SYNTHETIC DIPLOID HYBRIDS: RESPONSE TO BLACK SIGATOKA, THEIR MALE FERTILITY AND RELATEDNESS TO EAST AFRICAN HIGHLAND BANANAS.

SSALI REUBEN TENDO

A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MSC .CROP SCIENCE OF MAKERERE UNIVERSITY.

DECLARATION

I, SSALI Reuben Tendo, certify that this is my original work and that it has not been presented for examination for any award in any University.

DATE.....29/1/2007.....

Signature.....

SSALI REUBEN TENDO

This thesis has been submitted for examination with my approval as the University supervisor.

Signed.....

Date.....2-2-07.....

PROF. EM. RUBAIHAYO PATRICK

Dedication

I dedicate this thesis to my Parents Mr. and Mrs. Ssali of Kikonda, Bamunanika and to my mentor and friend Dr.W.K.Mugerwa and his family.

Acknowledgement

I am grateful to the Rockefeller Foundation which provided funds for this study through the National Banana Research Program-NARO.

My sincere thanks go to Prof. Em. P. Rubaihayo, Dept. of Crop Science, Makerere University, Dr.Micheal Pillay of the International Institute of Tropical Agriculture(IITA-Uganda) and Dr. W .K. Tushemereirwe, Head of National Banana Research Program, under whose leadership and supervision the investigations were conducted. Your guidance and valuable criticism right from the inception of the study to the end are highly appreciated. Special thanks go to Dr. Michael Pillay for allowing me to use the molecular laboratory at Namulonge for this study.

My appreciations also go to Messers L.Turyagenda, P.Komucunguzi and M.Nyine all staff of the molecular laboratory at Namulonge for their invaluable contributions and encouragement.
It is impossible to forget the input of my colleagues at the National banana research programme at Kawanda especially the breeding team, Batte Michael, Priver Namanya, Mwaka Henry, Tindamanyire Jimmy, Ajambo Joy, Alice Bukenya, Serwadda Stephen, Mugabo Sam and Betty Namagembe. It is always nice working with you. Thank you very much for your invaluable contributions and encouragement.

I will not forget the input of Mr. Ragama Phillip who not only helped me to analyse my data but also taught and encouraged me to do it myself.

Lastly but not least, I acknowledge my family members who comforted and encouraged me during this study.

Finally, I will forever be grateful to the almighty God, who has enabled me to successfully complete this study. O God, your love is better than life.

Table of Contents

List of Table

List of Figures

ABSTRACT

Banana diploids are used both as male parents and materials for genetic studies in breeding programmes. In this study a range of synthetic diploid hybrids were assessed for their response to black Sigatoka, male fertility and their genetic relationship to East African Highland banana, so as to evaluate the potential of using them in the improvement of the East African Highland Bananas. Synthetic diploid progeny obtained from 2x-2x, 3x-2x and 4x-2x crosses, were evaluated along with their parents in a completely randomized design for response to black Sigatoka and were rated for the production and viability of pollen. Genomic DNA isolated from the different genotypes was used in RAPDs analysis to estimate the genetic distances among the genotypes. Genotypes '2858K-8', '3550K-1', 'OPP2-861', 'OPP1-861', '12468S-3' and '3162K-3' were found to show hypersensitive response to black Sigatoka. Seven synthetic diploid banana genotypes'12506S-29', 'OPP2-861', '3162K-3', '3162K-1', '3162K-5' and '3146K-2' whose pollen rating was not significantly($p \leq 0.05$) different from that of the reference wild diploid banana were considered male fertile. All tested synthetic diploid hybrids except '1537K-1', '11025S-1' and '12515S-2' were found to be genetically closer to '*Calcutta4*', than to '*Enyeru*' the East African Highland Banana, indicating the potential to generate heterotic hybrids when crossed with East African Highland Bananas.

CHAPTER 1

Introduction

1.1 Background

Bananas and plantains (*Musa* spp.) are the fourth most important global food commodity after rice, wheat and maize (FAOSTAT, 2003). They are cultivated in over 100 countries covering about 10 million hectares, with an annual production of 97 million tons (FAOSTAT, 2003). In some countries, especially in Latin America and the Caribbean bananas are a major export commodity (FAOSTAT, 2003). However bananas are far more important as a starchy staple in local food economies (Stover and Simmonds, 1987). About 90% of the total world banana production is used as food for domestic consumption for both rural and urban consumers (INIBAP, 1999)

In Africa, bananas and plantain provide more than 25% of the energy requirements for about 70 million people of whom 20 million are from East Africa alone (Sharrock and Frison, 1998). Uganda ranks second after India in the world in banana production with an annual output of 9.84 million tons which accounts for 11.18% of the world's total production (INIBAP, 1999).

As a starchy staple food crop, bananas and plantain rank third in terms of total world production after cassava and potato (Chandelier, 1996). However, bananas and plantains are priced higher than either cassava or potato in many countries (Sharrock and Frison, 1998). Bananas are grown in a wide range of environments and produce fruit all year-round, thus bridging the 'hunger-gap' between other crop harvests (Sharrock and Frison, 1998). When grown under perennial production systems, they maintain soil cover throughout the year and the biomass is used for mulch and soil fertility. In mixed farming systems, bananas are used as ground shade and a nurse-crop for a range of shade-loving crops including cocoa, coffee and black pepper (INIBAP, 1999).

Ripe bananas are one of most rapidly digested foods, providing readily available energy and are thus recommended for people who require a lot of glucose to maintain adequate levels of muscle action (Sharrock and Lusty, 2000). Ripe bananas are also used in nutrition of infants and to avert various disorders like peptic ulcers, infant diarrhea and coeliac disease (Sharrock and Lusty, 2000). The banana is a good source of major elements like potassium, magnesium, phosphorous, calcium, iron and vitamins A, B_6 and C (Marriot and Lancaster, 1983). Other uses of bananas and plantain include production of juice, beers, wine and fibre which is used in the production of handicraft and shelter (INIBAP, 2000).

1.2 Problem

Unfortunately the actual yield potential of bananas in Uganda is usually not realized due to high pest and disease pressure, soil fertility decline, drought and poor management. For instance Van Asten *et al.*, (2004) reported smaller actual yields of 3-5 t $ha^{-1}yr^{-1}$ in

East African highland bananas in Uganda compared to the potential yield of 70 t $ha^{-1}yr^{-1}$ (Purseglove 1985). Banana weevils and a complex of parasitic nematodes are the major pests while fungal leaf spots, and banana streak virus are the most important diseases (Karamura, 1999) and recently banana *Xanthomonas* wilt has been reported in more than 33 districts of Uganda (http://www.banana.go.ug/disease/disease distributions.html).

Black Sigatoka, a wind-borne fungal disease caused by *Mycosphaerella fijiensis* Morelet, is considered the most important disease of bananas and plantains worldwide (Jeger *et al.*, 1995). Though it does not usually kill the plant, it causes heavy defoliation which severely suppresses fruit filling leading to reduced bunch weight accounting for about 37-50% yield loss in East African highland bananas (Tushemereirwe, 1996). Enhanced plant nutrition and removal of diseased leaves to reduce the inoculum are commonly used as control measures but host plant resistance is the most convenient and effective intervention to reduce yield loss at a low cost to the farmer (Jeger *et al.*, 1995).

The genetic improvement of bananas to produce cultivars with host plant resistance and other desirable agronomic traits is complicated by the long duration of 18 months for the crop to establish from seed to seed (Pillay *et al.*, 2002). Also the high cost and space ($9m^2$ per mat) requirements of bananas are limitations to the banana breeders (Rowe, 1984, Hilber 1997). The complex banana genetics, low genetic variability, polyploidy and the low levels of female and /or male fertility in most widely grown triploid clones once made banana breeding to be considered an impossible venture (Rowe 1984, Hilber 1997, Tezenas du Montcel *et al.*, 1996, Pillay *et al.*, 2002). Fortunately the ability of some triploid banana clones to produce seeds when pollinated has made banana improvement

possible (Hilber, 1997). For instance Ssebuliba (2002) reported 37 triploid female fertile breeding lines in East African Highland bananas. This implies that the improvement of East African bananas would rely on limited triploid female fertile parental lines.

1.3 Justification

The conventional breeding of bananas involves crossing the female parents usually triploid (3x) with diploid (2x) bananas as males (Ortiz *et al.*, 1995). Wild and/or cultivated diploid *Musa spp*, which produce viable pollen are used as male parents in breeding programmes.

Fortunately there is still a large variability among the diploids which is expressed in their morphological and organoleptic characteristics (Tezenas du Montcel *et al.*, 1996). Most wild diploid male and female fertile and their variability offers a large genetic base (Tezenas du Montcel *et al.*, 1996).

Furthermore, inheritance studies suggest that most traits of economic importance are more predictably inherited from the diploid male parents than from female parents with a higher ploidy status (Rowe and Rosales, 1990, Ortiz *et al.*, 1995). The diploid bananas can be a source of many desirable alleles. However diploid bananas can also pass on undesirable traits to the resultant progenies. For instance most wild diploid have eating characteristics which may not be acceptable by banana consumers due to their inferior organoleptic characteristics like off flavors, pulp color, non-parthenocarpic fruits and excess sap in their fruits (Hilber, 1997). Thus there is need to improve diploid bananas through phenotypic recurrent selection, so as to eliminate the deleterious alleles and enhance the accumulation of favorable alleles. Ploidy manipulations (i.e. the scaling up or down the number of chromosomes) in banana breeding offers an opportunity to

improve the crop at both the diploid and tetraploid levels where most of the clones are both male and female fertile. Furthermore, the disomic inheritance of diploid bananas makes the genetic analysis easier which facilitates and accelerates breeding by using information obtained from compatibility, quantitative genetics and heritability studies (Tezenas du Montcel *et al.*, 1996).

The synthesis of diploid hybrids from 4x-2x and 3x-2x crosses in improving East African Highland bananas increases the range of male parents (Vuylsteke *et al.*, 1997). These synthetic diploid hybrid bananas may combine both desirable and undesirable traits from their parents. For instance inferior bunch characters that are difficult to overcome were reported in *Musa ssp. burmannica* progenies when used as a source of resistance to black Sigatoka (Stover and Simmonds, 1987). The potential of using these synthetic diploid hybrids as male parents depends on their ability to produce viable pollen. Pollen production in *Musa spp.* depends mainly on genetic factors although environmental factors have been highlighted (Krishnakumar *et al.*, 1998).

Falconer and Mackay (1996) noted that the potential for a crop to respond to breeding programme depends on the nature and magnitude of genetic variability. Progress in improving East African Highland bananas to obtain high-yielding, pest and disease resistant hybrids will depend on identifying, and utilizing genes and gene combinations that will generate progenies with either enhanced heterosis and/ or genetic gain. This makes the precise selection of both male and female parents a critical step for determining success. Panther and Allen (1995) suggested that the use of genetic

relationships among individuals would increase the accuracy of predicting hybrid performance. Estimates of the genetic relationships among the synthetic diploid bananas (which are prospective male parents) with the East African Highland bananas (the prospective female parents) will provide essential information for streamlining the use of the diploids in the hybridization strategies.

1.4 Aim

The main aim of this study was to investigate synthetic diploid hybrids' response to black Sigatoka, their male fertility and relatedness to female fertile East African Highland bananas.

The specific objectives of the study were

1. To identify synthetic diploid hybrids with resistance to black Sigatoka.
2. To asses the quantity and quality of pollen produced by diploid hybrids.
3. To estimate the genetic relatedness of synthetic diploid hybrids and female fertile East African Highland bananas.

CHAPTER 2

Literature Review

2.1.0 Black Sigatoka

2.1.1Assessing banana genotypes for black Sigatoka response

The damage resulting from disease can be evaluated accurately by measuring its prevalence and intensity (Stover and Dickson, 1970). Several methods have been used to assess the response of banana genotypes to the black Sigatoka pathogen; these include measuring disease development time, the number of standing leaves(**NSL**) and the youngest leaf spotted(**yls**) at flowering (Vakili, 1968), calculating an index of the non-spotted leaf(**INSL**) (Ortiz *et al.,* 1997) and calculating the disease severity index (Gauhl,1994). The disease severity method estimates the amount of leaf area affected by the Sigatoka pathogens and can be expressed in percentages or grades. While the index of the non-spotted leaf measures the percentage of photosynthetic tissue available at flowering before fruit filling.

2.1.3 Black Sigatoka resistance in *Musa*_hybrids

The inheritance of black Sigatoka resistance in plantain–banana hybrids has been reported to be controlled by three independent loci with recessive/ additive effects. One is a major recessive gene ($\mathbf{bs_1}$) and along with two additive minor genes ($\mathbf{bsr_2}$ and $\mathbf{bsr_3}$) with dosage effects (Ortiz and Vuylsteke, 1994). The susceptible banana clones have the dominant gene (**BS**). This implies that the response of bananas to the black Sigatoka pathogen, *Mycosphaerella fijiensis,* is due to the interaction of these genes.

Thus four levels of black Sigatoka response are commonly observed in bananas (Table 1).

The effect of major recessive genes on a quantitative trait like *black Sigatoka* resistance variation can be explained by linkage or pleitropism and may act in additive or non additive manner (Falconer and Mackay, 1996). The non additive gene interaction can either be intralocus or between loci (Falconer and Mackay, 1996). In bananas a clear dosage effect has been observed for black Sigatoka response in favor of higher ploidy levels due to tri-and tetra-allelic interactions (Peloquin and Ortiz, 1992, Ortiz and Vuylsteke, 1994). This implies that a higher frequency of tetraploids have black Sigatoka resistance than either diploids or triploids. This is explained by the fact that higher ploidy levels allow the accumulation of the favorable recessive alleles for black Sigatoka resistance (e.g. **$BSbs_1bsr_2$**-in triploids clones **or $BSbs_1bsr_2bsr_3$** in tetraploid clones), which overcomes the action of the dominant susceptibility allele (**BS**). Unfortunately, the tetraploid bananas are highly male and female fertile and will readily set seed subsequently reducing fruit quality, with the exception of cases where self incompatibility occurs (Shepherd, 1999). For instance tetraploid hybrids derived from East African highland bananas have been reported to be highly female fertile (Ssebuliba *et al*, 2005).

Therefore diploid banana hybrids with resistance to black Sigatoka may be obtained when the resistance genes (**bs_1, bsr_2, and/ or bsr_3** are incorporated or preserved); but the ploidy manipulations (i.e. the scaling up or down the number of chromosomes) in banana breeding may not necessarily allow this (Ortiz 1997).

Table 1: Describing the response of banana genotypes to black Sigatoka resistance

Reaction	Description	Genetics of host plant response
Highly resistant	Leaf symptoms restricted because disease development does not reach final stages of necrosis (hypersensitivity)	**At the diploid level** ▪ Homozygosity of the major recessive gene (**bs_1**). **At the tetraploid level** ▪ Action of two major recessive allele (**bs_1**) with two additive genes **bsr_2** or **bsr_3**.
Partially resistant	Disease development slow, but can reach final necrotic stages; leaf spots do not coalesce (horizontal and durable resistance)	**At diploid level** ▪ Action of the major recessive gene (**bs_1**) and one of the 2 additive genes (**bs_1bsr_2 or bs_1bsr_3**). **At the tetraploid level** ▪ Action of one major recessive gene (**bs_1**) or ▪ Action of the major recessive gene (**bs_1**) with one or two of the minor additive genes (**bsr_2** or **bsr_3**).
Less susceptible	Complete disease development, but slower than in susceptible cultivars.	**At diploid level** ▪ Action of only the major recessive gene (**bs_1**) With **BS**(dominant allele for susceptibility) **At the tetraploid level** ▪ Action of only one of the minor recessive genes (**bsr_2** or **bsr_3**).
Susceptible	Fast and complete disease development.	**At diploid level** ▪ Action of only one of the minor recessive genes (**bsr_2** or **bsr_3**). With **BS**(dominant allele for susceptibility) **At the tetraploid level** ▪ Action of only one of the minor recessive genes (**bsr_2** or **bsr_3**).

Source: *Adapted from (Vuylsteke et al.,, 1997) IITA's Musa gene bank.*

2.2.0 Male fertility

The quality and quantity of pollen grains are important attributes to be considered in the selection of a male parent (Marrewijk, 1994; Dumpe and Ortiz, 1996). Both genetic and environmental factors have been observed to affect both the quality and quantity of pollen grains in banana genotypes (Krishnakumar *et al.,* 1992). Pollen viability and out put have been reported to vary along the rachis in some *Musa* cultivars including East African highland bananas (Sathiamoorthy, 1980, Krishnakumar *et al.,*1992, Ssebuliba,2002). This means that efficient artificial pollinations would depend on the position of the node from which pollen grains are obtained. However, Ssebuliba (2002) reported that the node number did not contribute significantly to variation in pollen stainability in wild diploid '*Calcutta4*' and diploid hybrids, although none of these diploid hybrids had East African Highland bananas in their pedigree. In the case of '*Pisang lilin*' the nodal position was found to be highly significant (Ssebuliba, 2002).

Pollen stainability is an *in vitro* method that tests for the viability of the pollen grains (Krishnakumar *et al.,* 1992). The rationale of pollen staining is to test the viability of the pollen since the extent of pollen abortion in the early development stages can not be estimated by observation (Fortescue and Turner, 2004). The effectiveness of pollen may be affected by pollen hydration, pollen germination, and pollen tube penetration through the stigma and growth through the style to the ovary (Shepherd, 1960). This implies that the pollen stainability tests indicate the potential of the cultivar to produce viable pollen - apparent male fertility (Krishnakumar *et al.,* 1992, O'Brien and McCully, 1981).

Several methods have been used in pollen staining to estimate pollen viability; these include aniline blue with lactophenol stain which acts as an acid dye and binds with molecules in the cytoplasm to stain fertile grains to dark blue without staining the sterile ones (Ashagari2000), acetocarmine jelly which acts as a basic dye binding with nucleic acids in the fertile pollen (Ashagari, 2000), Alexander's stain, contains malchite green which stains cellulose in pollen and acid fushin which stains pollen protoplasm (Alexander,1969) and the Iodine Potassium Iodide ($\mathbf{IKI_2}$) stain which instantly stains starch granules in the pollen endosperm (Scott 2001). The Iodine Potassium Iodide ($\mathbf{IKI_2}$) distinguishes the viability of the pollen grains based on the presence or absence of starch granules. The Iodine Potassium Iodide ($\mathbf{IKI_2}$) stain was more suitable for banana pollen which grows across long tubular flowers with long styles hence require high energy for the pollen tube to grow down the long styles for successful fertilization (Shepherd, 1960). The Iodine Potassium Iodide ($\mathbf{IKI_2}$) stain was also reported to be easier to use because the reaction occurs instantly (Scott, 2001).

Dumpe and Ortiz (1996) observed that the diploids had on average the highest pollen stainability as compared to the triploids and tetraploids with the stainability of *'Calcutta 4'* being almost 100%. Tenkouano *et al.*(1998) obtained similar results with pollen stainability of 49.1% in triploids, 90.1% in diploids and 90.0% in tetraploids. The germination of the pollen is a sure indication of the actual fertility of the pollen grains (Sharma and Sharma, 1972). The chief factors necessary for the artificial germination of pollen tubes are temperature, humidity and culture media. The underlying principle is to

provide a condition closely approaching the normal secretion of the stigma (Johnri and Vasil, 1961). The optimum temperature for germination is 20°C and the optimum humidity approaches saturation (Sharma and Sharma, 1972).

The amount of grains produced by banana genotypes is an important factor in enhancing the germination rate of pollen (Marrewijk, 1994). For instance pollen germinations both on culture medium and stigma have indicated that the aggregation of pollen grains has a stimulatory effect on the germination and length of the pollen tubes (Posonen and Kapyla,1998). This phenomenon which is referred to as the 'population effect'; has been attributed to extra-cellular proteins and enzymes whose effective concentration occurs only when the pollen grains are aggregated (Marrewijk, 1994). In addition to population effect, high quantities of pollen have been reported to contain high percentages of viable pollen (Dumpe and Ortiz, 1996). An edible banana '*Maraw*' with 25% pollen stainability at moderate production levels was a functional male parent that produced true viable seeds when its pollen was used in crosses with '*Mbi Egome*' as female triploid plantain. On the other hand, '*Tuu Gia*' had scanty but good pollen (75% stainability) and did not yield any seed despite its consecutive crossing to several female fertile accessions over a period of one year (Dumpe and Ortiz, 1996). Ortiz *et al.* (1998) also obtained higher numbers of seeds with *'Calcutta 4'* (the parent with the highest amount of pollen) than with '*Pisang lilin*' and '*Galeo*' (cultivated diploids with lower pollen quantities). In addition to the population effect, high quantities of pollen have been reported to contain high percentages of viable pollen as Dumpe and Ortiz (1996) obtained a significant correlation between pollen production and stainability in both landraces and hybrids. Low

quantity pollen in '*Solanum*' species also showed a significantly lower percentage of 'good' pollen than did both medium and high pollen amounts (Janssen and Hermsen, 1976).

2.3.0 Genetic relatedness

Progress in breeding for high yielding and disease resistant *Musa* hybrids depends on the ability to detect and access gene combinations most likely to produce enhanced yields and disease resistance (Tenkouano *et al.,* 1999). This in turn requires effective selection of prospective male and female parents. Therefore knowledge of the genetic diversity in available germplasm is fundamental for the optimum designing of breeding programs, the efficiency of which can be increased if superior crosses are pre-established. This *apriori* choice of parents is based on the fact that heterosis is a relative measure of two generations-the parental and the progeny (Falconer 1989). Panther and Allen (1995) suggested that the use of genetic relationships among individuals would increase the accuracy of predicting hybrid performance.

Different methodologies are available to investigate genetic relationships; analysis based on morphological, biochemical and pedigree data can be used for this purpose. However the morphological characteristics are limited mainly by environmental influence and therefore do not always express genetic relationships and the differences revealed may not be comprehensible in terms of genetic distances (Smith & Smith, 1989). Data from molecular markers like RAPDs AFLPs, SSRs and RFLPs overcome most of these limitations (Oliveira *et al.*, 2004). Characteristics such as 1) an almost unlimited number

of markers 2) absence of environmental influence 3) a great number of polymorphic loci 4) access to contributions of both parents and 5) a possibility of comparing genotypes based on the DNA, make molecular markers a powerful tool for genetic diversity estimates (Oliveira *et al.*, 2004). DNA marker systems based on Polymerase chain reaction are particularly suited to this application in plant breeding (Rafalski *et al.*, 1995). Among these systems Random Amplified polymorphic DNA (RAPDs) has been proven useful in assessing genetic diversity in *Musa* (Ude *et al.*, 2003, Pillay *et al.*, 2001). One of the advantages of the RAPD method is that the arbitrarily designed primers can potentially anneal to homologous sequences in the entire genome, providing greater opportunities to uncover polymorphic regions (Williams *et al.*, 1990)

The genetic distance which is a measure of the relatedness of genotypes, can be used as a selection criterion for parental genotypes based on dissimilarity (Panther and Allen 1995). Most *Musa* genotypes are genetically heterozygous because they were derived from natural hybridization and therefore their breeding value may not be predictably reflected by their own performance (Ortiz and Vuylsteke 1994).

Pillay *et al* (2001) demonstrated the use of RAPD markers to readily dissect genetic differences between closely related 29 highland bananas and provided a basis for the selection of parents for improvement of this germplasm. In the same study RAPDs showed that the highland bananas are closely related with a narrow genetic base. Nevertheless, there were sufficient RAPD polymorphisms that were collectively useful in distinguishing the cultivars. The dendrogram was divisible into a major cluster composed of all the AAA highland banana cultivars and *'Agbagba'* (AAB) and a minor cluster

consisting of *'Kisubi'* (AB), *'Kamaramasenge'* (AB) and '*Calcutta 4*' (AA). Several subgroups were recognized within the major cluster. However the RAPD data did not separate beer and cooking banana cultivars. Damasco *et al.* (1996) and Pancholi *et al.* (1996) were able to distinguish normal and dwarf off-type banana plants using the Random Amplified Polymorphism DNA technique (RAPD).

CHAPTER 3

Materials and methods

3.1.0 Experiment 1. Investigation of the response of synthetic genotypes to black Sigatoka (*Mycosphaerella fijiensis* Morelet)

3.1.1 Study area

The study of the response of synthetic diploid hybrids to black Sigatoka was conducted at Kawanda Agricultural Research Institute (KARI) $0^0$25'N $32^0$32'E, 1190 above sea level, with a moist sub-humid climate and a mean annual rainfall of 1250 mm per annum, bi-modally distributed; the main wet season being March-May and uncertain short rains in September-November.

3.1.2 Plant materials used in the study

Twenty four synthetic diploid hybrids were obtained from interspecific and interploidy crosses made from 1999 to 2001 (Table 2) by the breeding programmes at IITA-Uganda and NARO at Namulonge and Kawanda, respectively. The seeds from these crosses were harvested and the embryos germinated *invitro* according to the embryo rescue method described by Vuylsteke (1998).

Synthetic diploid hybrids were sorted out using a Partec II ploidy analyzer (Partec GmbH, Munster,Germany), based on relative fluorescence intensity of stained nuclei as described by Singh's (2003) modification of a method by Dolezel, *et al.*, (1997). Two plants of each genotype were planted in the field including the 9 male (diploid) parents, 14 female parents, standard black Sigatoka resistant variety *'Calcutta 4'* and susceptible cultivar *'Mbwazirume'*.

Table 2: Crosses used to obtain the diploid hybrids under study

cross type	Female parent	Male parent	Genotype
	861S-	-	OPP1-861
Open Pollination	OPP1861	-	OPP2-861
	OPP1-861	SH3362	3550K-1
	7197-2	8075-7	3146K-3
	8075-5	9128-3	11043S-1
2x-2x	7197-2	8075-7	3146K-2
	Kabucuragye	Calcutta4	1537K-1
3X-2X	Nyamwezi	Calcutta4	861S-1
	401K-1	SH3362	11374S-5
	FHIA17	OPP1-861	3162K3
	FHIA17	OPP1-861	3162K-1
	FHIA17	OPP1-861	3162K-5
	365K-1	7197-2	11025S-1
	917K-2	8075-7	2858K-8
	222K-1	8075-7	8608S-1
	1438K-1	SH3217	12528S-1
	401K-1	SH3217	2866K-4
	917K-2	SH3142	12591S-2
	917K-2	7197-2	12506S-3
	917K-2	7197-2	12538S-1
	917K-2	8075-7	2858K-5
	917K-2	SH3362	12658S-1
	917K-2	SH3362	12468S-3
4X-2X	222K-1	7197-2	2714K-7

A modification of the complete randomized design by Orjeda (1998) was used for the study, in which '*Mbwazirume*'; the susceptible local variety was used both as a spreader and a guard row. Stand management such as weeding, gap filling, manuring, deleafing, mulching and de-trashing were applied uniformly (Tushemereirwe, 2003b).

3.1.3 Index of non spotted leaf

The response of the genotypes to black Sigatoka was measured using a method described by Vakili (1968) and modified later by Craenen (1998), which involves recording the youngest leaf with at least ten necrotic spots (Yls) and the number of standing leaves (NSL) at flowering. The data were used to calculate the index of non-spotted leaf from the formula by Craenen (1998):

$$INSL = \frac{100(Yls - 1)}{NSL}$$

Where,

INSL = index of non spotted leaves,

YLS = youngest leaf spotted,

NSL = Number of standing leaves.

This index measures the amount of photosynthetic tissue available before fruit filling and shows the degree of resistance of plants to the disease. A high value of the index shows greater resistance.

3.1.4 Disease severity

The Sigatoka disease severity was recorded at 6 months after planting and flowering stages as per the International Musa Testing Programme guidelines, which is based on a severity scoring system described by Stover and Dickson (1970) and later modified by Gauhl. (1994): 0=no symptoms 1=less than 1 % of lamina with symptoms 2=1–5% of lamina with symptoms 3=6–15% of lamina with symptoms 4=16–33% of lamina with symptoms 5=34–50% of lamina with symptoms 6=51–100% of lamina with symptoms.

The infection index (I.I) was calculated for each plant using a formula by Gauhl (1994):

Infection index (I.I) = [Σ (n b)/ (N-1) T*100]

Where

n=number of leaves in each grade

b= grade

N=number of grades used (7)

T= total number of leaves scored

3.1.5 Statistical analysis

Duncan's multiple range tests was used to compare the number of standing leaves, youngest leaf spotted, index of youngest leaf spotted at flowering and the severity index at 6 months and flowering using SAS software(SAS institute Inc, 2004). Principle components were computed pair wise for each genotype to the susceptible check Mbwazirume using the index of non-spotted leaf (INSL), severity at 6months and at flowering were to make a biplot with GGE software (Waikai,2001).

3.2.0 Experiment 2. Investigation of the male fertility in synthetic diploids hybrids.

3.2.1 Materials

Diploid banana genotypes selected for studies on male fertility, included 24 synthetic diploids hybrids obtained from 2x-2x, 3x-2x and 4x-2x described earlier in Table.2 and the 4 diploids which have been used successfully as male parents by the breeding programmes at IITA and NARO.

3.2.2 Examination of pollen

The pollen collected from the genotypes under study was examined for pollen quantity, stainability and germination.

Pollen quantity: 8-10 anthers were excised from the male bud of each genotype (early in the morning 7-8 a.m) and rated for pollen production on a scale of 0-4. i.e. 0-No pollen, 1-low pollen, 2-little pollen, 3-Moderate pollen, 4-much pollen using a modification of a method developed by Mukasa and Rubaihayo (1993).

Pollen stainabilty: The 8-10 anthers collected between 7 and 8 a.m had pollen scraped immediately onto the microscope slides. The Iodine Potassium Iodide (IKI_2) stain (containing 0.5 gms of iodine(I_2) and 1 gm of potassium iodide(KI_2)dissolved in 100 mls of deionised distilled water), was used to stain pollen grains on a microscope slide and pollen viability estimated by counting the stained pollen after 2-5 minutes under a light microscope at X100 magnification(Scot, 2001). About 20 pollen grains from 6 selected areas were counted per slide for each genotype. Only viable pollen grains stained immediately to black with Iodine Potassium Iodide (IKI_2) stain (Scott, 2001); the percentage of stained pollen grains was calculated from the pollen counts.

Pollen germination: Pollen obtained from the excised anthers was dusted on the cover glass for each genotype and a drop of nectar in water (1:9 v/v) was placed on a clean microscope slide. The labeled microscope slide was hanged inside a 3000ml plastic trough containing 20mls of water (as moisture chamber) to ensure that the relative humidity was near saturation. The room temperature was adjusted to 20°C using a

'Carrier' air conditioner and a thermometer was used to monitor the temperature in the moisture chamber. After 3 hours, the pollen tubes had grown to suitable length. The percentage of pollen germination was recorded for 20 pollen grains from 6 microscope areas for each slide (Sharma and Sharma, 1972).

Statistical analysis: Duncan's multiple range test was used to compare the means. Correlation analysis was performed to determine the relationship between pollen quantity, stainability and germination.

3.3.0. Experiment 3. Investigation of the Genetic relatedness among synthetic diploid banana hybrids and East African Highland bananas.

3.3.1 Materials and methods

Diploid banana genotypes were selected for the study on the genetic relatedness with East African highland bananas. Genomic DNA was extracted from 46 genotypes including 38 synthetic diploids hybrids, 4 highland bananas and 5 tetraploid hybrids were used in the RAPD analysis.

3.3.2 DNA extraction and Quantification

The DNA was extracted from the young unfurled cigar leaf using a method described by Crouch *et al.*, (1998). The DNA quality was checked in a 0.8% agarose gel. The DNA concentration was calculated by measuring the absorbance at 260nm with U.V spectrophotometer and the DNA samples standardized to the concentration of 40 ng/μl in TE buffer and stored in a refrigerator (4^0C for use in subsequent assays while the stock DNA samples are stored at -20^0 C).

3.3.4 DNA amplification

The RAPD analysis was done using a modification of the method in Pillay *et al.*, (2001).The Genomic DNA from the samples was used as a template for PCR(polymerase chain reaction) amplification, using 18 randomly selected primers (Table 3). A single 10-mer oligonucleotide with an arbitrary sequence was used for each PCR amplification. The amplification was performed in a System *MC2* "DNA-Technology" cycler. The amplification reaction volumes were adjusted to 15 μl solutions containing 3μl of 40ng/μl template DNA, 0.24μl of Taq-polymerase from *ABgene*, 2.0μl of the primer, 1.5μl of PCR-reaction buffer(10X) from *ABgene* and 1.5μl of 100mM dNTPs. The Amplification products were resolved by electrophoresis on 1.5% agarose gels in 1xTBE buffer. The DNA gels were stained with 0.5μgl/μl of Ethidium Bromide and then photographed under U.V light using Polaroid film.

3.3.4 Molecular data evaluation

Only well visualized bands were used for the genetic divergence analysis. Bands with the same migration distance were considered homologous. RAPD fragments for each primer were scored as present (1) or absent (0). The matrix raw data was used to calculate genetic similarities among the banana genotypes. Estimates of genetic similarities (GS) among all genotypes were calculated according to Jaccard's similarities co-efficient (Murguia and Villasenor, 2003):

$$GS_{ij}=a/(a+b+c),$$

where GS_{ij} corresponds to the genetic similarities between genotype ***I*** and genotype ***j***, **a** is the number of polymorphic bands present in both genotypes ***I*** and ***j***, **b** is the bands

Table 3: Sequences of the primers used in generating amplification products for the genotypes in the study.

Primer	Primer Sequence 5'>3'
OpB09	GTAGTAATTGC
OPB08	GTCCACACGG
OPQ01	ATCGCGATTC
OPR09	TGAGCACGAG
OPR10	CCATTCCCCA
OPR03	ACACAGAGGG
OPA18	AGGTGACCTG
OPR12	ACAGGTGCGT
OPR06	GTCTACGGCA
OPQ09	GGCTAACCGA
OPB17	AGGGAACGAG
OPP13	ATCGGATCAG
OPB13	TTCCCCCGCT
OPA08	GTGACGTAGG
OPP05	TCGAGGTACG
OPP03	CTGGCTTAGC
OPR11	GTAGCCGTCT
OPQ07	GCCATGCATC

present in genotype ***I*** but absent in ***j*** and **c** is the number of bands present in genotype ***j*** but absent in ***i***. The Jaccard's coefficient has been preffered in plant breeding and evolution studies with dominant markers like RAPDs, due to its good comparison capacity in analyzing genotypes of the same species, when more similarities are expected as compared to inter-specific comparisons where more diversity is seen (Williams *et al.*, 1990).

Cluster analysis based on the similarity matrix was carried out using the un-weighted pair group method with arithmetical averages-UPGMA, as was suggested by Sneath and Sokal (1973). The cophenetic coefficient between the similarity matrix and the

cophenetic values matrix was also calculated to measure the goodness of fit of the cluster analysis. All preliminary clustering procedures were performed using NTSYSpc software, version 2.02 (Rohlf,1997) The Bootstrap method was used to verify if the number of loci used was sufficient to obtain genetic distances with good precision (King et al.,1993, Hallden et al.,1994) using WINBOOT software to generate a consensus dendogram.

Chapter 4

Results and discussion

4.1 Response of Synthetic diploid banana hybrids to black Sigatoka (*Mycosphaerella fijiensis* Morelet).

The results of the host response to black Sigatoka as measured by the number of standing leaf (NSL), youngest leaf spotted (Yls), the index of non spotted leaf (INSL) at flowering and the severity infection index at both the pre-flowering (6 months after planting) and at flowering stages are presented in Table 4. The results show a significant($p<0.05$) variation for the index of non spotted leaf(INSL) at flowering ranging from 49.40% on *'Mbwazirume'* to 100% on genotypes '2858K-8', '3550K-1', 'OPP2-861', 'OPP1-861' and '12468S-3'. The mean Sigatoka severity infection index also varied greatly($p<0.05$) among the genotypes at both the pre-flowering and flowering stages ranging from 0% on *'Calcutta4'*, '2858K-8', '3550K-1', 'OPP2-861', 'OPP1-861' and '12468S-3' to 42.47% in 'SH3362'(Table 4). These results indicate that the overall varietal response to black Sigatoka is partly affected by the genotype.

Since disease severity is a measure of the damage caused by the black Sigatoka pathogen (*Mycosphaerella fijiensis* Morelet), the genotypes ('2858K-8', '3550K-1', 'OPP2-861', 'OPP1-861' and '12468S-3') observed to have 0% severity infection index at both the pre-flowering and flowering stages, indicate a hypersensitive varietal response to the pathogen.

Table 4: Black Sigatoka infection index (I.I) and Index of Non-spotted leaf (INSL) for the genotypes under evaluation.

Genotype	Pedigree		NSL	Yls	INSL	Severity Infection index		Response
	female	male				6 months	flowering	
OPP1-861	861S-	-	10.00abc	0.00b	100.00a	22.01abc	0.00c	R
3550K-1	OPP1-861	SH3362	10.00abc	0.00b	100.00a	27.38abc	0.00c	R
Calcutta4	-	-	9.80abc	5.2ab	95.60ab	0.00c	1.72bc	R
2858K-8	917K-2	8075-7	8.50abc	0.00b	100.00a	32.64abc	0.00c	R
OPP2-861	OPP1861	-	7.00abc	0.00b	100.00a	22.22abc	0.00c	R
12468S-3	917K-2	SH3362	6.00abc	0.00b	100.00a	19.55abc	0.00c	R
3162K3	FHIA17	861S-1	11.00ab	11.00a	90.91ab	9.52abc	1.52bc	PR
3162K-5	FHIA17	861S-1	9.00abc	5.67ab	81.90ab	7.29bc	9.92abc	PR
3146K-3	7197-2	8075-7	8.67abc	5.33ab	79.80ab	24.03abc	11.62abc	PR
12528S-1	1438K-1	SH3217	8.25abc	6.75ab	69.73ab	42.42ab	12.96abc	PR
365K-1	Kabucuragye	Calcutta4	8.00abc	7.00ab	75.00ab	25.00abc	10.42abc	PR
11043S-1	8075-5	9128-3	8.00abc	7.00ab	75.00ab	23.33abc	12.50abc	PR
8075-7	SH3362	Calcutta4	8.0abc	4.00ab	85.17ab	14.44abc	4.30abc	PR
2866K-4	401K-1	SH3217	8.00abc	7.00ab	75.00ab	32.02abc	10.42abc	PR
12506S-3	917K-2	7197-2	8.00abc	7.50ab	81.25ab	35.85abc	7.82abc	PR
7197-2	SH3362	Long Tavoy	7.50abc	7.50ab	86.61ab	24.90abc	4.66abc	PR
12591S-2	917K-2	SH3142	7.20abc	4.00ab	75.00ab	13.10abc	11.11abc	PR
12538S-1	917K-2	7197-2	7.00abc	6.00ab	71.43ab	23.33abc	4.76abc	PR
660K-1	Enzirabahima	Calcutta4	7.00abc	4.50ab	94.45ab	9.52abc	1.85bc	PR
SH3217	SH2095	SH2766	6.00abc	6.00ab	83.33ab	31.72abc	5.56abc	PR
3146K-2	7197-2	8075-7	6.00abc	3.75ab	75.36ab	13.10abc	10.58abc	PR
2714K-7	222K-1	7197-2	6.00abc	4.00ab	78.93ab	28.33abc	14.66abc	PR
1438K-1	Entukura	Calcutta4	5.00bc	3.00ab	91.67ab	23.34abc	2.78abc	PR
11374S-5	401K-1	SH3362	12.00a	12.00a	91.67ab	31.25abc	4.17abc	S
3162K-1	FHIA17	861S-1	9.00abc	7.00ab	66.67ab	1.19c	11.00abc	S
11025S-1	365K-1	7197-2	8.71abc	6.71ab	61.19ab	21.80abc	16.74abc	S
8608S-1	222K-1	8075-7	8.50abc	7.00ab	55.84ab	25.00abc	18.06abc	S
376K-7	Nantc	Calcutta4	8.00abc	6.00ab	62.50ab	21.43abc	22.92abc	S
5105-1	Calcutta4	Psang lillin	7.80abc	6.60ab	71.79ab	32.22abc	13.69abc	S
1537K-1	Kabucuragye	Calcutta4	7.50abc	6.50ab	71.95ab	28.33abc	14.16abc	S
MBW	-	-	7.00abc	4.80ab	49.40b	40.72abc	24.93a	S
ENYERU	-	-	7.00abc	5.00ab	57.14ab	37.88abc	23.81ab	S
861S-1	Nyamwezi	Calcutta4	7.00abc	6.00ab	71.43ab	39.74abc	14.29abc	S
917K-2	Enzirabahima	Long Tavoy	7.00abc	5.00ab	57.14ab	28.33abc	23.81ab	S
SH3362	SH3142	SH3217	7.00abc	5.50ab	64.29ab	42.42ab	19.05abc	S
2858K-5	917K-2	8075-7	6.75abc	5.50ab	63.89ab	32.64abc	17.42abc	S
12658S-1	917K-2	SH3362	6.00abc	5.00ab	66.67ab	36.81abc	16.67abc	S
222K-1	Nfuuka	Calcutta4	6.00abc	5.00ab	66.67ab	32.64abc	11.00abc	S
401K-1	Entukura	Calcutta4	6.00abc	5.00ab	66.67ab	20.00abc	11.00abc	S
Pr>F			*0.689*	*0.404*	*0.044*	*0.002*	*0.008*	
R^2(%)			*39.97*	*45.29*	*53.84*	*88.25*	*61.04*	
C.V (%)			*13.0*	*6.7*	*12.2*	*13.0*	*7.2*	

NSL=Number of standing leaves, Yls= youngest leaf spotted, R=resistant, PR=partially resistant, S=susceptible

Subsequently, these hypersensitive genotypes showed 100% index of non-spotted leaf, which is a measure of the amount of photosynthetic area available at flowering (Orjeda, 1998). This collaborates with the findings of Vuylsteke and Ortiz (1995) in evaluating plantain-derived diploid hybrids. This implies that these hypersensitive genotypes have an inherent mechanism to restrict the development of the black Sigatoka symptoms to the final stage of necrosis as suggested by Ortiz and Vuylsteke (1994).

4.1.1 Principal Component analysis

The positions of the diploid genotypes on the first and second components derived from the pair wise comparison of Index of Non-Spotted Leaf (INSL), severity at 6 months and severity at flowering of each genotype with reference to the susceptible cultivar 'Mbwazirume' are shown in Fig.1. The first component accounted for 70% of the variation in response to black Sigatoka among the genotypes, while the second principal component accounted for 29% of the variation. This implies that the variation among the genotypes as measured by infection index at both pre-flowering and flowering and the index of non-spotted leaf could adequately accounted for by only the first and second components and the biplot was ideal to separate the genotypes on the basis of their response to black Sigatoka. The Eigenvectors for number of standing leaves (NSL) and youngest leaf spotted(Yls) were too short to make any separation of the genotypes indicating that the index of non-spotted leaf (computed from both NSL and Yls) is a more informative parameter for black Sigatoka response than either NSL or Yls.

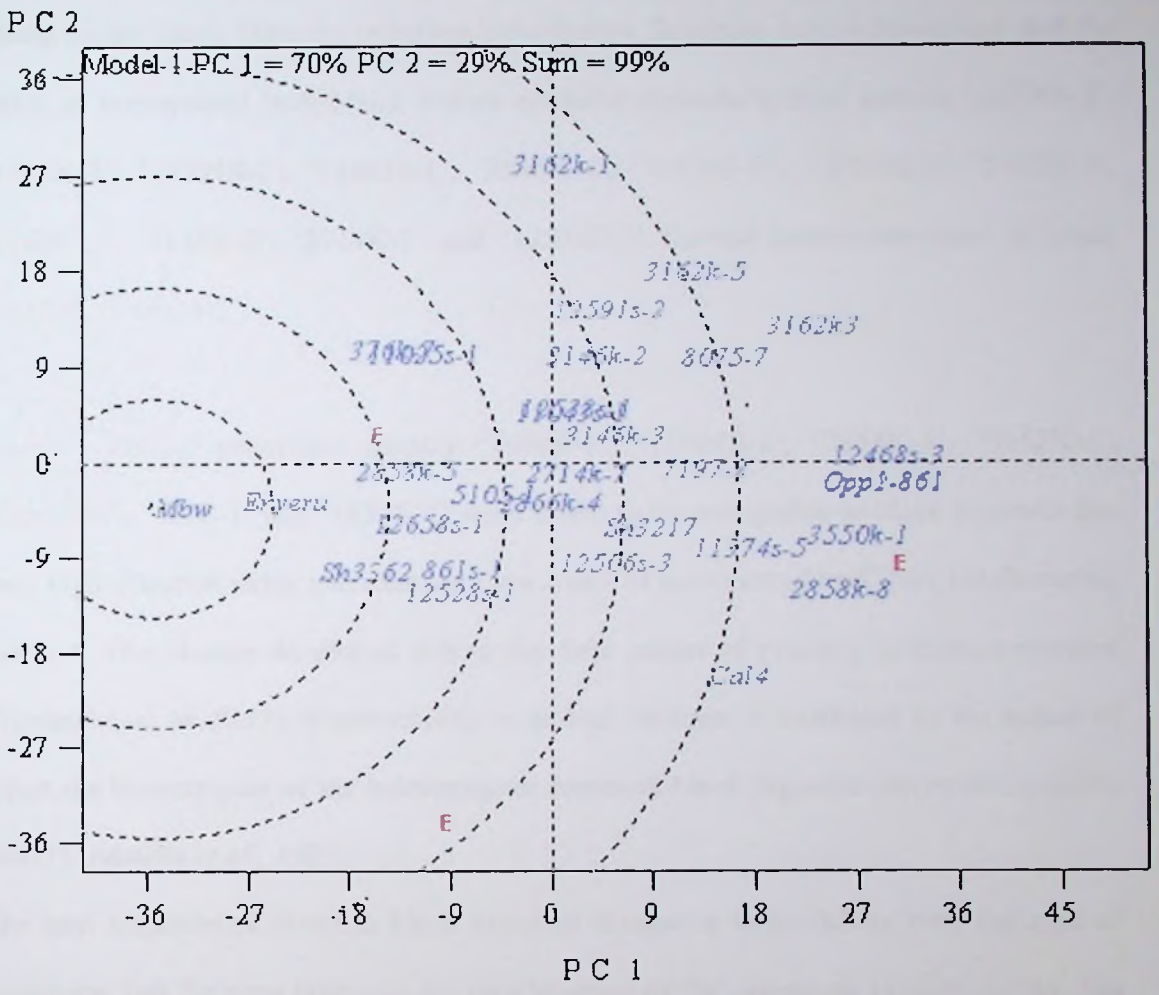


Figure 1: A Biplot comparing the response of the genotypes under evaluation to black Sigatoka using INSL, and severity index at 6 months and flowering with reference to Mbwazirume the susceptible cultivar.

A biplot of the principle component 1 and principal component 2 separated *'Enyeru'* (a landrace almost equally susceptible to black Sigatoka to *'Mbwazirume'* while the five hypersensitive genotypes ('2858K-8', '3550K-1', 'OPP2-861', 'OPP1-861', and '12468S-3') were grouped together with *'Calcutta 4'* based on the indices used for the biplot for resistance.

Based on the black Sigatoka infection index(before flowering and at flowering) and the index of non-spotted leaf(INSL), twelve synthetic diploids hybrids namely '12506S-3', '3146K-3', '12591S-2', '11043S-1', '2866K-4', '11374S-5', '3162K-3', '3162K-5', '3162K-1', '3146K-2', '2714K-1' and '12538S-1' showed partial resistance to black Sigatoka (Table 4).

Seven synthetic genotypes namely '8608S-1', '11025S-1', '2858K-5', '3162K-1', '12658S-1', '861S-1' and '1537K-1' were found to be susceptible to black Sigatoka due their high infection index and relatively low index of non-spotted leaf(INSL) at flowering Table 4. The disease developed fast to the final stages of necrosis in these genotypes (Vuylsteke *et al.*,1997). Susceptibility in diploid bananas is attributed to the action of either the homozygous or the heterozygous dominant black Sigatoka susceptibility allele (**BS**) (Vuylsteke *et al.*, 1997).

The host response of *Musa* to black Sigatoka disease is indicated by both the type of symptoms and the time taken for the development of the symptoms (Vakili, 1968). The type of symptoms is an indicator of the stage at which lesion development is arrested. The development of disease symptoms on the leaves of hypersensitive/highly resistant genotypes like '2858K-8', '3550K-1', 'OPP2-861', 'OPP1-861', and '12468S-3' do not reach the finals stages of necrosis. This restricted development of leaf symptoms in *Musa* to *M.fijiensis* has been attributed to physiological and biochemical factors following the penetration of host tissues by fungal hyphae (Hoss *et al.*, 2000). The high concentration of total sugars, wax, phenols, and lignin coupled by the activity of phenylalanine

ammonia lyase, ascorbic acid oxidase catalase and polyphenol oxidase in resistant *Musa* clones have been reported to be responsible for this restricted symptom development (Krishnamoorthy *et al.*,2004). Craenen and Ortiz (1997) using statistical and genetic analysis of the frequency distribution of host response to black Sigatoka in plantain-banana hybrids suggested that hypersensitivity in *Musa* clones was due to the expression of the homozygous major recessive gene (bs_1).

The results showed that in some genotypes the disease symptoms progressed to the final stages of necrosis. However, there was significant ($p \leq 0.05$) variation in the extent of necrosis in both the amount of damage caused by the pathogen as measured by the severity infection index and the rate for the symptoms to develop by genotype as shown by the youngest leaf spotted (Yls) at flowering in Table 4. Vuylsteke and Ortiz (1995) suggested that the less leaf spot damage in *Musa* is due to the slow or delayed disease symptoms. This implies that the age of the leaf on which mature lesions appear is an important criterion for host response to black Sigatoka in *Musa*. This implies that the more susceptible the genotype is the shorter the incubation period, and the younger the leaf on which mature lesions appears. Since leaf emission varies by genotype the youngest leaf spotted (Yls) was modified by Craenen (1998) by computing the index of non-spotted leaf,(INSL) which measures the percentage of photosynthetic area available to the plant at flowering. Orjeda (1998) suggested that bananas require more than 70% of active leaf foliage at flowering for proper development of the banana fruits. Genotypes like '12506S-3', '3146K-3', '12591S-2', '11043S-1', '2866K-4', '11374S-5', '3162K-3', '3162K-5', '3162K-1', '3146K-2', '2714K-1' and '12538S-1' showed partial

resistance to black Sigatoka (Table 4). In some instances the lesions did not coalesce. Ortiz and Vuylsteke (1994) attributed partial response to the co-expression of the major recessive gene ($\mathbf{bs_1}$) with one of the minor genes $\mathbf{bsr_2}$ or $\mathbf{bsr_3}$ in diploid bananas. Since diploid bananas are normally used as parental material in breeding programmes, the additive gene action of the $\mathbf{bs_1}$ locus in both the resistant and partially resistant genotypes makes these genotypes a plausible source of resistance in banana improvement. Especially when generating progenies of higher ploidy levels and when these additive genes are interlocus (Falconer and Mackay, 1996) because higher ploidy levels with cumulative numbers of the favorable recessive alleles can overcome the action of the host's dominant Black Sigatoka susceptibility allele (**BS**) (Vuylsteke *et al.*, 1997).

4.2 Male fertility in synthetic diploid hybrids

4.2.1 Pollen output rating

The results of pollen output in synthetic diploid hybrids are shown in Table 5 as pollen rating. The results show that mean pollen quantity ratings among the diploid genotypes varied significantly ($p \leq 0.05$) ranging from 1.0 on genotypes '11025S-1' and '12506S-1' to 4.0 on *'Calcutta 4'* and '2506S-29' (Table 5). This implies that variations for pollen output exist among the genotypes and can affect germination of the pollen during fertilizations (Posonen and Kapyla,1998). The analyses of variance for pollen quantity rating showed that the node from which the anthers were excised did not significantly ($p \leq 0.05$) contribute to the variation in pollen rating of the genotypes (Table 6).

This suggests that there is no limit to the nodal position from which pollen grains should be collected when using these synthetic diploids for artificial pollination.

Table 5: Mean pollen rating, stainability and pollen germination in synthetic diploids.

Genotype	Pedigree		Pollen rate	Pollen stainability	pollen Germination
	female	male			
12506S-29	917K-2	7197-2	4.00a	88.03ab	83.05ab
OPP2-861	Opp1-861	-	3.80ab	93.39a	87.98ab
3162K-5	Fhia17	OPP1-861	3.60abc	28.65ih	52.85cdef
3162K-3	Fhia17	OPP1-861	3.50abcd	90.00ab	82.23ab
3162K-1	Fhia17	OPP1-861	3.50abcd	100a	65.79cde
3146K-3	7197-2	8075-7	3.00abcde	95.41a	81.67ab
3146K-2	7197-2	8075-7	2.75abcdef	97.38a	81.46ab
12468S-1	917K-2	SH3217	2.56bcdefgh	56.87defg	48.07defg
3162K-2	FHIA17	OPP1-861	2.33cdefghi	96.77a	87.02ab
1537K-1	Kabucuragye	Cal4	2.30cdefghi	50.71efgh	39.57efgh
12658S-1	917k-2	SH3362	2.29cdefghi	74.16abcde	16.60ij
2866K-4	401K-1	SH3217	2.17defghi	92.62a	88.18ab
2858K-5	917K-2	8075-2	2.13defghi	82.67abc	1.67j
8606S-1	222K-1	8075-7	2.00efghi	53.02defgh	32.22ghij
3550K-1	OPP1-861	SH3362	2.00efghi	77.56abcd	85.50ab
2714K-7	222K-1	7197-2	1.75efghi	50.00efgh	53.94cdef
12538S-1	917K-2	7197-2	1.60efghi	43.61fgh	27.18ghi
12506S-3	917K-2	7197-2	1.33fghi	34.43ghi	29.54ghi
12528S-12	1438K-1	SH3217	1.29ghi	28.00hi	15.44ij
12591S-1	917K-2	SH3142	1.17hi	39.17fgh	25.00hi
11025S-1	365K-1	7197-2	1.00i	35.19ghi	10.98ij
12506S-1	917K-2	7197-2	1.00i	12.96i	12.64ij
8075-7	SH3362	Cal4	2.67abcdefg	88.51ab	94.04a
861S-1	Nyamwezi	Cal4	22.29cdefghi	65.22bcdef	650.07bcd
7197-2	SH3362	Long tavoy	2.00efghi	95.00a	93.01a
5105-1	Calcutta4	Psang Lilin	1.80efghi	57.80cdefg	55.97cde
OPP1-861	861S-1	-	1.80efghi	90.00ab	74.96abc
Calcutt4	-	-	4.00a	96.67a	95.66a

Genotypes with the same letter within a column are not significantly different at (p<0.05)

A high coefficient of determination R^2 of 76.07% for pollen rating among the synthetic diploids implied that there was minimal contribution of other factors other than genotype and node to the variation in pollen quantity among the hybrids. Such factors would include weather conditions such as high solar radiation and high temperatures that significantly ($p \leq 0.05$) affected pollen quantity in a range of diploid bananas as Ortiz *et al.*, (1998).

Table 6: ANOVA for pollen rating in synthetic diploids.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Node (genotype)	70	51.38	0.734	1.04	0.4283
Genotype	27	75.16	2.78	3.96	<.0001
Cv%=3.6	R^2%=76.06				

'*Calcutta4*', a highly male fertile wild diploid banana, which has been widely used in *Musa* breeding, was used as the reference genotype for pollen output in this study. The results show that diploid genotypes '12506S-29', 'OPP2-861', '3162K-3', '3162K-1', '3162K-5' and '3146K-2' had pollen rating not significantly different from that of the reference genotype. This implies that these genotypes could be used in crosses with female fertile East African Highland bananas, tetraploid hybrids and diploid bananas.

The quantity of pollen produced by the different cultivars is important for banana breeding where both male and female fertility are a limiting factor. Johnri and Vasil (1961) suggested that genotypes that produced small amounts of pollen grains stand less chances of successful pollinations because of the 'population effect', i.e., the need for a minimum density of pollen grains to get proper germination. Aggregation of pollen grains was reported to have a stimulatory effect on the germination and length of pollen tubes (Posonen and Kapyla, 1998), which was suspected to be due to certain growth stimulating substances that diffuse out of the pollen grains and enhance pollen germination (Posonen and Kapyla, 1998).

The mean pollen rating for the parental genotypes of the synthetic diploid hybrids which ranged from 1.80 to 2.76 implies that the synthetic diploid hybrid pollen output values of 1.80 can be used for successful fertilizations, although genotypes with a high proportion of viable pollen will be favored (Krishnakumar *et al.,* 1992).

4.2.2 Pollen stainability

Pollen stainability also varied significantly ($p \leq 0.05$) among the genotypes ranging from 12.96% on genotype '12506S-1' to 100% on '3162K-1' as shown in Table 5. This indicates that the proportion of living pollen from the total pollen output varies for the different genotypes. Pollen stainability for the reference genotype *'Calcutta 4'* was 96.67% and genotypes '3162K-1','3162K-2', '3162K-3', '3146K-2', '3146K-3', '8075-7', '7197-2', '12506S-29', 'OPP2-861', '2858K-5', '3550K-1' and '12658S-1'did not show significantly different pollen stainability. This implies that these genotypes have a high proportion of their pollen viable which increases the chances for a successful fertilization. Genotypes '8075-7', '7197-2' and 'OPP1-861' have been used as male parental materials for some of the genotypes under evaluation. The mean pollen stainability for the parental genotypes ranged from 57.8% on '5105-1' to 95.0% on '7197-2'. This suggests that pollen stainability of 57.80% is capable of successful fertilization.

Besides pollen quantity, male fertility in bananas is also affected by the proportion of living pollen grains. The proportion of living pollen is affected by pollen abortions due to either genetic or environmental factors like hydration (Singh, 2003). Assuming that the non-viable pollen grains have no starch granules, the Iodine Potassium Iodide ($\mathbf{IKI_2}$) stain distinguishes the viability of the pollen grains based on the presence or absence of

starch granules. Starchy pollen grains are favored in *Musa* breeding because the pollen tubes growing down the long styles of the female tubular flowers will have high energy demands (Grayum, 1985). Franchi *et al* (1996) suggested that starch in the pollen is hydrolyzed to form sucrose and other oligosaccharides, which protect pollen membranes against dessication. This implies that the genotypes with a high proportion of their pollen viable will remain active by the time it gets to the stigma which increases the chances for a successful fertilization. Breeders can therefore use these genotypes to introgress genes for their desirable attributes into female fertile East African Highland bananas.

4.2.3 Pollen germination

The mean pollen germination of the synthetic diploid genotypes on nectar: water (1:9 V/V) varied significantly ($p \leq 0.05$) ranging from 1.67% on '2858K-5' to 95.66% on *'Calcutta 4'* as shown in Table 5. This indicates that the ability for the pollen to germinate and grow towards the ovule is different for various genotypes. Mean pollen germination for genotypes '2866K-4', 'OPP2-861S-1', '3162K-2', '3550K-1', '12506S-29', '3162K-3', '3146K-2' and 'OPP1-861' were not significantly different with that of *'Calcutta4'* as a reference. This implies that pollen from these genotypes has equal potential to germinate on the stigma and grow towards the ovules during germination. The effectiveness of pollen may be affected by pollen hydration, pollen germination, and pollen tube penetration through the stigma and growth through the style to the ovary (Shepherd, 1960). Pollen germination reflects the functional competency of the genotype as a progenitor (Fortescue and Turner, 2004).

The correlation coefficients of the means of the pollen rating, stainability and germinations of the synthetic diploids are shown in Table 7.

Table 7: Correlation coefficients for means pollen rate, stainability and germination.

	Pollen Rate	Pollen Stainabilty	Pollen germinations
Pollen Rate	-	0.12092 (0.5499)	0.19810 (0.3122)
Pollen Stainability	0.12092 (0.5499)	-	0.76448 (<.0001)
Pollen Germinations	0.19810 (0.3122)	0.76448 (<.0001)	-

The results showed that pollen stainability was significantly ($p \leq 0.05$) correlated to pollen germination however there is no consistent relationship between the stainability and germinability values. This indicates that the proportion of viable pollen is a significant factor in the pollen germinability but it can not be used to predict the functional competency of the genotype. The pollen rating did not correlate with either pollen stainability or pollen germination. This suggests that pollen rating was not a significant factor in pollen stainability and germination. This is contrary to an earlier study by Dumpe and Ortiz (1996) which reported that high quantities of pollen produced high percentage rates of viable pollen and germination. This implies that selecting male parents only on the basis of pollen quantity may not be sufficient.

4.3 Genetic relatedness in the synthetic diploid banana hybrids and East African Highland bananas.

4.3.1 Genetic polymorphism

The RAPD analysis of synthetic diploid hybrids gave between 6 and 13 polymorphic bands (Table 8). The size range of the amplified products differed according to the primers used, but all were in the range of 300bp-2000 bp. The amplification of the 46 different genotypes with the 21 primers produced 154 alleles. The polymorphism of the alleles was analyzed and 111 (72%) were found polymorphic (Table 8).

Table 8: Sequences of the primers generating amplification products for the genotypes in the study.

Primer	Primer Sequence 5'>3'	No. of scorable bands	No. of polymorphic bands
OpB09	GTAGTAATTGC	7	5
OPB08	GTCCACACGG	8	7
OPQ01	ATCGCGATTC	8	5
OPR09	TGAGCACGAG	9	3
OPR10	CCATTCCCCA	13	11
OPR03	ACACAGAGGG	9	8
OPA18	AGGTGACCTG	9	7
OPR12	ACAGGTGCGT	9	5
OPR06	GTCTACGGCA	10	9
OPQ09	GGCTAACCGA	8	4
OPB17	AGGGAACGAG	10	9
OPP13	ATCGGATCAG	5	2
OPB13	TTCCCCCGCT	7	3
OPA08	GTGACGTAGG	10	8
OPP05	TCGAGGTACG	8	6
OPP03	CTGGCTTAGC	6	4
OPR11	GTAGCCGTCT	8	7
OPQ07	GCCATGCATC	10	8
Total		154	111

An allele is said to be polymorphic when it is present in one genotype but absent in another. By comparison, in a survey assessing 16 *'Sukali Ndiizi'* bananas collected from different parts of Uganda Pillay *et al.*, (2001) found no polymorphism with RAPD primers. While Ude *et al.*, (2003) reported 88.3% polymorphic RAPD bands among 25 plantain cultivars from the core collection at Onne in South Eastern Nigeria suggesting that the use of many RAPD primers can uncover great levels of polymorphism. The high level of polymorphism in RAPDs fragments from this study indicates that a high proportion of alleles are not identical in state in the *Musa* population used in this study indicating breeding increases genetic diversity (Helm et al., 1997).

The results of a cluster analysis based on similarity matrices obtained with the unweighted pair group method using arithmetic averages (UPGMA) and relationships between cultivars are presented in a dendogram (fig 2). The cophenetic coefficient which shows the approximation of the dendogram to the similarity matrix was 0.83 indicating that the dendogram fits the similarity matrix.

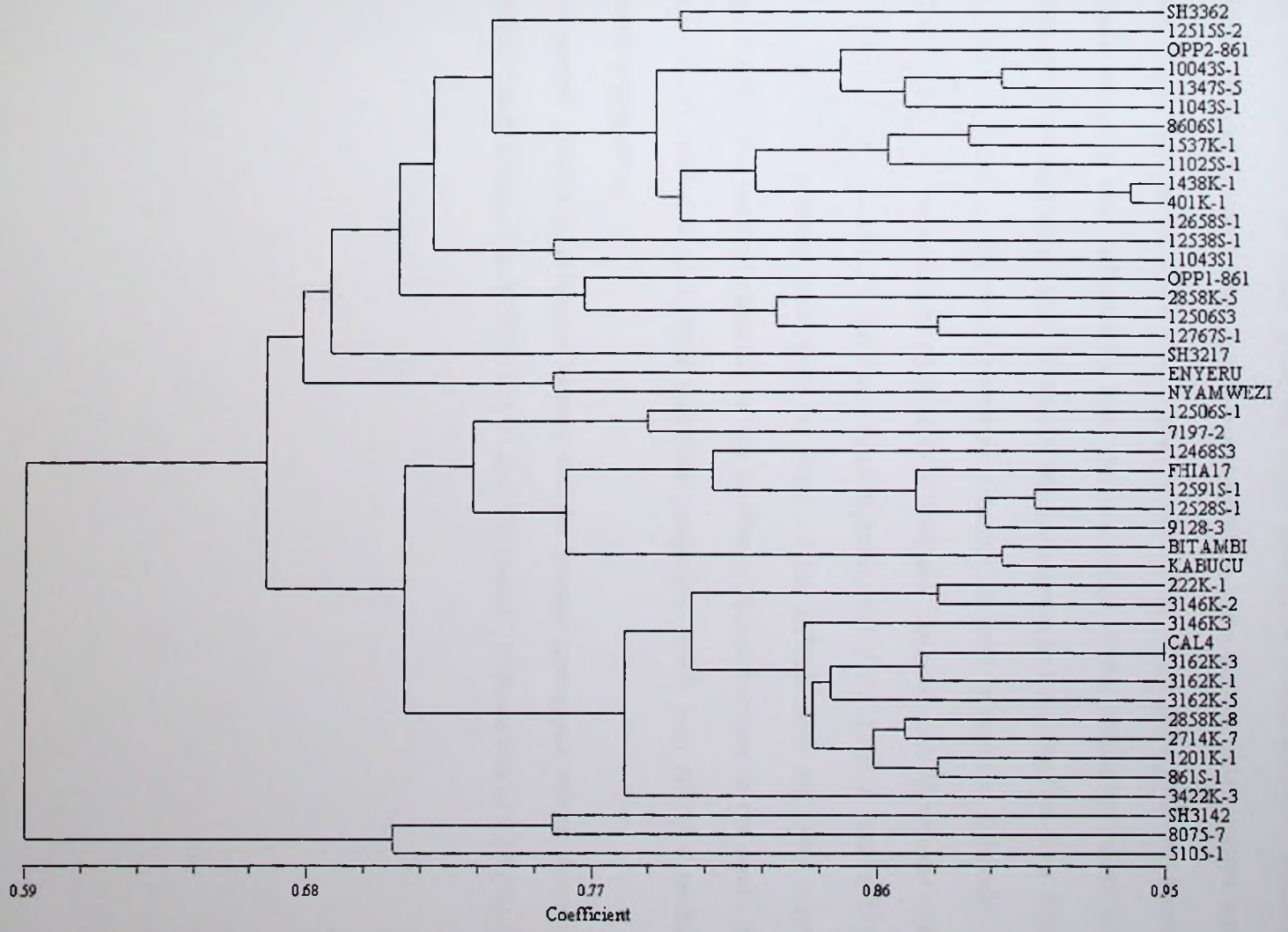


Figure 2: Genetic relationships among the synthetic diploid hybrids with their parental materials

The results showed no major grouping of the genotypes, which indicates absence of distinct clusters amongst these genotypes suggesting close genetic relationships among them. In the dendogram, there was no precise separation of the genotypes into heterotic groups, which is also reflected by both the low range of the similarity values and a moderate mean similarity value. However, the dendogram showed the four East African Highland Bananas in the study clustered together on two occasions, *'Bitambi'* and *'Kabucuragye'* with a similarity coefficient of 0.90 and *'Enyeru'* and *'Nyamwezi'* with a similarity coefficient of 0.76. Similarly three diploids, '8075-7', '5105-1' and 'SH3142' which are male parental genotypes for some of the hybrids also clustered together implying that the parental materials of the synthetic diploid bananas in the study were more closely related to each other than their progenies, which may reflect a moderate genetic segregation.

The genetic similarity estimates among the 46 banana genotypes varied from 0.48 ('8075-7' and *'Enyeru'*) to 0.95('3162k-3' and *'Calcutta4'*) with a mean of 0.55 (fig.3).

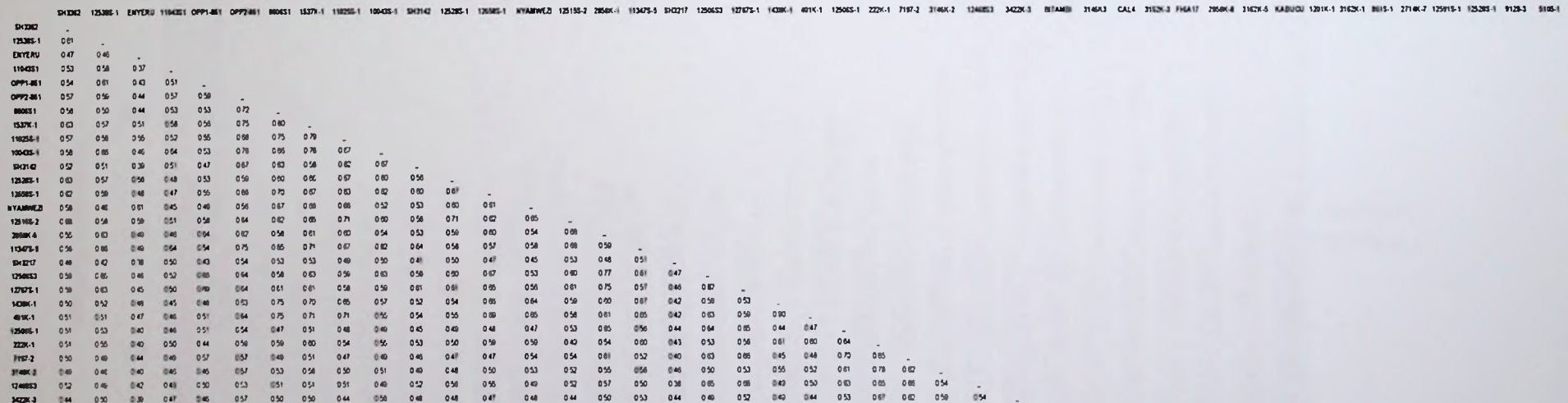


Figure 3. Similarity coefficients of banana genotypes under evaluation

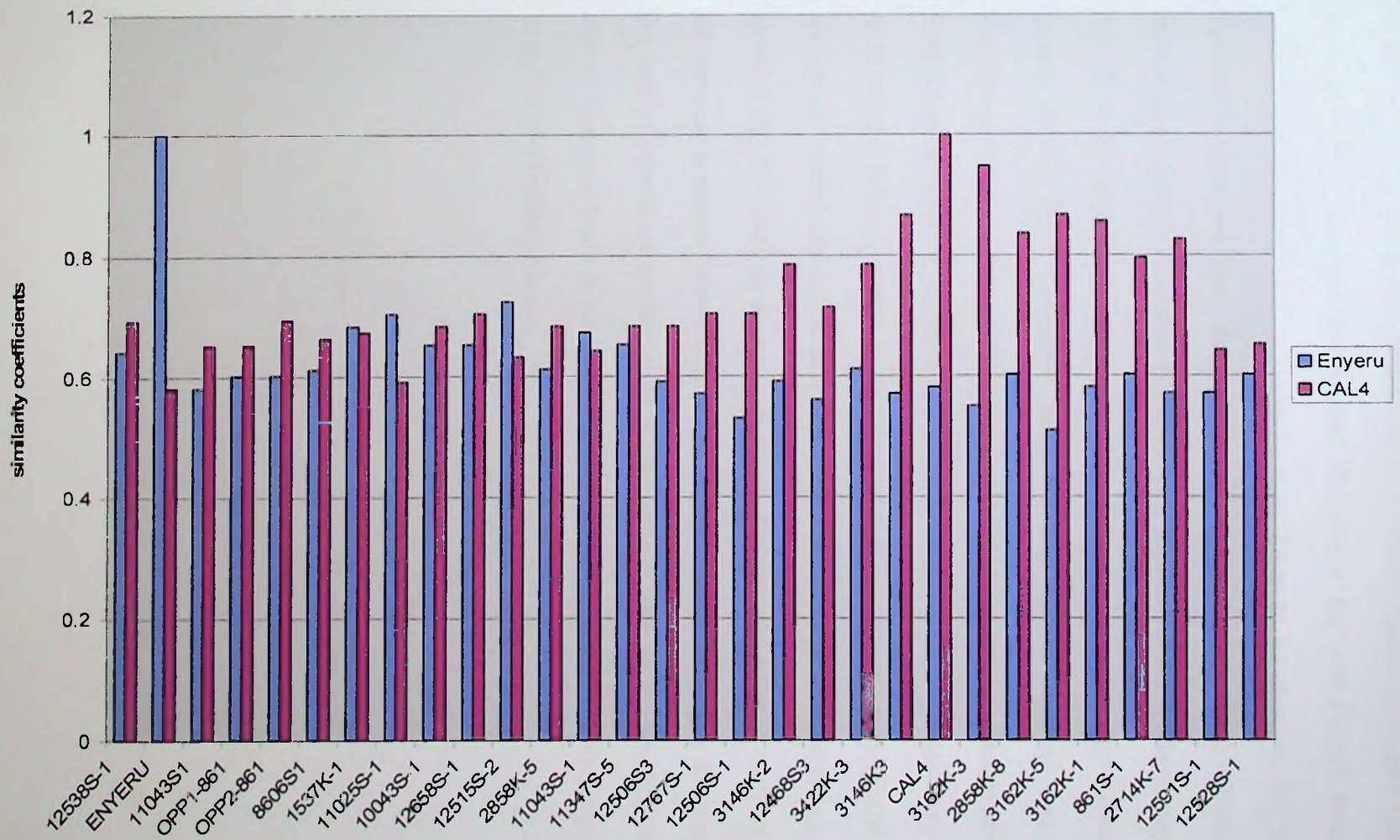


Figure 4. A bar graph comparing the similarity coefficients of the synthetic diploid hybrids with East African Highland banana genotype Enyeru and wild diploid banana genotype *Calcutta 4*

In comparison of the genetic similarities of synthetic diploid genotypes with *'Enyeru'*, the East African Highland landrace and *'Calcutta 4'* the wild diploid; three genotypes ('1537K-1', '11025S-1' and '12515S-1') were found to be closer to the East African highland banana genotype, *'Enyeru'* than *'Calcutta 4'* while the 25 synthetic diploid banana were closer to *'Calcutta 4'*(fig.4). This implies that the synthetic diploid banana hybrids in this study have more in the allelic differences with the East African Highland banana *'Enyeru'* than *'Calcutta4'*. Since the East African Highland bananas are the prospective female parents in the banana breeding programmes, these synthetic diploid banana hybrids have the potential for the synthesis of heterotic hybrids when used as the male parents. Falconer (1989) argues that heterosis is associated with genetic divergence and genetic relatedness can be used as a solid criterion for parental selection. This study revealed that the synthetic diploids are genetically variable amongst each other however the variation is narrow, which implies that the inter-diploid crosses in this population will not lead to significant genetic gains in the resultant progenies. However, the synthetic diploid banana hybrids in the study can be used as progenitors in the improvement of East African Highland banana.

Chapter 5

Conclusions and recommendations

Black Sigatoka, a fungal leaf spot disease is one of the major banana production constraints in East African Highland bananas causing heavy defoliation which severely suppresses fruit filling leading to yield decline of about 37-50%. Diploid bananas are used as male parents in *Musa* improvement to develop hybrids with resistance to black Sigatoka which is the most promising strategy for managing the disease among small holder farmers who grow the bulk of the crop. Unfortunately *Musa* improvement has often been limited by low levels of male fertility among the prospective parents. Therefore the utilization of these synthetic diploid banana hybrids as male parents relies on their ability to produce viable pollen in adequate amounts. Also the low genetic variability in East African highland bananas is a limitation in generating progenies with either enhanced heterosis and/ or genetic gain. Therefore this research contributed to banana breeding by showing that synthetic diploid banana hybrids can be utilized as male parents and the findings lead us to the following conclusions:

- The synthetic diploid banana hybrids were categorized into three levels of black Sigatoka response i.e. highly resistant, partially resistant and susceptible.
- Six synthetic diploid banana genotypes('2858K-8', '3550K-1', 'OPP1-861', 'OPP2-861' '12468S-3' and '3162K-3' showed hypersensitivity and were therefore categorized as highly resistant.

- While eleven synthetic diploids hybrids namely '12506S-3', '3146K-3', '12591S-2', '11043S-1', '2866K-4', '11374S-5', '3162K-5', '3162K-1', '3146K-2', '2714K-1' and '12538S-1' showed partial resistance to black Sigatoka.
- Ten genotypes namely '3550K-1', '861S-1', 'OPP1-861', '3146K-3', '12506S-29', '2866K-4', 'OPP2-861', '3162K-3', '3162K-1', '3162K-5' and '3146K-2' showed sufficient pollen germination, stainability and pollen rating to be used as male parents.
- The synthetic diploid banana hybrids under study had more allelic differences with the East African Highland banana, *'Enyeru'* than with 'Calcutta4'.

Broad based, improved diploid *Musa* germplasm combining male fertility with pest/disease resistance will be a major component to the success of banana breeding. Therefore the following recommendations can be considered to effectively use these synthetic diploid bananas:

- Since the inheritance of black Sigatoka resistance in bananas is polygenic, both the resistant and partially resistant synthetic diploid genotypes are recommended to be used as a source of resistance to black Sigatoka in improving East African Highland bananas.
- The male fertile synthetic diploids should be further characterized for other desirable traits like nematode and weevil resistance to ensure that resultant progenies combine resistance to all these production constraints.
- Also, the increased use of molecular markers will accelerate the process of parental and recurrent selection and hence facilitate the development of new hybrids.

References

Alexander, M.P. 1969. Differential staining of aborted and non aborted pollen. Stain Techno. 44:117-122.

Ashagari, J. 2000. Estimation of pollen viability of mestusulon treated dyers wood for herbicide efficacy evaluation. J.Agr.Sci.Tech. 2:85-93

Buddenhagen, I.W. 1987. Disease susceptibility and genetics in relation to breeding of bananas and plantains. In: Banana and plantain Breeding Strategies. Persely GJ & De Langhe EA (Eds.) ACIAR Proceedings No. 21:95-109, Canberra.

Chandelier, S. 1996. The nutritional value of bananas In: Bananas and Plantains. Gowen S (Ed). Chapman and Hall. Pp 468-480.

Craenen, K. 1998. Technical Manual on Black Sigatoka Disease of Banana and Plantain. International Institute of Tropical Agriculture, Ibadan, Nigeria.

Craenen, K. and Ortiz, R. 1997. Effect of black Sigatoka resistance locus ***bs1*** and ploidy level on fruit and bunch traits in plantain-banana hybrids.Ephyitica. 87:97-101

Crouch, H.K., Crouch, J.H., Jarret, R.L., Cregan, P.B. and Ortiz, R. 1998. Segregation of microsatelite loci from haploid and diploid gametes in *Musa*. Crop Sci 38:211-217.

Damasco O., Graham G., Henry R., Adkins S., Smith M., and Godwin D. 1996. Random amplified polymorphic DNA (RAPD) detection of dwarf off-types in micropropagated Cavendish (*Musa spp.* AAA) bananas. Plant Cell Reports, 16: 118-123.

Dolezel, J., Lysak, M.A.,Van den Houwe, I., Dolezelova, M. and Roux, N. 1997. Use of flow cytometry for rapid ploidy determination in *Musa* species. INFOMUSA 6:6-9,

Dumpe, B.B. and **Ortiz, R. 1996.** Apparent male fertility in *Musa* germplasm. Hortscience.31:1019-1022.

Falconer, D.S. 1989. *Introduction to Quantitative Genetics*. 3rd edn. Longman, London, England.

Falconer, D.S. and Mackay, T.F.C. 1996. Introduction to quantitative genetics. 4th edition Longman England.

FAOstat. 2003. Agricultural Production Statistics Database. Food and Agricultural Organisation of the United Nations.

Fortescue, J.A. and Turner, D.W. 2004. Pollen fertility in *Musa*: Viability in cultivars grown in southern Australia. Journal of Australian agricultural research. 55:1085-1091

Foure, E. 1987. Varietal reactions of banana and plantain to black leaf streak. In: Banana and Plantain Breeding Strategies. Persley GJ and De Langhe EA (Eds.) 21:110- 113, ACIAR Proceedings Caniberra.

Franchi, G.G., Bellani, L. Nepi, M. and Pacini, E. 1996. Types of carbohydrate reserves in pollen: localization, systematic distribution and ecophyisiological significance. *Flora* 191:143-159.

Gauhl, F. 1994. Epidemiology and ecology of black Sigatoka (*Mycosphaerella fijiensis* Morelet) on plantain and banana in Costa Rica. INIBAP Montpellier, France.

Gold, C.S., Ogenga-Latigo., Tushemereirwe, W.K., Kashaija. I., and Nankinga, C. 1993. Farmer perceptions of banana pest constraints in Uganda: Results of a rapid rural appraisal In: Biological and integrated control of highland banana and plantain pests and diseases. Gold CS and Gemmil B (eds). Proceedings of a research coordination meeting, Cotonou, Benin12-14th November 1991.

Gold, C.S., and **Messian, S. 2000.** The banana weevil *cosmopolites Sordidus.* INIBAP fact sheets.

Gowen, S.R. 1996. The source of nematode resistance, the possible mechanisms and potential for nematode tolerance in *Musa sp.* In: New Frontiers In Resistance Breeding For Nematodes, Fusarium And Sigatoka. INIBAP pp 45-49.

Grayum, M.H. 1985. Evolutionary and ecological significance of starch storage in pollen of the Araceae. American Journal of Botany72:1565-1577.

Gower, J.C. 1985. Measures of similarity, dissimilarity and distance. In: Encyclopedia of statistical sciences and distance. Kutz S and Johnson NL (eds). Pp 397-407Willey, NewYork, U.S.A. Vol.5, .

Hallden, C., Nilson, N.O., and Rading, I.M. 1994. Evaluation of RFLP and RAPD markers in comparison of Brassica Nupus breeding lines. Theor. Appl. Genet. 88:123-128.

Hilber, M. 1997. Breeding a better banana. IDRC reports archive. Vol 22, No.1Ottawa, Canada

Helms, T., Orf, J., Valad, G. and McClean, P. 1997. Genetic variance coefficient of parentage and genetic distance of six soybean populations. Theor Appl Genet 94:20-26

Horry, R.P., Sharrock, S.L. and Frison, E.A. 1998. Present situation of biogenic resources for the betterment of society. In: MEMORIAS XII Arizanga IHL (ed). Pp 679-691 Reunion ACORBAT, Ecuador.

Hoss, R., Helbig, J. and Bochow, H. 2000. Function of Host and fungal metabolites in resistance resource response of Banana and plantain in the black Sigatoka disease path system (*Musa sp-Mycosphaerella fijiensis*). J.Phytopathology : 0931-1785.

INIBAP. 1999. Networking Banana and plantain: INIBAP Annual Report 1998. Montpellier, France. 64pp.

INIBAP. 2000. The many uses of *Musa*. MusaDoc. IPGRI 2000 Rome, Italy.

Janssen, A.W.B. and Hermsen, J.G. 1976. Estimating pollen fertility in *Solanum* species and haploids. Euphitica 25:577-586.

Jeger, M.J., Eden-Green, S., Tresh, J.M., Johnson, A., Waller, J.M. and Brown, A.E. 1995. Banana Diseases. *In*: Bananas And Plantains. Gowen S (Ed). Pp 317-381. Chapman and Hall, London, U.K.

Johnri, B.M. and Vasil, I.K. 1961. Physiology of pollen. The Botanical Review 27:325-381.

Karamura, E.B. 1999. Workshop Objectives. *In*: Mobilizing IPM For Sustainable Production In Africa. Frison E.A., Gold C.S., Karamura E.B. And Sikora R.A. (Eds.) Pp5-9 Proceedings Of A Workshop On IPM Held In Nelspuit, South Africa-23-28 November 1998.

King, G., Nienhuis, J. and Hussey, C. 1993. Genetic similarities among ecotypes of Arabidopsis thaliana estimated by analysis of restriction Fragment length polymorphism- Theor. Appl.Genet. 86:1028-1032.

Krishnakumar, M.P., Valsalakumari, P.K. and Aravindakshan, M. 1992. Pollen production, fertility and viability in different nodes of the banana cultivars. Agricultural Research Journal of Kerala 30:53-57.

Krishnamoorthy, V., Kumar, N., Angappan, K. and Soorianathasundraram,. 2004. Evaluation of new banana hybrids against black Leaf streak disease. Infomusa Vol.13, No.1.

Marrewijk, G.A.M. 1994. Flowering biology and hybrid varieties. International course on applied plant breeding, Department of Plant Breeding, Agricultural University Wageningen, The Netherlands.

Marriot, H. and **Lancaster, P.A. 1983.** Bananas and plantains. In: Handbook of tropical foods. Chan HT(ed). pp.85-143 Marcel Dekker, New York.

Mourichion, X., Carlier, J. and **Foure E. 1997.** Sigatoka Leaf Spot Diseases. INIBAP fact sheets.

Mukasa, S.B. and Rubaihayo, P.R. 1993. Male fertility in Uganda banana germplasm. African Crop Science Journal 1(2):87-93.

Murguia, M. and Villasenor, J .L. 2003. Estimating the effect of the similarity coefficients and cluster algorithm on biogeographic classification. Ann. Bot. Fennici 40: 415-421.

O'Brien, T.P. and McCully, M.E. 1981. The study of plant structure: principles and selected methods. Termarcarphi: Australia

Oliveira, K.M. Laborda, P.R. Garcia, A.A.F., Zagatto P., M.E.A. and Souza, A.P. 2004. Evaluating genetic relationships between tropical Maize inbred lines by AFLP profiling. Heriditas 140:24-33

Orjeda, G. 1998. Evaluation Of Musa Germplasm For Resistance To Sigatoka Diseases And Fusarium Wilt. INIBAP Technical Guidelines3. International Plant Genetic Resources Institute, Rome, Italy. International Network For The Improvement Of Bananas And Plantains, Montpelier, France. ACP-EU Technical Centre For Agricultural And Rural Cooperation, Wageningen, The Netherlands.

Ortiz, R. and Vuylsteke, D. 1994. Inheritance of black Sigatoka resistance in plantain banana (*Musa spp.*) hybrids. Theor. Appl. Genet.89:146-152.

Ortiz, R. 1997. Secondary Polyploidy, Heterosis And Evolutionary Crop Breeding For Further Improvement Of The Plantain And Bananas (Musa Spp.L) Genome. Theor Appl Genet. 94:1113-1120.

Ortiz, R. 1997. Occurrence and inheritance of 2n pollen in *Musa*. Annals of Botany. 79:449-453.

Ortiz, R., Ferris, B. and **Vuylsteke, D.R. 1995.** Banana And Plantain Breeding. *In*; Bananas And Plantains Gowen, S. (Ed). Chapman and Hall, London, U.K. pp130-137.

Ortiz, R., Ulburghs, F. and Okoro, J. 1998. Seasonal variation of apparent male fertility and 2n pollen production in plantain and banana. HortScience 33:146-148.

Ortiz, R., Vuylsteke, D., Ferris, R.S.B., Okoro, J.U., Guessan, A.N., Hemeng, O.B., Yeboah, D.K., Afreh-Nuamah, K., Ahiekpor, E.K.S., Fouré, E. Adelaja, B.A., Ayodele, M., Arene, O.B., Ikiediugwu, F.E.O., Agbor, A.N., Nwogu, E., Okoro, G., Kayode, I.K., Ipinmoye, S., Akele, S. and Lawrence, A. 1997. Developing new plantain cultivars for Africa. Plant Varieties and Seeds 10: 39-57.

Panther, D.M. and Allen, F.L. 1995. Using best linear predictions to enhance breeding for yield in soybean II. Selection of superior crosses from a limited number of yield trials. Crop Sci. 35:405-410

Pancholi, N.C., Wetten, A. and Caligari, P.D.S., 1996. Detection of levels of somaclonal variation in *Musa* using molecular markers. In: Meeting on tropical plants. EUCARPIA, Meeting on tropical plants, Montpellier, pp 11-15.

Peloquin, S.J. and Ortiz, R. 1992. Techniques for introgressing unadapted germplasm to breeding population In: Plant breeding in the 1990's CAB int. Stalker, T. P. and Murphy, J. P. (eds) Pp 485-507 Wallingford, Oxon, U. K.

Pillay, M. Hartman, J. and Tenkouano, A. 2002. "Bananas and plantains: future challenges in *Musa* breeding" *In:* Crop Improvement, Challenges in the Twenty-First Century. Kang, M.S. (Ed.), Pp223-252 Food Products Press, New York.

Pillay, M., Goodwin, E., Nwakanman, D.C., Ude, G. and Tenkaouno, A. 2001. Analysis of genetic diversity and relationships in East African banana germplasm. Theor.Appl Genet.102:965-970. Springer-Verlag.

Pinochet, J. 1996. Review Of Past Research On *Musa* Germplasm And Nematode And Nematode Interaction. *In: New* Frontiers In Resistance Breeding For Nematodes, Fusarium And Sigatoka. INIBAP.

Posonen, H.L. and Kapyla, M. 1998. Pollen-pollen interactions in *Betula Pendula invitro*. New Pathologist 138:481-487

Purseglove, J. W. 1985. Tropical crops. Mono-cotyledons. English Language society. Longman pp.345-347

Rafalski, J.A. Tingy, S.V. 1993. Genetic diagnostics in plant breeding. RAPDs and Microsatelites. Trends Genet. 9: 275-280.

Rafalski, J.A., Morgante, M., Powell, W., Vogel, J.M. and Tingey, S.V. 1995. Generating and using DNA markers in plants. *In:* Non-mammalian genomic analysis: a practical guide. Birren B Lai E (eds) Academic press, California, USA, pp75-134.

Rohlf, F.J. 1997. NTSYSpc-Numerical taxonomy and multivariate analysis system. Exerter publication, New York.

Rowe, P. 1984. Breeding Bananas And Plantains. Plant Breeding Reviews.2: 135-155.

Rowe, P. and Rosales F. 1990. *Musa* Breeding At FHIA. *In*: The Improvement And Testing Of *Musa*: A Global Partnership.

Rukazambuga, N.D.T.M., Gold, C.S. and Gowen S.R. 1998. Yield Loss In East African Highland Bananas (*Musa spp.* AAA-EA) Caused By Banana Weevil Cosmopolites Sordidus Germer. Crop Protection 17: 581-589.

SAS. (2004) SAS Institute Inc. version 8, Cary, NC. USA

Sathiamoorthy, S. and Rao, V.N. 1980. Pollen production in relation to genome and ploidy in banana clones In: Proceedings of National Seminar, Banana Production Technology. Tamil Nadu Agricultural University, Coimbatore, India, pp. 46-49.

Sathiamoorthy, S., Rao, V.N. and Bhakthavatsalu C. 1984. Banana germplasm and breeding in India. Current problems on fruits and vegetables India, pp. 121.

Scott, Z. 2001. Starchy pollen in commelinoid Monocots. Annals of Botany. 87:109-116.

Ssebuliba, R.N. 2002. Fertility In East African Bananas. PhD Thesis Submitted to Makerere University, Uganda.

Ssebuliba, R.N. Rubaihayo, P., Tenkouano, A., Makumbi, D., and Talengera 2005. Genetic diversity among East African Highland bananas for female fertility. African Crop Science Journal 13(1):13-26

Sharma, A.K. and Sharma, A. 1972. Chromosome techniques. Butterworths and company London. University press. Pp190-193.

Sharrock, S. and Frison E. 1998. *Musa* production around the world- trends, varieties and regional importance. INIBAP Annual Report 1998, 42–47. INIBAP, Montpellier , France .

Sharrock, S. and Lusty, C. 2000. Nutritive value of banana. Pp. 28- 31. In: *Networking Banana and Plantain: Annual report 1999.* Montpellier, France.

Shepherd, K. 1960. Seed fertility of edible bananas. Journal of Horticultural Science 35:6-20

Shepherd, K. 1999. Cytogenetics of the genus Musa. International network for the improvement of Banana and Plantain. Montpelier, France.

Singh, J.R. 2003. Plant cytogenetics. CRC press Washington D.C (2nd edition).pp 90-91

Smith, J.S.C. and Smith, O.S. 1989. The description and assessment of distance between inbred lines of maize: II the utility of morphological, biochemical and genetic descriptors and a scheme for testing distinctiveness between inbred lines. Maydica 34151-161.

Sneath, P.H.A. and Sokal, R. 1973. Numerical Taxonomy. Freeman San Francisco California.

Speijer, P.R. 1993. Plantain and banana research at the International Institute of Tropical Agriculture. Hortscience 28: 873- 874, 970-971.

Speijer, P.R. and Kajumba, C. 1996. Yield loss from plant parasitic nematodes in East African highland bananas (*Musa* AAA) MusAfrica 10:26

Speijer, P.R. and De Waele, D. 1997. Screening Of Musa Germplasm For Resistance And Tolerance To Nematodes. INIBAP Technical Guidelines 1. International Plant Genetic Resources Institute, Rome, Italy. International Network For The Improvement Of Bananas And Plantains, Montpelier, France. International Institute For Tropical Agriculture, Ibadan, Nigeria.

Stover R.H. and Dickson J.D. 1970. Leaf spot of bananas caused by Mycosphaerella musicola-methods of measuring spotting prevalence and severity. Tropical Agriculture TRINIDAD 47:289-302.

Stover, R.H. and Simmonds, N.W. 1987. Bananas. Tropical agriculture series 3rd edition. Longman scientific and technical publications.Pp72.

Tenkouno, A., Ortiz, R. and Vuylsteke, **D. 1998.** Combining ability for yield and plant phenology in plantain derived populations. Kluwer academic publishers Euphytica104: 151-158.

Tenkouano, A. ,Crouch, J.H., Crouch, H.K., Vuylsteke, D. and Ortiz, R. 1999. Comparison of DNA marker and pedigree-based methods of genetic analysis of

plantain and banana (Musa spp.) clones. Estimation of genetic relationships. Theor Appl Genet 98:62-68

Tezenas Du Montcel, H., Carreel, F., Bakry, F. and Horry, J.P. 1996. Improve The Diploids: The Key To Banana Breeding. In: New Frontiers In Resistance Breeding For Nematodes, Fusarium And Sigatoka. Inibap pp119-128.

Tushemereirwe, W.K. 1996. Factors influencing expression of leaf spot diseases of highland bananas in Uganda. PhD Thesis University of Reading, UK.

Tushemereirwe, W.K. and Bagabe, M. 1999. Review Of Disease Distribution And Pest Status In Africa. Pp139-147. *In*: Mobilizing IPM For Sustainable Production In Africa. Proceedings Of A Workshop On IPM Held In Nelspuit, South Africa-23-28 November 1998. (Frision.E.A, C.S.Gold, E.B Karamura And R.A.Sikora, Eds.).

Tushemereirwe, W.K., Kangire, A. Smith, J., Ssekiwoko, F., Nakyanzi, M., Kataama, D., Musitwa, C. and Karyaija, R. 2003a. An outbreak of bacterial wilt on banana in Uganda. Infomusa Vol.12 No.2. Pp6-8.

Tushemereirwe, W.K., Kashaija, I.N., Tinzara, W., Nankinga, C. and New, S. 2003b. Banana production Manual. Second edition.

Tushemereirwe, W.K. and Waller, J.M. 1993. Black leaf streak (*Mycosphaerella fijiensis*) and associated diseases bananas in Uganda. *Plant pathology* 42:471-472

Ude, G., Pillay, M., Ogundiwin, E. and Tenkouno, A. 2003. Genetic diversity in an African plantain core collection using AFLP and RAPD markers. Theor. Appl. Genet.107:248-255

Van Asten, P.J.A. Gold, C.S., Wedt, J., De Waele, D., Oketch, S.H.O., Ssali, H. and Tushemereirwe, W.K. 2004. The contribution of soil quality to banana yield problems and its relation with other banana yield loss factors in Uganda. Proceedings African crop science society conference.

Vakili, N.G. 1968. Response of *Musa acuminata* species and edible cultivars to infection by *Mycosphaerella musicola*. Trop. Agr. (Trinidad) 45: 13-22.

Vuylsteke, D. and Ortiz, R. 1995. Plantain- derived diploid hybrids (TMP2x) with black Sigatoka resistance. Hortscience 30: 147- 149.

Vuylsteke, D. 1998. Shoot-tip culture for the propagation, conservation and distribution of *Musa* germplasm. International Institute of Tropical Agriculture. Ibadan. Nigeria.

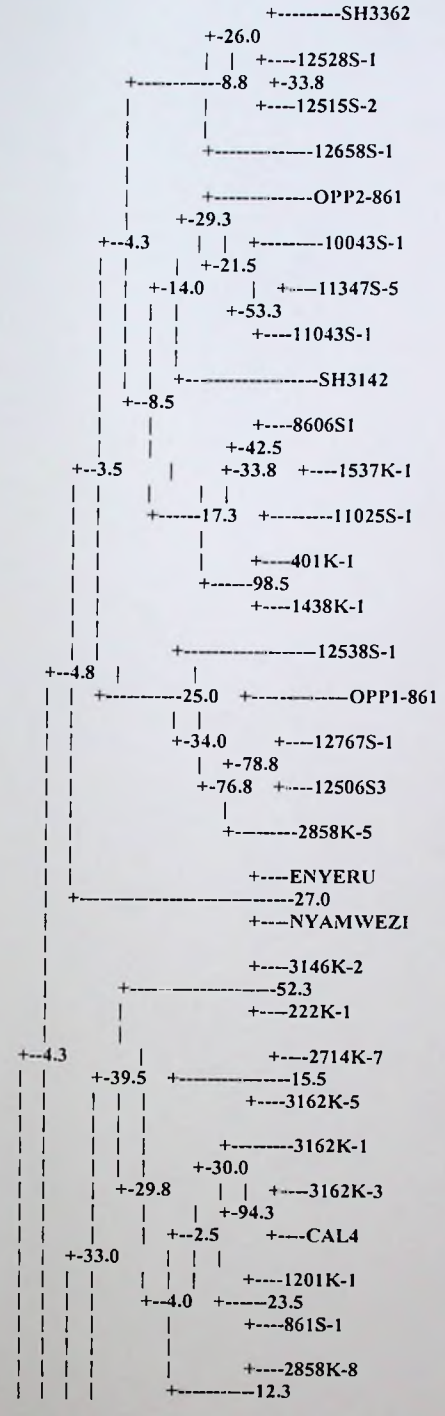
Vuylsteke, D., Ortiz, R., Ferris. R.S.B. and Crouch, J.H. 1997. Plantain improvement. Plant breeding reviews. 14:267-320

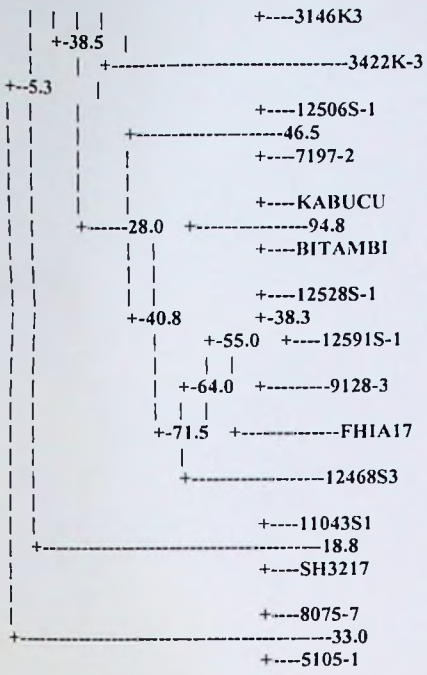
Waikai, Y. 2001. GGEbiplot pattern explorerCRs: A Graphical Tool for Breeders, Geneticists, and Agronomists. CRC Press.

Williams, J.G.K., Kubelik, A.R., Livak. K.J., Rafalski, J.A. and Tingey, S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res. 18*: 6531-6535.

Appendix 1

A Consensus cluster for the synthetic diploid hybrids




3146K3
38.5
3422K-3
5.3
12506S-1
46.5
7197-2
KABUCU
28.0
94.8
BITAMBI
12528S-1
40.8
38.3
55.0
12591S-1
64.0
9128-3
71.5
FHIA17
12468S3
11043S1
18.8
SH3217
8075-7
33.0
5105-1