematodes, they are not necessary free from viruses (Viljoen *et al*., 2004). Banana Streak Virus (BSV) is wide spread among banana and plantain germplasm and it is difficult to obtain BSV-free plantlets through tissue culture. Although indexing for the absence of viruses before multiplication of plant material through tissue culture has been successful for many viral diseases, it still remains a challenge for BSV in bananas (Dahal *et al.,* 1998).

Tissue culture is also associated with generation of genetic variation. Variants can be induced during the *in vitro* propagation of bananas by means of prolonged sub-culturing in the presence of growth regulators, by mutagenesis through chemical treatment or by irradiation (Bhagwat and Duncan 1998a; Xu *et al*., 2006). Through the tissue culture process disease resistant somaclonal variants are generated and can be selected (Jain, 2001). Oh *et al*. (2007) demonstrated that some genomic regions of *Musa* show higher rearrangement and mutational rates than others under stresses imposed by tissue culture. Induction of somaclonal variation through tissue culture is another way of broadening; otherwise narrow genetic pool of natural hybrids of bananas. However, generation of somaclonal variation with disease resistant resultant plants is a tedious process with low success rates. Banana cultivar improvement by somaclonal variation was demonstrated in Taiwan by Hwang and Ko (1988) who selected somaclones resistant to *F. oxysporum*. No somaclonal variants have been found so far with good tolerance to Sigatoka, Banana weevil, nematodes or virus diseases like Banana Bunchy Top (Viljoen *et al*., 2004).

Another technique of banana genetic improvement that uses tissue culture principles is the use of protoplasts. Protoplasts are totipotent, naked cells lacking cellwalls capable of reforming a cell wall and later initiate either a callus or a somatic embryo. In banana, protoplasts are obtained from *in vivo* tissues or *in vitro* cultures. The development of protoplast system allows regeneration of plants from single cell/protoplast and is considered as a complimentary technique to conventional breeding in its applications to somaclonal variation, genetic transformation and somatic hybridisation. Nakedness of protoplast renders them useful for uptake of foreign genetic material and production of somatic hybrids through protoplast fusion (Haicor *et al*., 2004).

Banana is now easily amenable to *in vitro* culture and plants are regenerated from various explants through organogenesis (Cronauer and Krikorian, 1986), embryogenesis (Cote *et al*., 1996), anther culture (Kerbelec, 1996) and even from cultured protoplasts (Panis *et al.*, 1993). Through genetic engineering, it has become possible to confer new traits on banana plant using particle bombardment (Sagl *et al*., 1995) or *Agrobacterium*-mediated transfer (May *et al*., 1995). Protoplasts facilitate direct transformation of plant cells by DNA microinjection and electroporation (Haïcour *et al*., 2004). Through protoplast fusion, useful traits can be transferred, even if detailed genetic or molecular knowledge of genes encoding for these desired traits is lacking (Jones, 1988). Therefore protoplast fusion is a complementary tool to increase nuclear and cytoplasmic variability and to confer desirable agronomic traits (Haïcour *et al*., 2004).

It is obvious that tissue culture in its many forms and applications is a needed instrument for advancement of banana production worldwide.

Cytogenetics and genomics of bananas

Limited knowledge in the genetics of banana cells (cytogenetics) and the function of genes (genomics) is partly to blame for minimal advancement in genetic improvement of *Musa* spp. With most cultivated clones highly susceptible to banana pests and diseases caused by pathogenic fungi, bacteria and viruses, banana production has been under threat and classical breeding of improved disease resistant material has been hampered by complicated genetic system of *Musa* (Doleel *et al.,* 2004). Classical breeding approaches coupled with biotechnological and molecular techniques can result into a formidable tool for *Musa* improvement.

Cheesman (1932) reported initial efforts to characterise nuclear genome of *Musa* at the chromosomal level. Division of genus *Musa* into four sections (*Eumusa, Rhodochlamys, Callimusa* and *Australimusa*) was a result of correct determination of chromosome numbers. This classification is based on basic chromosome number and morphological characteristics (Cheesman, 1947). Doleel *et al.* (1997) demonstrated that rapid and reliable ploidy screening in *Musa* could be performed using DNA flow cytometry. The method led to accurate classification

of some clones. For instance, several well-known clones previously thought to be tetraploids were found to be triploids (Horry *et al*., 1998). Additionally, accurate determination of ploidy is essential even in breeding because choice of successful hybridisation cultivar partners depends on knowledge of the candidates' ploidy.

Over the years there has been a shift in chromosome studies from purely cytogenetic level to molecular level resulting in an era of molecular cytogenetics (Pillay *et al*., 2004). New techniques for genome analysis have been developed, such as restriction fragment length polymorphism (RFLP) (Botstein *et al*., 1980), randomly amplified polymorphic DNA (RAPD) (Williams *et al*., 1990), amplified fragment length polymorphism (AFLP) (Vos *et al.,* 1995), and genomic *in situ* hybridisation (GISH) (Le *et al*., 1989). These tools have simplified the possibility of examining in greater detail the structure of plant genome. GISH is a technique, which uses labeled genomic DNA as a probe to differentiate chromosomes from different species by DNA *in situ* hybridisation. This technique is effective in distinguishing chromosomes from different plant genomes (Jiang, 1994). Significant progress has been made in the analysis of nuclear genome at molecular level but knowledge of the genome at the nuclear and chromosomal level is still sketchy.

Integration of existing *Musa* genetic maps and physical maps of *Musa* genome has been enhanced by accumulation of sufficient numbers of cytogenetic markers across the *Musa* genome leading to an integrated genome map. An integrated genome map is an indispensable tool for effective breeding as well as for map-based cloning of genes of interest. Molecular cytogenetical methods that permit physical mapping of DNA sequences to *Musa* chromosomes have been developed and are useful in obtaining physical chromosome landmarks and to characterise various repetitive DNA sequences. Furthermore, understanding of chromosomal structure provides a basis for more targeted application of mutation techniques for breeding of improved cultivars of bananas and plantains (Doleel *et al*., 2004). Knowledge in the cytogenetics and genomics of *Musa spp* serves as a valuable base for future manipulation of *Musa* genes in addressing some of its challenges whose solutions would depend on genetic engineering. Conventional breeding also benefits from this knowledge since reproductive fertility of bananas is dependent on correct cytogenetics and genomics of gametes.

Genetic transformation of bananas

Genetic transformation offers an alternative way of improving *Musa* and some success has been registered using microprojectile bombardment of embryogenic cell suspensions (Sagi *et al*., 1995; Becker *et al*., 2000). *Agrobacterium*-mediated transformation however, offers some advantages over direct gene transfer methodologies such as particle bombardment and electroporation. *Agrobacterium*-mediated transformation has the advantages of transferring only one or few copies of DNA fragments carrying the genes of interest at higher efficiencies yet at low cost and the transfer of very large DNA fragments with minimal rearrangement (Shibata and Liu 2000). Low copy number of inserted genes is preferred, because it results in higher expression of desired traits in the transformants. Protocols have been developed for *Agrobacterium*-mediated transformation of embryogenic cell suspensions as well as using shoot tips from various cultivars of *Musa* (Ganapathi *et al*., 2001; Tripathi *et al*., 2002). Detection of *Fusarium wilt* at molecular level has been enhanced by use of improved electrochemiluminescence polymerase chain reaction (ECL-PCR) method (Wei and Zhou, 2008). Stable and transient expression of introduced genes has been observed in a range of banana cultivars, including Williams and Grande naine (AAA Cavendish), Gros Michel (AAA dessert banana) Three hand planty, French somber and Currare (AAB plantain), Bluggoe, Cardaba and Monthan (AAB cooking banana), Rastali (AAB, sweet-acid) and FHIA-23 (AAAB, tetraploid hybrid) (Escalant *et al*., 2002).

Banana plants have been transformed with anti-fungal genes by the Katholeike Universiteit Leuven (KULeuven), Centro de Investigacióny de Estudios avanzados del IPN (CINVESTAV/CATIE) and SYNGENTA (Escalant *et al*., 2002). Genes for resistance to Banana Bunchy Top and Banana Bract Mosaic-viruses and genes controlling the ripening process have been used by Queensland University of Technology (QUT) and SYNGENTA respectively, to transform bananas (Escalant *et al*., 2002). Remy *et al*. (1998a) have reported progress on the development of new lines, through genetic modification, resistant to Fusarium wilt and Black Sigatoka diseases. Several field trials have been carried out with success (Aguilar and Kohlmann, 2006). Dauwers (2007) reported a field trail in Uganda of genetically modified (GM) banana with resistance to Black Sigatoka disease. The short communication indicated that the

GM banana was produced at the Laboratory of Tropical Crop Improvement in Leuven in Belgium. At KULeuven, testing of plants transformed with anti-fungal and virus-resistance genes had been on going in green houses (Escalant *et al*., 2002). Subramaniam *et al*. (2006) demonstrated resistance to Fusarium wilt disease in transgenic Pisang Rastali (AAB) by showing higher increased activities of enzymes that are associated with disease resistance in plants such as chitinase, β-1,3-glucanase, peroxidase and polyphenol oxidase than in non transgenic banana plantlets.

Morphological, biochemical and molecular markers in banana

Several methods have been used to investigate diversity in *Musa* germplasm. Morpho-taxonomic characters were the first to be developed and optimized for bananas and 119 descriptors were defined to characterise *Musa* germplasm (IPGRI, 1999). The *Musa* descriptors have been widely used in various morphological investigations. For example, morphological studies of 105 accessions of *M. balbisiana* in the Philippines showed a wide genetic variation (Sotto and Rabara, 2000). However, many morphological characteristics show a high degree of plasticity and continuous variation due to high influence of the environment (Wan *et al*., 2005). Besides, morphological markers are limited in number and they do not often reflect genetic relationships because of interactions with the environment, epistasis and largely unknown genetic control of the traits (Smith and Smith, 1989). In contrast, DNA markers are abundant and are not influenced by the environment or developmental stage of a plant, rendering them useful for genetic relationships studies (Reddy *et al.,* 2002).

Morphological characterisation has shaped the present understanding of diversity and classification of banana. For instance the genomic groupings of banana and plantains were first coined using morphological characters (Simmonds and Shepherd, 1955) hence morphological markers have a a role in diversity studies of bananas and should not be disregarded but rather should be complimented by other more precise recent biochemical and molecular markers. For instance, Cheniany *et al*. (2007) reported that two diploid wheat species (*Triticum boeoticum* Bioss. and *Triticum urartu* Thumanian ex Gandilyan) were proved to be two separate species

using isozyme variation reflecting their distinct gene pools and substantiating their previous specific recognition using hair length differences on leaf brades despite their overall morphological similarity.

Various types of biochemical and molecular markers have been used to reveal the evolution and systematic relationship between *Musa* cultivars and species (Valarik *et al*., 2002). Great divergence among *Musa* species and a high level of differentiation of *M. acuminata* was demonstrated by Jarret and Litz (1986) and Horry and Jay (1998) who used biochemical markers based on isozymes and flavonoids, respectively. But, Isozymes and flavonoids are expressed only in certain tissues and depend on the developmental stages of the plant, making data collection and comparison between laboratories difficult (Ude *et al*., 2002). Furthermore, because of redundancy in the DNA code that dictates protein sequences, all changes in a gene may not result in a change in the overall charge of the protein expressed; yet protein electrophoresis depends on differences of net charges of samples. Consequently many genetic variants are not detected by protein-based markers like isozymes and allozymes.

Molecular markers, though expensive in terms of both their discovery, synthesis and execution have been developed for bananas and enabled detailed analysis of genetic relationship between various accessions of *Musa* (Jarret *et al*., 1992; Kaemmer *et al*., 1997; Grapin *et al*., 1998) and construction of genetic linkage maps (Fauré *et al*., 1993). Application of molecular markers provides the only tools that are able to reveal polymorphism at the DNA level, which are sufficient to detect genetic variability between individuals and within populations (Kresovich *et al*., 1995, Simmons *et al*., 2007). Several DNA markers have been used to study genetic diversity in banana. These include; Restriction Fragment Length Polymorphism (RFLP) (Bhat *et al.,* 1995), Randomly Amplified Polymorphic DNA (RAPDs) (Uma *et al*., 2005; Nsabimana and Staden, 2007) microsatellites or Simple Sequence Repeats (SSRs) (Ning *et al*. 2007, Changadeya et al. 2012), Amplified Fragment Length Polymorphism (AFLP) (Loh *et al*, 2000), Sequence-tagged microsatellite sites (STMS) (Grapin *et al.,* 1998), Inter-retrotransposon amplified polymorphism (IRAP) (Nair *et al*., 2005), Genomic in situ hybridisation (GISH) (D'Hont *et al.,* 2000), PCR product restriction fragment length polymorphisms (PCR-RFLP) (Nwakanma *et al*., 2003) and Inter simple sequence repeats (ISSR) (Venkatachalam *et al*., 2008). RFLP has been

useful in *Musa* diversity studies, but this technique is expensive and technically very demanding (Crouch *et al*., 1999a). Consequently, *Musa* researchers have concentrated on the application of PCR-based markers such as RAPD, SSRs, ISSR, PCR-RFLP and AFLP for genetic analysis (Ude *et al*., 2002; Simmons and Webb, 2006). RAPD and AFLP markers show sufficient polymorphism for genotype discrimination but SSR and AFLP markers are more highly reproducible. AFLP markers possess both high reproducibility and discriminatory capability. AFLP can distinguish between two cultivars used with a minimum of one selective primer pair (Hautea *et al*., 2004). It can also detect variation in DNA profiles of induced mutant clones which are otherwise morphologically indistinguishable, and detect variation between the induced mutant parent clones and their derived suckers (Hautea *et al*., 2004). Nonetheless, all markers have proved useful in various applications, hence the need to keep and use the whole "tool box" of markers.

RAPD markers were used to investigate genetic diversity and phylogenetic relationships of 29 East African banana cultivars and two outgroup taxa, *M. acuminata* Calcutta 4 and Agbagba. The results revealed that East African bananas are closely related with a narrow genetic base (Pillay *et al*., 2001). RAPD polymorphisms were sufficient and useful in distinguishing the cultivars. A dendrogram derived from the RAPD data was divisible into a cluster composed of all AAA highland banana cultivars and Agbagba (AAB) and a minor cluster consisting of Kisubi (AB), Kamaramasenge (AB) and Calcutta 4 (AA). However, RAPD data did not separate beer and cooking banana cultivars both of AAA genome composition (Pillay *et al*., 2001). The findings provide a basis for breeders to identify diverse cultivars for breeding. RAPD technique has advantages, which include its speed, low DNA template requirements and technical simplicity, making it a convenient tool for genetic variation determination in bananas (Pillay *et al*., 2001). However, RAPD markers being dominant markers, have problems distinguishing bands that represent two alleles at a homozygous locus from bands that represent only a single allele at a heterozygous locus. Consequently, it is impossible to determine exact genetic similarity between two cultivars that share a band at the same position (Yonemoto *et al*., 2006). In separate study reported by Uma *et al*. (2006), who used RAPD markers, 16 accessions of *Musa balbisiana* were clustered into four against seven clusters obtained through morpho-taxonomic characterisation. Uma *et al.* (2006) indicated that in general characterisation using

RAPD strengthens diversity analysis in *Musa* accessions but needs refinement using more random primers for more specific results and also using AFLP markers. In their study, 34 RAPD primers produced reproducible bands but only four primers among them showed polymorphic bands.

Ude *et al*. (2002) reported that using AFLP marker on 28 accessions of *Musa acuminata* (AA) Colla and *Musa balbisiana* (BB) Colla, Neighbour-joining and principal co-ordinate (PCO) analyses using Jaccard's similarity coefficient produced four major clusters that closely corresponded with the genome composition of the accessions (AA, BB, AAB, and ABB). The AFLP data distinguished between wild diploid accessions and suggested new subspecies relationships in *M. acuminata* complex that are different from those based on morphological data. The data suggested three subspecies within the *M. acuminata* complex (ssp. *burmannica* Simmonds, *malaccensis* Simmonds and *microcarpa* Simmonds. The reported subspecies are contrary to those based on SSRs and RFLP which are four; *banksii*, *zebrine*, *malaccensis* and *burmannica/burmanicoides* (Carreel *et al*., 1994; Grapin *et al.,* 1998). A study done by Noyer *et al*. (2005) used both AFLP and SSR to assess genetic diversity among 30 plantains constituting a representative sample of phenotypic diversity. A total of nine microsatellite loci showed very low polymorphism within the plantain group rendering the group difficult to differentiate. Similarly eight AFLP data would not differentiate the plantain group. Data from SSR and AFLP suggests that these plantain cultivars may have arisen from vegetative multiplication of a single seed hence their high similarity.

Creste *et al*. (2004) showed the usefulness of microsatellites markers for the analysis of genetic variability, the distribution of the variability and genetic relationship present within *Musa* germplasm in Brazil. The study detected a large number of alleles suggesting a large genetic variability present and evidence of the multi-allelic nature of microsatellite. The study also revealed similarity between diploid and triploid accessions implying possibility of potential crosses to maximize the recovery of typical fruit qualities required in Brazil's (AAB, Pome and Silk dessert banana). Analyses have been done using SSR loci to compare the genetic similarity of full-sib 2X and 4X hybrids, and parental genotypes. Microsatellite markers detected high levels of polymorphism in full-sib *Musa* breeding populations. The implication of such analyses

rest on the fact that progress in breeding *Musa* hybrids with ever increasing yields will depend on the ability of breeders to identify and access the genes and gene combinations most likely to generate enhanced heterosis in yield components (Crouch *et al*., 1999b). A study conducted to investigate genetic variability using nine B-genome derived SSR primers between 40 *Musa* genotypes maintained at the IITA, Ibadan, Nigeria produced high genetic polymorphism generating a total of 23 alleles. Hierarchical cluster analysis showed clusters of diploid cultivars separated from triploids with only two exceptions (Oriero *et al*., 2006). The ability of SSR markers to reveal high diversity was also demonstrated in another study by Changadeya et al (2012) which yielded a total of 176 alleles among Malawian cultivars. This capacity of SSR is useful in aiding the choice of suitable parents for breeding in *Musa* genetic improvement.

Conclusion

Genetic improvement of bananas and plantains has a role in increasing global banana production. Given the genetic system complexity and general biology of bananas that hamper conventional breeding, there is need to invest more resources towards mutation breeding, tissue culture and genetic transformation. The DNA probes, especially microsatellites should be employed to establish records of cultivar relationships and determine genetic structure of various banana and plantain cultivar populations. This is particularly important for cultivars found in both primary and secondary centres of diversity.

References

Aguilar, F.X. and Kohlmann, B. 2006. Willingness to consume and produce transgenic bananas in Costa Rica. *International Journal of Consumer Studies,* 30 (6): 544-551.

Argent, G.C.G. 1976. The wild bananas of Papua New Guinea, *Notes from the Royal Botanic Garden*, 35: 77-114.

Arias, D., Dankers, C., Liu, P., Pilkauskas, P. 2003. The World Banana Economy 1985-2002. Food and Agriculture Organisation of the United Nations, Rome, Italy.

Bartos, J., Alkhimova, O., Dolezelova, M., De Langhe, E., Dolezel J. 2005. Nuclear genome size and genomic distribution of ribosomal DNA in *Musa* and *Ensete* (Musaceae): taxonomic implications. *Cytogenetics and Genome Research*, 109:50–57.

Becker, D.K., Dugdale, B., Smith, M. K., Harding, R. M., Dale, J.L. 2000. Genetic transformation of cavendish banana (*Musa sp*. AAA group) cv. Grand Nain via microprojectile bombardment. *Plant Cell Reports,* 19: 229-234.

Bhagwat, B. and Duncan, E.J. 1998a. Mutation breeding of banana cv Highgate (*Musaspp*. AAA Group) for tolerance to *Fusarium oxysporum F.spp cubense* using chemical mutagens. *Scientia Horticulturae*, 73: 11-22.

Bhat, K.V., Jarret, R.L., Rana, R.S. 1995a. DNA profiling of banana and plantain cultivars using random amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP) markers. *Electrophoresis,* 16: 1736-1745.

Botstein, D., White, R.L., Skolnick, M., Davis, R.W. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms, *American Journal of Human Genetics,* 32: 314-331.

Bremer K., Chase M. W., Stevens P. F. (eds.) 1998. An ordinal classification for the families of flowering plants. *Annals of the Missouri Botanical Garden,* 85 (4): 531-553.

Buddenhagen, I.W. 1986. Disease susceptibility and genetics in relation to breeding of bananas and plantains. pp 95-109. In: *Banana and Plantain Breeding Strategies*. Persley, G.J. De Langhe, E.A. (eds.). ACIAR, Canberra 21.

Carreel, F. 1995. Etude de la diversité génétique des bananiers (genre *Musa*) à l'aide des marqueurs RFLP, PhD Thesis, Institut National Agronomique Paris-Grignon.

Carreel, F., Faure, S., Gonzalez de Leon, D., Lagoda, P.J.L., Perrier, X., Bakry, F., Tezenas du Mantcel, H., Lanaud, C., Horry, J.P. 1994. Evaluation de la diversité

génétique chez les bananiers diplo (*Musa* spp), *Genetics Selection Evolution,* 26: 125-126.

Carreel, F., Leon, D.G., Lagoda, P., Lanaud, C., Jenny, C., Horry J.P., Montcel, H.T. 2002. Ascertaing maternal and parental lienage within *Musa* by chloroplast and mitochodrial DNA RFLP analyses. *Genome,* 45: 679-692.

Changadeya, W., Kaunda, E and Ambali, A. Molecular characterization of *Musa* L. cultivars cultivated in Malawi using microsatellite markers. *African Journal of Biotechnology* Vol. 11 (18), (2012) pp. 4140-4157.

Cheesman, E. E. 1947. The classification of the bananas, *Kew Bulletin* 2: 97-117.

Cheesman, E.E.1948. Classification of the bananas III. Critical notes on species. a. *Musa balbisiana*. *KewBulletin* 3: 11-16.

Cheesman, E.E. 1932. Genetical and cytological studies in *Musa*. In: Certain hybrids of the Gros Michel banana; II. Hybrids of the Mysore banana, *Journal of. Genetics,* 26: 219-312.

Cheniany, M., Ebrahimzadeh, H., Salimi, A., Niknam, V. 2007. Isozyme variation in some populations of wild diploid wheats in Iran. *Biochemical Systematics and Ecology,* 35: 363-371.

Cote, F.X. Domergue, R., Monmarson, s., Schwendimon, J., Teisson, C. 1996. Embryogenic cell suspension from the male flower of *Musa* AAA cv. Grande Naine (AAA), *Plant Physiology*, 97: 285-290.

Cronauer, S.S. and Krikorian, A.D. 1986. pp 233-252. Banana (*Musa* spp.). In: *Biotechnology in agriculture and forestry*. Bajaj, Y.P.S. (ed.). Springer Verlag, Berlin 1.

Crouch, J.H., Crouch, H.K., constandt, H., van Gysel, A., Breyne, P., Van Montagu, M., Jarret, R.L., Ortiz, R. 1999a. Comparison of PCR-based molecualr marker analyses of Musa breeding populations. *Molecular Breeding,* 5: 233-244.

Crouch J.H., Crouch H.K., Tenkouano A. and Ortiz R. 1999b.VNTR-based diversity analyses of 2 x and 4x full-sib Musa hybrids. *EJB Electronic Journal of Biotechnology*, 15: 99–108.

Crouch, J.H., Vuylsteke, D., Ortiz, R. 1998a. Perspectives on the application of biotechnology to assist the genetic enhancement of plantain and banana (*Musa* spp.) *Electronic Journal of Biotechnology*, 1:1-19.

Dahal, G., Hunghes, J. D.A., Lockhart, B.E.L. 1998. Status of banana streak disease in Africa: problems and future research needs. *Integrated Pest Management Review,* 3: 85-97.

Dauwers, A. 2007. Uganda hosts banana trial. *Nature* on –line publication 447: 1042.

De Langhe, E. 2000. Diveristy in the genus *Musa*: its significance and its potential. *Acta Horticulturae*, 540: 89-97.

De Vries, A.C., Ferwerda, J.D., Flach, M. 1967. Choice of food crops inrelation to actual and potential production in the tropics. *Netherlands Journal of Agricultural Science,* 15: 241-248.

D'Hont, A., Paget-Goy, A., Escoute, J., Carreel, F. 2000.The interspecific genome structure of cultivated bananas, *Musa* spp. revealed by genomic DNA *in situ* hybridisation, *Theoretical and Applied Genetics,* 100:177-183.

Doleel, J., Lysák, M.A., Van den houwe, I., Dolezlová, M. and Roux, N. 1997. Use of flow cytometry for rapid ploidy determination in *Musa* species, *InfoMusa,* 6: 6-9.

Doleel, J., Valarik, M., Vrana, J., Lysak, M.A., Hoibova, E., Bartos, J., Gasmanova, N., Doleelova, M., Safao, J., Simkova, H. 2004. Molecular cytogenetics and cytometry of bananas (*Musa* spp.). pp 229-244. In: *Banana improvement: Cellular, Molecualr Biology and Induced Mutations*. Jain S.M. and Swennen, R. (eds.). Science Publishers, Inc. Enfield, NH, USA.

Dolezel, J. and Bartos, J. 2005. Plant DNA flow cytometry and estimation of nuclear genome size. *Annals of Botany*, 95: 99–110.

Donini, B. and Sinnino, 1998. Somaclonal variants and induced mutations in crops improvement. Jain, S.M., Brar, D.S. and Ahloowalia, B.S. (eds.). Kluwer Academic Press, UK.

Escalant, J.V., Sharrock, S., Frison, E. 2002. The genetic improvement of *Musa* using conventional breeding, and modern tools of molecular and cellular biology. pp 1-11. INIBAP Montpellier, France.

FAO, 2002. Agrobiodiversity Conservation and the role of Rural Women: an expert consultation report. Bangkok, Thailand.

FAOSTAT. 1979-2005. Production. Statistics for Crops. United Nations, Food and Agriculture Organisation.

FAO Statistical database, FAOSTAT (2007) http://faostat.fao.org/.

FAO Statistical database, FAOSTAT (2008) http://faostat.fao.org/.

Fauré, S., Noyer, J.L., Horry, J.P., Bakry, F., Lanaud, C., De Leon D.G. 1993. A Molecular marker-based linkage map of diploid bananas (*Musa acuminata*). *Theoretical and Applied Genetics,* 87: 517-526.

Frison, E.A. and Sharrock, S.L. 1998. Banana Streak Virus: a unique virus-Musa interaction? In: *Proceedings. Workshop of the PROMUSA Virology working group*. Montpellier, 1997. Frison, E.A. and Sharrock, S.L. (eds.). INIBAP, Montpellier, France.

Frison, E.A. and Sharrock, S.L. 1999. The economic, nutritional and social importance of bananas in the world. pp 21-35. In: *Bananas and Food Security*. Proceedings. Symposium. Douala, 1998. Picq, C., Fouré, E. and Frison, E. A. (eds.). INIBAP, Montpellier, France.

Fukuda, M., Itoh, H., Ohshima, T., Saidoh, M., Tanaka, A. 2004. New application of ion beams to material, space and biological science and engineering. pp 813-859. In: *Charged particles and photon Interactions with Matter: Chemical, physicochemical, and biological Consequences with Applications*. Hatano, Y and Mozumder, A. (eds.). Marcel Dekker Inc. New York-Basel.

Ganapathi, T.R., Higgs, N.S., Balint-Kurti, P.J., Arntzen, C.J., May, G.D., Van Eck, J.M. 2001. Agrobecterium-mediated transformation of the embryogenic cell suspensions of the banana cultivars Rasthali (AAB). *Plant Cell Reports,* 20: 157-162.

George, E.F. 1993. Plant tissue culture techniques. pp 3-36. In: *Plant propagation by tissue culture*. Part 1: The technology, Exegetics Ltd., Edington, Wilts, England.

Gill, L.S., 1988. Taxonomy of Flowering Plants. Africana FEP Publishers, Onitsha, Nigeria.

Grapin, A., Noyer, J.L., Dambier, D., Carreel, F., Lanaud, C., Baurens, F.C., Lagoda, P.J.L. 1998. Diploid Musa acuminata genetic diversity with Sequence Tagged Microsatellites Sites. *Electrophoresis,* 19: 1374-1380.

Haïcour, R., Assani, A., Matsumoto, K., Guedira, A. 2004. Banana protoplasts. pp 111-125. In: *Banana improvement: Cellular, Molecular Biology and Induced Mutations*. Jain, S.M. and Swennen, R. (eds.). Science Publishers, Inc. Enfield, NH, USA.

Hartl, D.L. and Clark, A.G. 1989. Principles of Population Genetics. Second Edition. Sinauer Associates, Inc., Publishers. Sunderland.

Hautea, D.M., Molina, G.C., Balatero, C.H., Coronado, N.B., Perez, E.B., Alvarez, M.T.H., Canama, A.O., Akuba, R.H., Quilloy, R.B., Frankie, R.B., Caspillo, C.S. 2004.

Analysis of induced mutants of Philippine bananas with molacualr markers. pp 45-57. In: *Banana improvement Cellular, Molecular Biology and Induced Mutations*. Jain, S.M. and Swennen, R. (eds.). Science Publishers, Inc.Enfield, NH, USA.

Heslop-Harrison, J.S. and Schwarzacher, T. 2007. Domestication, Genomics and the future for banana. *Annals of Botany*, 100 (5): 1073-1084.

Horry, J.P. and Jay, M. 1998. Distribution of anthocyanins in wild and cultivated banana varieties. *Phytochemistry,* 27: 2667-2672.

Hwang, S.C. and Ko, W.H. 1988. Mutants of Cavendish banana resistant to race-4 of *Fusarium oxysporumf sp. Cubense*. *Plant Protection Bulletin*, 30: 386-392.

International Plant Genetic Resources Institute (IPGRI), 1999. Descriptors for banana (*Musa* spp). http://198.93.227.125/descript/banwebe.pdf.

Jain S.M. 2001 Tissue culture- derived variation in crop improvement. *Euphytica,* 118: 153-166.

Jarret, R. L. and Litz, R.E. 1986. Isozymes as genetic markers in bananas and plantains. *Euphytica,* 35: 539-549.

Jarret, R.L., Gawel N., Whittemore, A., Sharrock, S. 1992. RFLP-based Phylogeny of Musa species in Papua New Guinea. *Theoretical and Applied Genetics,* 84: 579-584.

Jiang, J., Friebe, B. and Gill, B.S. 1994. Recent advances in alien gene transfer in wheat, *Euphytica*, 73: 199-212.

Jones, D.R. 2000. Introduction to banana, abaca and enset. In: *Diseases of Banana, Abaca and Enset.* Jones, D.R. (ed.). CAB International, Wallingford, UK.

Jones, D.R. and Diekmann, M. 2000. Quarantine and the safe movement of *Musa* germplasm. pp 409-423. In: *Diseases of banana, Abaca and Enset*. Jones D.R. (ed.). CABI Publications, Oxon, United Kingdom.

Jones, M. 1988. Fusing plant protoplasts. *Trends in Biotechnology*, 6: 153-158.

Kaemmer D., Fischer, D., Jarret, R.L., Baurens, F.C., Grapin, A., Dambier, D. 1997. Molecular breeding in the genus *Musa:* A strong case for STMS marker technology. *Euphytica,* 96: 49-63.

Kerbelec, F. 1996. Etablissement d'une technique d'androgenèse pour l'amélioration génétique du bananier (*Musa* spp.) Thèse de Doctorat, Ecole Nationale Agronomique de Rennes.

Kresovich, S., Szewc-Macfadden, A.K., Blick, S., McFerson, J.R. 1995. Adundance and characterisation of simple-sequence repeats (SSRs) isolated from a size-fractionated genomic library of *Brassica napus* L. (rapeseed). *Theoretical and Applied Genetics,* 91: 206-211.

Laisnez, l. 2005. *Musa spp*. in Malawi: Morphological characterisation of local varieties. MSc. Thesis. Departement Biotechnologische Wetenschappen, Landschapsbeheer en Landbouw, Gent, Belgium.

Le, H.T., Armstrong, K.C., Miki, B. 1989. Detection of rye DNA in wheat-rye hybrids and wheat translocation stocks using total genomic DNA as a probe. *Plant Molecular Biology Reporter,* 7: 150-158.

Lo, J.P., Kiew, R., Set, O., Gan, L.H., Gan, Y.Y. 2000. Amplified Fragment Length Polymorphism fingerprinting of 16 banana cultivars (Musa cvs.). *Molecular Phylogentics and Evolution,* 17 (3): 360-366.

May, G.D., et al., 1995. Generation of transgenic banana (*Musa acuminata*) plants via *Agrobacterium*-mediated transformation. *Biotechnology,* 13: 486-492.

Micke, A. 1991. “induced mutations for crop improvement”, Gamma field Symp. No 30 Institute of Radiation Breeding, NIAR, MAFF, Japan. pp. 1-21.

Nair, A.S., Teo, C.H., Schwarzacher, T., Harrison, P.H. 2005. Genome classification of banana cultivars from south India using IRAP markers. *Euphytica*, 144: 285-290.

Ning, S.P., Xu, L.B., Lu, Y., Huang, B.Z., Ge, X.J. 2007. Genome composition and genetic diversity of Musa germplasm from China revealed by PCR-RFLP and SSR markers. *Scientia Horticulturae*, 114: 281-288.

Noyer, J.L., Causse, S., Tomekpe, K., Bouet, A., Baurens, F.C. 2005. A new image of plantain diversity assessed by SSR, AFLP and MSAP markers. *Genetica*, 124: 61-69.

Nsabimana, A. and van Staden, J. 2006. Ploidy investigation of bananas (*Musa* spp.) from the National Banana Germplasm Collection at Rubona-Rwanda by flow cytometry. *South African Journal of Botany,* 72: 302-305.

Nsabimana, A. and Van Staden, J. 2007. Assessment of genetic diversity of highland bananas from the National Banana Germaplasm Collection at Rubona, Rwanda using RAPD markers. *Scientia Horticulturae,* 113: 293-299.

Nwakanma, D.C., Pillay, M., Okoli, B.E., Tenkouano, A. 2003. PCR-RFLP of the ribosomal DNA internal transcribed spacers (ITS) provides markers for the A and B genomes in *Musa* L. *Theoretical and Applied Genetics,* 108: 154-159.

Oh, T.J., Cullis, M.A., Kunert, K., Engelborghs, I., Swennen, R., Cullis, C.A. 2007. Genomic changes associated with somaclonal variation in banana (*Musa* spp.). *Physiologia Plantarum,* 129:766–774.

Oriero, C.E., Odunola, O.A., Lokko, Y., Ingelbrecht, I. 2006. Analysis of B-genome derived simple sequences repeat (SSR) marker in *Musa* spp. *African Journal of Biotechnology*, 5 (2): 126-128.

Ortiz R, and Vuylsteke D. 1995. Factors influencing seed set in triploid *Musa* spp. L. and production of euploid hybrids. *Annals of Botany*, 75: 151–155.

Ortiz, R. 1997. Genetic and phenotypic correlations in plantain-banana euploid hybrids. *Plant Breeding*, 116:487-491.

Ortiz, R. and Vuylsteke, D. 1994. Genetics of apical dominance in plantain (Musa spp., AAB group) and improvement of suckering behaviour. *Journal of the American Society for Horticultural Science*, 119 (5): 1050-1053.

Ortiz, R. and Vuylsteke, D. 1996. Recent advances in *Musa* genetics, breeding and biotechnology. *Plant Breeding Abstracts,* 66: 1355-1363.

Ortiz, R. and Vuylsteke, D. 1998. PITA-14: A black Sigatoka-resistant tetraploid plantain hybrid with virus tolerance. *Hortscience,* 33 (2): 360-361.

Panis, B., Van Wauwe, A. and Swennen, R. 1993. Plant regeneration through direct somatic embryogenesis from protoplasts of banana (*Musa* spp.), *Plant Cell Reports,* 12: 403-407.

Panis, B., Strosses, H., Remy, S., Sagi, L., Swennen, R. 2004. Cryopreservation of banana tissues: support for germplasm conservation and banana improvement. pp 13-21. In: *Banana improvement: Cellular, Molecualr Biology and Induced Mutations*. Jain S.M. and Swennen, R. (eds.) Science Publishers, Inc. Enfield, NH, USA.

Pillay, M., Hartman, J., Dimkpa, C., Makumbi, D. 2003. Establishing the genome of 'Sukali Ndizi'. *African Crop Science Journal*, 11 (2): 119-124.

Pillay, M., Ogundiwin, E., Nwakanma, D.C., Ude, G. 2001. Analysis of genetic diversity and relationships in East African banana germplasm. *Theoretical and Applied Genetics,* 102: 965-970. Springer-Verlag.

Pillay, M., Tenkouano, A., Ude, G., Ortiz, R. 2004. Molecular characterisation of genomes in *Musa* and its applications. pp 271-286. In: *Banana Improvement: Cellular, Molecular Biology, and Induced Mutations*. Jain, S.M. and Swennen, R. (eds.). Science Publishers. Enfied, NH, USA.

Pollefeys, P., Sharrock, S, Arnaud, E. 2004. Preliminary analysis of the literature on the distribution of wild Musa species using MGIS and DIVA-GIS Montpellier, France, INIBAP.

Raboin, L.M., Carreel, F., Noyer, J.L., Baurens, F.C., Horry, J.P., Bakry, F., Tezenas DuMontel, H., Ganry, J., Lanaud, C., Lagoda, P.J.L. 2005. Diploid ancestors of triploid export banana cultivars: molecular identification of 2*n* restitution gamete donors and n gamete donors. *Molecular Breeding*, 16:333–341.

Reddy, P.M., Saral, N., Siddiq, E.A. 2002. Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. *Euphytica,* 128: 9-17.

Remy, S., Buyens, B.P., Cammue, B.P.A., Swennen, R., Sagi, L. 1998a. Production of transgenic banana plants expressing antifungal proteins. pp. 433-436. In *Acta Horticulturae* 490. Sauco, V.G. (ed.). International Society for Horticultural Science, Leuven, Belgium.

Reyes-Borja, W.O., Sotomayor, I., Garzon, I., Vera, D., Cedeno, M., Castillo, B., Tanaka, A., Hase, Y., Sekozawa, Y., Sugaya, S., Gemma, H. 2007 Alteration of resistance to black sigatoka (*Mycosphaerella fijiensis* Morelet) banana by in vitro irradiation using carbon ion-beam. *Plant Biotechnology* 24: 349-353.

Roux, N.S. 2004. Mutation induction in *Musa*–review. pp 23-32. In: *Banana improvement: Cellular, Molecualr Biology and Induced Mutations*. Jain S.M. and Swennen, R. (eds.). Science Publishers, Inc. Enfield, NH, USA,

Roux, N.S., Toloza, A., Dolezel, J., Panis, B. 2004. Usefulness of embryogenic cell suspension cultures for the induction and selection of mutants in *Musa* spp. pp 33-43. In *Banana improvement Cellular, Molecular Biology and Induced Mutations*. Jain, S.M. and Swennen, R. (eds.). Science Publishers, Inc.Enfield, NH, USA.

Rowe, P. and Rosales, F. 1993. Diploid breeding at FHIA and the development of Goldfinger. Info*Musa*, 2: 9-11.

Sagi, L., Panis, B., Remy, S., Schoofs, H., De Smet, K., Swennen, R., Cammue, B. 1995. Genetic transformation of banana (*Musa spp*.) via particle bombardment. *Biotechnology*, 13: 481-485.

Sharrock, S. 1989. Collecting *Musa* in Papua New Guinea. pp 140-157. In: *Identification of Genetic Diversity in the Genus Musa*. Jarret, R., (ed.), INIBAP, Los Banos, Philippines.

Sharrock, S. 1997. INIBAP annual report 1996. Montpellier. France. pp 42-44.

Shepherd, K. and Ferreira, F.R. 1984. The PNG Biological Foundation Banana Collection at Laloki, Port Moresby, Papua New Guinea, South-east Asia. *Newsletter*, 8: 28-34.

Shibata, D. and Liu, Y.G. 2000. Agrobeacterium-mediated plant transformation with large DNA fragments. *Trends Plant Science*, 5: 354-357.

Simmonds, N.W. 1962. The Evolution of the bananas. Tropical Science Series, Longmans, London.

Simmonds, N.W. 1966. Bananas. 2nd ed. Tropical Agricultural Series, Longman, New York.

Simmonds, N.W. and Shepherd, K. 1955. The taxonomy and origins of the cultivated bananas. *The Botanical Journal of the Linnean Society of London,* 55: 302-312.

Simmons, M. P. and Webb, C.T. 2006. Quantification of the success of phylogenic inference in simulations. *Cladistics*, 22: 249-255.

Simmons, M.P., Zhang, L.B., Webb. C.T., Muller, K. 2007. A penalty of using anonymous dominant markers (AFLPs, ISSRs and RAPDs) for phylogenetic inference. *Molecular Phylogenetic Evolution*, 42: 528-542.

Smith, J.S.C. and Smith, O.S. 1989. The description and assessment of distances between inbred lines of maize. II. The utility of morphological, biochemical and genetic descriptors and a scheme for testing of distinctiveness between inbred lines. *Maydica*, 34: 151-161.

Sotto, R.C. and Rabara, R.C. 2000. Morphological diversity of *Musa balbisiana* Colla in the Philippines. *InfoMusa*, 9: 28-30.

Ssebuliba, R., Talengera, D., Makumbi, D., Namanya, P., Tenkouana, A., Tushemereirwe, W., Pillay, M. 2005. Reproductive effeciency an dbreeding

potential of East African highland (Musa AAA-EA) bananas, Field crops Research. Http://www.sciencedirect.com/science/journals.

Ssebuliba, R., Talengera, D., Makumbi, D., Namanya, P., Tenkouno, A., Tushemereirwe, W., Pillay, M. 2006. Reproductive efficiency and breeding potential of East African highland (*Musa* AAA-EA) bananas. *Field Crops Research*, 95: 250-255.

Strosse, H., Van den Houwe, I., Panis, B. 2004. Banana cell and tissue culture-review. pp 23-32. In: *Banana improvement: Cellular, Molecualr Biology and Induced Mutations*. Jain S.M. and Swennen, R. (eds.). Science Publishers, Inc. Enfield, NH, USA.

Subramaniam, S., Maziah, M., Sariah, M., Puad, M.P., Xavier, R. 2006. Bioassay method for testing Fusarium wilt disease tolerance in transgenic banana. *Scientia Horticulturae,* 108: 378-389.

Swennen, R. and Vuylsteke, D. 1991. Bananas in Africa: diversity, uses and prospects for improvement. In: Ng, N.Q. Perrino, P. Attere, F. and Zedan, H. (eds.). *Crop Genetic Resources of Africa,* 2: 151-159.

Swennen, R., Vuylsteke, D. and Hahn, S. K., 1989. Combating the black Sigatoka threat to plantains. *IITA Research Brief,* 9 (2): 2-4.

Tripathi, L. 2003. Genetic engineering for improvement of *Musa* production in Africa. *African Journal of Biotechnology,* 2 (12): 503-508

Tripathi, L., Hughes, Jd'A., Tenkouano, A. 2002. Production of transgenic *Musa* varieties for sub-Saharan African. International Symposium on the Molecular and Cellular Biology of Banana. Http://www.promusa.org/publications/leuven-abstracts.pdf.

Ude, G., Pillay, M., Nkwakanama, D., Tenkouano, A. 2002. Genetic diversity in Musa acuminata Colla and Musa balbisiana Colla and some of their natural hybrids using AFLP markers. *Theoretical and Applied Genetics,* 104: 1246-1252.

Uma, S., Siva, S.A., Saraswathi, M. S., Durai, P., Sharma, T.V.R.S., Singh, D.B., Selvarajan, R., Sathiamoorthy, S. 2005. Studies on the origin and diversification of Indian wild banana (Musa balbisiana) using arbitrarily amplified DNA markers. *Journal of Horticultural Science Biotechnol*ogy, 80: 575-580.

Uma, S., Siva, S.A., Saraswathi, M.S., Manickavasagam, M., Durai, P. Selvarajan, R., Sathiamoorthy, S. 2006.Variation and interspecific relationships in Indian wild *Musa*

balbisiana (BB) population as evidenced by random amplified polymorphic DNA. *Genetic Resources and Crop Evolution*, 53: 349-355.

Valarik, M., Simkova, H., Hribova, E., Safar, J., Dolezelova, M., Dolezel, J. 2002. Isolation characterisation and chromosome localization of repetitive DNA sequeunces in bananas (*Musa* spp.). *Chromosome Research*, 10: 89-100.

Van Harten, A.M. 1998. Mutation Breeding: Theory and Pratical Applications, Cambridge University Press, Cambridge.

Venkatachalam, L., Sreedhar, R.V., Bhagyalakshmi, N. 2008. The use of genetic markers for detecting DNA polymorphism, genotypes identification and phylogentic relationships among banana cultivars. *Molecular Phylogenetics and Evolution,* 47: 974-985.

Viljoen, A., Kunert, K., Kiggundu, A. and Escalant, J.V. 2004. Bitechnology for sustainable banana and plantain production in Africa: the South African contribution. *South African Journal of Botany*. 70 (1): 67-74

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Pelema, J., Kuiper, M. and Zabeau, M. 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acid Research,* 23: 4407-4414.

Vrydaghs, L and De Langhe, E. 2003. Phytoliths: An opportunity to rewrite history. pp 14-17. In: INIBAP Annual Report 200. Montpellier, France.

Vuylsteke, D. and Swennen, R. 1992. Biotechnology approaches to plantain and banana improvement at IITA. pp 143-150. In: *Biotechnology Enhancing Research on Tropical Crops in Africa*. Thottappily, G., Manti, L.M., Mohan Raj, D.R. and Moore, A.W. (eds.) CTA/IITA, Ibadan, Nigeria.

Vuylsteke, D., Ortiz, R., Ferris, R.S.B., Crouch, J.H. 1997. Plantain improvement. *Plant Breeding Review,* 14: 267-320.

Vuylsteke, D., Ortiz, R., Ferris, S., Swennen, R. 1995. PITA-9: A black sigatoka-resistant hybrid from the 'fals-horn' plantain genepool. *HortScience,* 30: 395-397.

Vuylsteke, D., Swennen, R., Ortiz, R. 1993a. Development and performance of black sigatoka resistant hybrids of plantains (*Musa spp*., AAB group). *Euphytica*, 65: 33-42.

Vuylsteke, D.R., Swennen, R.L.,Ortiz, R. 1993b. Registration of 14 improved tropical *Musa* plantain hybrids with black sigatoka resistance. *HortScience* 28: 957-959.

Wan. Y., Watanabe, J.A., Yi, S.S., Htaik, T., Win, K., Yamanaka, S., Nakamura, I., Watanabe, K.N. 2005. Assessment of Genetic diversity among the major Myanmar banana landraces. *Breeding Science*, 55: 365-369.

Wei, J. and Zhou, X.M. 2008. An improved electrochemiluminescence polymerase chainreaction method for the detection of Fusarium wilts. *Chinses Chemical Letters*, 19: 959-961.

Williams, J.G.K., et al., 1990. DNA polymorphisms amplified by arbitrary primers are useful genetic markers, *Nucleic Acid Research,* 18: 6531-6535.

Wilson, S. M. 1988. Phytolith analysis at Kuk, an early agricultural site in Papua New Guinea. *Archaeology in Oceania*, 20: 90-96.

Wong, C., Kiew, R., Argent, G., Ohn, S., Lee, S.K., Gan Y.Y. 2002. Assessment of the validity of the sections in Musa (Musaceae) using AFLP. *Annals of Botany*, 90: 231-238.

Xu, L., Li, Z.Y., Du, Z.J. 2006. A pilose fruit mutant in banana (Musa spp. 'Williams'). *Scientia Horticulturae*, 107: 315-318.

Yonemoto, Y., Chowdhury, A.K., Kato, H., Macha, M.M. 2006. Cultivars indentification and their genetic relationships in *Dimocarpus longan* subspecies based on RAPD markers. *Scientia Horticulturae*, 109: 147-152.