Breeding investigations for black Sigatoka resistance and associated traits in diploids, tetraploids and the triploid progenies of bananas in Uganda

By

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Thesis abstract

Reduced banana yield owing to black Sigatoka *Mycosphaerella fijiensis* Morelet is a threat to the livelihoods of Ugandan subsistence farmers who depend entirely on the banana crop for food security. The objectives of this investigation were to: (i) assess farmers' knowledge of black Sigatoka disease in central Uganda; (ii) document the qualities farmers would desire in the banana genotypes to be developed for black Sigatoka resistance; (iii) appraise the methods for assessing black Sigatoka resistance in diploid banana populations; (iv) determine the phenotypic variation for black Sigatoka resistance and agronomic traits in diploid and tetraploid bananas; (v) determine the influence of tetraploid and diploid parents on the black Sigatoka resistance and agronomic traits in the triploid progenies; and (vi) evaluate 2x by 2x banana progenies for yield and black Sigatoka resistance.

A survey that focused on low and medium banana production zones in Uganda established that there was limited awareness of black Sigatoka disease as a constraint on banana production in the areas surveyed. It was also established that farmers liked local bananas because of their superior taste, early maturity, and marketability. There were farmers who had been exposed to new black Sigatoka resistant materials but never liked these new banana materials because of poor taste and lack of market. Farmers desired new banana materials with good taste on cooking, heavy bunches, resistance to pests and diseases, drought tolerance, and early maturing capacity in that order. The results indicated that the banana farmers in Uganda attached more importance to food quality attributes than to production attributes especially when considering new banana materials. This suggested that farmers mainly grow bananas for consumption.

Three black Sigatoka assessment methods, youngest leaf spotted, disease development time and area under disease progress curve (AUDPC) were appraised using a diploid population. All the three methods were able to classify the diploid accessions into resistant and susceptible clones. The cultivar rankings of AUDPC correlated strongly with the rankings of disease development time. The cultivar rankings of AUDPC correlated positively with the rankings of youngest leaf spotted method. The youngest leaf spotted at flowering and AUDPC predicted significantly total number of leaves at flowering (R^2 = 0.53). Overall AUDPC had the highest

coefficient of determination (R^2=0.84) in assessment of banana diploids for black Sigatoka resistance indicating that it accounted for the highest variation in disease response observed among the diploid clones. From this investigation it was recommended that AUDPC should be used to assess resistance on black Sigatoka in *Musa* species.

A phenotypic analysis on the diploid and synthetic tetraploids, and a molecular analysis using RAPD markers on the tetraploid population were conducted. Results indicated that the diploid population had significant ($P<0.001$) variation for plant height, plant girth, days from flowering to harvest, bunch weight, number of suckers, youngest leaf spotted, total leaves at flowering, area under disease progress curve, and number of functional leaves at harvest. Principal component analysis showed that plant height and girth explained most of the variation observed in the diploid population. In the tetraploid population, significant differences were observed for plant height, plant girth, and number of suckers ($P<0.05$). In the tetraploids principal component analysis, indicated that youngest leaf spotted and total leaves at flowering had higher loadings on principal component one. Genetic distances computed from RAPD markers indicated limited genetic variability in the tetraploid population.

Another investigation was also carried out to determine the influence of tetraploid and diploid parents on black Sigatoka resistance and agronomic traits in the triploid progenies generated from tetraploid-diploid crosses. The results indicated that diploids transferred black Sigatoka resistance to triploid progenies as measured by disease development over time, the number of functional leaves at flowering and at harvest. On the other hand, the female synthetic tetraploids influenced plant height and bunch weight in the triploid progenies generated from tetraploid-diploid crosses as observed from triploid progeny correlations and parent-offspring regressions. Therefore, it is important to select tetraploids with heavy bunch weights to generate high yielding triploids in tetraploid-diploid crosses.

Lastly, this thesis investigated the relationship between bunch weight and black Sigatoka resistance traits in 2x by 2x progenies generated using a random polycross design. Phenotypic correlations revealed strong positive relationships between bunch weight with total leaves at flowering, youngest leaf spotted, plant girth, and

days from planting to flowering among the 2x by 2x progenies. Linear regression analysis indicated that girth, total fingers and finger length significantly predicted bunch weight (R^2=0.67). However, days from planting to flowering, and total leaves at flowering had strong indirect effects on bunch weight via plant girth. The results imply that selection for parents with good combining ability for girth, finger length and total fingers can improve bunch weight in a diploid population.

Declaration

I, Alex Barekye declare that:

1. The research reported in this thesis, except where otherwise indicated, is my own original research.
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As the candidate's supervisors, we agree to the submission of this thesis

Professor Pangirayi Tongoona ………………………………Date……………………….

Dr. John Derera ………………………………………………..Date ………………………

Professor Mark D. Laing ………………………………………Date ……………………...

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Dedication

This thesis is dedicated to

My dear wife
Grace

And

Our children
Jonathan and Anna

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Introduction

Significance of bananas for food security in Uganda

The importance of bananas in Africa, and Uganda in particular, is indicated by the production figures, the role of bananas in the diet and the extent of cultivation. Sub-Saharan Africa produces about 35% of the world's bananas and plantains, and it is estimated that 25% of the carbohydrate of approximately 70 million people in Africa comes from bananas (INIBAP, 2001; FAOStat, 2007). Uganda alone accounts for about 15% of global output, with an annual banana production estimated at 10 million tonnes. It is estimated that 75% of Ugandan farmers grow bananas on 1.5 million hectares (Zake et al., 2000) or 38% of utilised arable land (FAO, 2004). The bananas produced are mainly for local consumption with an estimated per capita consumption of 300 kg $person^{-1}$ $year^{-1}$ - the highest in Africa (INIBAP, 2001).

The banana is the most important food security crop in Uganda. The areas where banana production is the main farming activity are never hit by famine because the crop produces fruits throughout the year, and high banana yields ensure a continuous supply of dietary carbohydrate. In addition, the perennial nature of banana makes it the crop least affected by drought or social instability. Above all, it is potentially the highest yielding and least labour demanding food to produce, provided the plantation lasts for more than 5 years to enable the farmer to recover the initial high cost of establishing it.

Bananas are important not only for food security in Uganda. In terms of rural revenue, the banana is the second most important cash crop after coffee, contributing 5-22% of the national agricultural rural revenue (Embrechts et al., 1996). Supportive data show that banana is the second best crop after coffee for a return on family labour in south western Uganda (Ssenyonga et al., 1999).

The banana crop also occupies an important position in the socio-economy of the country. A culture based on banana cultivation and use in various ways has evolved over time, making the banana an indispensable crop in the lives of Ugandans. For example, banana food (Matooke) and banana juice have cultural functions at some stages of wedding ceremonies and funeral rites. Some of the East African highland

banana cultivars are even used for medicinal purposes in south-western and central Uganda.

Almost all components of the banana plant have found some use in the daily lives of the Ugandan farm household. The fruits are steamed or boiled and consumed as the main meal. They may be ripened for use as a dessert, squeezed to provide juice, which is drunk fresh, or fermented as local beer/wine. The wine may be distilled into a spirit (Waragi), which is processed and sold locally, or exported as "Uganda Waragi". The banana pulp may be dried and powdered for use in bakery products, though this option is yet to be developed. Furthermore, the dried pulp may be stored as a food reserve to guard against famine.

The vocabulary of the indigenous Ugandan languages reflects the importance of bananas. Whereas bananas are usually simply bananas in other languages, Ugandans recognise various categories of bananas; the commonly grown bananas are referred to as cooking bananas, brewing bananas, sweet bananas, and roasting bananas. East African highland bananas popularly known as “matooke”, represent 90% of the bananas grown in Uganda (Gold et al., 2002), are unique in taste, and food colour, and are found only in the Great Lakes region of Eastern Africa. Banana consumers attach such importance to “matooke” bananas that in Luganda, one of the Bantu languages, the word “matooke” means food. Thus when a meal is served without “matooke”, it literally means there is no food.

Constraints on banana production in Uganda

Despite the importance of banana to Uganda, its production is below its potential and declining. Currently banana production stands at an average of about 8 to 10t ha^{-1} although there is the potential for producing 60t ha^{-1}. Banana bunch weights at farm level have dropped from 60kg to 10kg, or even less. Plantation life has been shortened from 30 to 50 years to 4 years. This severe decline has put banana consumers at risk of food insecurity as banana is such an important staple food. The low banana yields have been due to a number of factors, including pests (banana weevils and banana nematodes), drought, poor soils, and diseases (*Fusarium* wilt, banana bacterial wilt, and black Sigatoka), among others (Gold et al., 1994; Tushemereirwe et al., 2003).

However, black Sigatoka is the major constraint causing yield reduction on cooking bananas particularly in central Uganda where it is now considered a threat to food security. The losses happen because the disease attacks the banana leaves, causing a decrease in the functional leaf area. Reduction in the functional leaf area results in a drastic decline in quality, and quantity of the fruit. Fruits from infected plants ripen prematurely before proper filling. Black Sigatoka has been reported to cause a yield loss of up to 37% on bananas in Uganda (Tushemereirwe, 1996). Because the disease is most devastating in humid and warm conditions below 1200m above sea level, its major impact has been felt in central Uganda where these conditions are predominant. Black Sigatoka has caused a severe decline in banana production in this area to the extent that the banana consumers have shifted to cassava production (Rubaihayo and Gold, 1993). However, cassava needs replanting every season, unlike bananas so it cannot effectively sustain the daily food requirements of farmers of the area. For sustainable food security in central Uganda, there is a need to address black Sigatoka on bananas.

There has been one failed intervention to address the problem of black Sigatoka. As a medium term intervention against reduced yields, disease resistant materials were accessed from international breeding programmes and given to farmers particularly those in central Uganda (Nowakunda et al., 2000; Rutherford and Gowen, 2003; Nowakunda and Tushemereirwe, 2004). These farmers grew the local bananas alongside the disease resistant materials. However, ultimately, they never adopted the new disease resistant high yielding materials.

The research questions that arose from the failure of this intervention were whether farmers understood the impact of black Sigatoka on East African highland bananas, and also whether there were important traits in the susceptible East African highland bananas that were missing in the black Sigatoka resistant materials leading to their rejection. One of the objectives of the research project being reported therefore became to assess farmers' perceptions of black Sigatoka disease and their preferences for important traits they would like to have in black Sigatoka resistant materials to pre-empt the rejection of future varieties.

Methods for assessing black Sigatoka resistance in bananas

There is need to assess black Sigatoka resistance in order to identify resistant genotypes that would be developed through breeding. However, existing methods have limitations. Vakili (1968) suggested using youngest leaf spotted, done at flowering, to assess black Sigatoka resistance among *Musa* genotypes. However, diploid materials need to be assessed before flowering so that they can be analysed and the best selected for participation in a recurrent selection programme. An alternative, stable and reliable method to achieve this should be developed.

Other methods have been tried. Mobambo et al. (1997) investigated disease development in *Musa* germplasm at different ages under natural conditions. They noted that disease incubation and evolution times were unable to predict yield in *Musa* genotypes. Twizeyimana et al. (2007) used in-vitro plantlets and detached leaves to screen *Musa* genotypes for black Sigatoka resistance. This method could not be adopted because the development of disease symptoms in detached leaves was inconsistent with symptom development in intact plants (Liu et al., 2007). In other crops, disease assessment is normally done by computing disease severity data using a formula (Shaner and Finney, 1977) to convert the disease severity into area under disease progress curve (AUDPC). The AUDPC has been widely used to assess damage by rusts (Holland and Munkvold 2001; Kushwaha et al., 2007) in legumes, and leaf spots (Asea et al., 2002) in cereal crops. Another objective of this research investigation was therefore to compare the efficiency of AUDPC, youngest leaf spotted and disease development time methods in discriminating banana diploid genotypes according to black Sigatoka resistance. This is necessary in order to identify an early and reliable assessment technique that can identify small differences among the *Musa* genotypes. This will help in early selection of materials for the recurrent selection programme.

Banana germplasm in Uganda

The unique East African highland bananas are homogeneous, although small differences have occurred among them as a result of somatic mutations (De Langhe, 1969). Karamura (1998) characterised the East African highland bananas using

morphological traits categorising them into five major clone sets: *Musakala, Nfuuka, Nakitembe, Nakabululu*, and *Mbidde*. Tugume et al. (2002) carried out a molecular characterisation and found no major differences between the molecular and morphological characterisation of the East African highland bananas. Ssebuliba et al. (2006) evaluated the different clone sets for female fertility and some cultivars from the *Nfuuka* clone set were found to be female fertile. These clones were crossed with a common male parent, Calcutta 4. A number of tetraploids were generated, evaluated, and 16 were selected for further improvement (Ssebuliba et al., 2006). These 16 genotypes had disease resistance, high yield and some had acceptable food qualities such as colour, taste, texture, and aroma. However, they were not a perfect match for East African highland bananas. The tetraploids could not be promoted to farmers because they had residual fertility which would cause them to form seeds and this would make them unacceptable to farmers.

Because the synthetic tetraploid banana materials conserved some unique traits from East African highland bananas, they have been used with improved diploids to generate secondary triploids[1] to be promoted to farmers. Considering how these materials were generated, and their importance as breeding materials, an objective of the research presently being reported on became to identify the amount of genetic variation for black Sigatoka resistance and agronomic traits within these tetraploids. It was also important to carry out a genetic analysis of these tetraploids to find out how much of the disease and agronomic traits tetraploids pass on to secondary triploids.

In banana breeding, diploids are used as sources of disease resistance (Vakili, 1968; Swennen and Vuylsteke, 1993; Vuylsteke, 2001). To make progress, especially in the improvement of quantitative traits, it is essential to have large genetic variation in the breeding or base population. An objective of the study presently being reported on was thus to establish how much variation in disease resistance and agronomic traits existed in the diploids and how these traits were transmitted to triploids when diploids were crossed with tetraploids. A final focus was the determination of the relationship

[1] Secondary triploids are derived from natural triploid banana: 3x by 2x=4x; 4x by 2x=3x (secondary triploids)

between bunch weight and black Sigatoka and agronomic traits in progenies of inter-diploid crosses as the basis for designing better strategies for improving diploids.

Objectives of the study

The overall aim of this breeding investigation was to contribute to improved food security by assessing farmer desired traits, the variation for black Sigatoka resistance in banana breeding populations, and inheritance of traits in banana diploids and tetraploids that would assist in developing acceptable black Sigatoka resistant triploid bananas.

The specific objectives of the study were to:

a) assess farmers' knowledge of black Sigatoka disease in central Uganda, and document the qualities they would desire in the banana genotypes to be developed for black Sigatoka resistance;

b) compare the efficiency of three methods: youngest leaf spotted, disease development time, and area under disease progress curve, in the assessment of black Sigatoka resistance in a diploid population;

c) determine the phenotypic variation for black Sigatoka resistance and agronomic traits in diploid (AA) and tetraploid (AAAA) bananas;

d) determine the influence of tetraploid and diploid parents on black Sigatoka resistance and agronomic traits in the triploid progenies generated from tetraploid-diploid crosses, and

e) determine the relationship between bunch weight and black Sigatoka resistance traits in 2x by 2x progenies.

Hypotheses tested

The following hypotheses were tested:

a) farmers in central Uganda are aware of the importance of black Sigatoka disease, and have some specific desirable traits they want to be incorporated in new banana cultivars in addition to black Sigatoka

resistance. These traits might not be known by the banana improvement programme in Uganda;

b) youngest leaf spotted, disease development time, and area under disease progress curve methods are equally effective in discriminating the banana genotypes according to black Sigatoka resistance;

c) there is a large genetic variation for black Sigatoka resistance and desired agronomic traits in the diploid (AA) and tetraploid (AAAA) populations of bananas in Uganda which can be exploited to generate new cultivars with farmer preferred traits;

d) the diploids and synthetic tetraploids are equally important in determining agronomic and black Sigatoka resistance traits in triploid progenies generated from tetraploid-diploid crosses, and

e) there are strong and positive relationships between bunch weight and black Sigatoka resistance traits in 2x by 2x progenies.

Thesis structure

This thesis is structured as follows:

Chapter One	Literature review
Chapter Two	Assessment of farmers' perceptions of black Sigatoka disease and preferred traits in bananas in Uganda
Chapter Three	Appraisal of methods for assessing black Sigatoka resistance in diploid banana populations
Chapter Four	Variation in diploid (AA) and tetraploid (AAAA) banana populations for black Sigatoka resistance and agronomic traits
Chapter Five	Genetic studies of black Sigatoka resistance and associated traits in tetraploid-diploid banana crosses
Chapter Six	Evaluation of 2x by 2x banana progenies for yield and black Sigatoka resistance
Chapter Seven	Overview of the investigation and implications

The thesis is written in a composite format and therefore there is overlap of both information and references across chapters.

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Chapter one
Literature review

1 Introduction

Black Sigatoka is a major disease reducing banana yields in Uganda. There are cultural and chemical control measures available, but they have not been effective in eliminating the disease. The use of host plant resistance is considered the most effective way of controlling the disease. However, the development of disease resistant banana genotypes requires knowledge and understanding of black Sigatoka disease, the banana breeding populations, and the challenges of improving the banana populations for black Sigatoka resistance. It is also essential to review the progress made on banana improvement, so that lessons could be learnt about the banana improvement process. This literature review has had to focus largely on the work done on plantains owing to limitations of the literature available on bananas. However, as the same production constraints and breeding approaches occur for both bananas and plantains, combining the available literature on bananas and plantains enabled the identification of the gaps to be addressed in the investigation being reported on.

1.1 Origin of bananas

Knowledge of the origin, diversity, genomic and ploidy differences of bananas and plantains is very important in *Musa* improvement. Their centre of origin is believed to be in Southeast Asia. This proposed centre of origin maintains the greatest diversity of wild *Musa* species (Simmonds, 1962; Purseglove, 1972). The two wild *Musa* species, *Musa acuminata* Colla, (designated as A genome) and *Musa balbisiana* Colla, (designated as B genome) are the origins of edible bananas (Simmonds 1962;1966). Although there could be other sources of A genome, Cavendish bananas (AAA) were confirmed, (using amplified fragment length polymorphism markers) to have their origin in *Musa acuminata* (Roboin et al., 2005). The two species, *M. acuminata* and *M. balbisiana,* hybridised to form a wide range of ploidies such as AB, ABB, AAB, AABB, and AAAB bananas. The existing cultivars are usually classified into three genome groups; AAA, AAB and ABB. However, the varieties grouped in the same genomic category can be very different (Simmonds, 1962; Stover and Simmonds, 1987). Thus

the AAA group consists of both sweet dessert bananas that are eaten raw when ripe and bananas of the East African highlands that require cooking before they can be eaten. Likewise, the plantains dominate the AAB group, which also contains dessert bananas such as Silk and Prata. In contrast, varieties in the ABB group are predominantly used for cooking although some may be eaten raw as dessert bananas usually when overripe. Because the greatest diversity of plantains (AAB) are found in West Africa and cooking and beer bananas (AAA) in the East African highlands, these two areas are referred to as secondary centres of diversity of these specific bananas (Purseglove, 1972; Swennen and Vuylsteke, 1991).

There is no clear distinction in definition between bananas and plantains. Bananas are referred to as the sweeter fruits that can be eaten uncooked, while plantains are referred to as the starchy fruits that must be cooked before being eaten (Simmonds, 1966; Swennen and Vuylsteke, 1987). Plantains are characterised by the orange yellow colour of the pulp when ripe, while colour in edible bananas commonly varies from white to cream/yellow and there are even varieties with bright orange colour for example Fe'i bananas. Plantains have less sugar than bananas and, on average, have a moisture content of about 65%, while bananas have 83% moisture of fresh weight. Hydrolysis converts starches faster in bananas than in plantains; this could explain why we have beer bananas but not beer plantains. Plantains have also been reported to have higher dietary fibre and thicker skins (fruits) than bananas (Grab Em Snacks, 2009).

1.2 Black Sigatoka disease and *Musa* species

In order to contribute to the development of host plant resistance, the following areas of knowledge about black Sigatoka were reviewed: history, symptoms, disease epidemiology and spread, impact, black Sigatoka resistance in *Musa* species, mechanisms of resistance to black Sigatoka, and the different methods that have been used to assess the disease.

1.2 1 History of black Sigatoka

The origin of black Sigatoka (*Mycosphaerella fijiensis* Morelet) is believed to be the same centre of diversity of bananas in Southeast Asia (Stover, 1978). From its centre

of origin, the disease is believed to have spread to other regions through the movement of infected material across continents (Rivas et al., 2004). The disease was first observed in the Sigatoka district, Fiji, in 1963 (Rhodes, 1964) hence the name. Subsequently, black Sigatoka was observed in Honduras in 1972 (Mourichon and Fullerton, 1990) and in southern Mexico and Central America (Stover, 1980; 1983) between 1977 and 1980. In Africa, the disease was first observed in Zambia in 1973 and Gabon in 1978 (Gauhl et al., 2000). Black Sigatoka later spread to surrounding countries and it was observed in the Democratic Republic of Congo in 1988 (Mobambo and Naku, 1993). In East Africa, the disease was first observed in Burundi and Rwanda in 1986 (Sebasigari and Stover, 1988); then in 1990 the disease was reported in Uganda (Tushemereirwe and Waller, 1993).

Molecular characterisation has enhanced the understanding of the *Mycosphaerella fijiensis* pathogen. For instance, Hayden et al. (2003) studied the genetic structure of *Mycosphaerella fijiensis*, the causal agent of black Sigatoka, and detected a great diversity of the pathogen in Papua New Guinea and the Pacific islands, thus confirming its origin. By contrast, low levels of genetic diversity were detected in Africa and Latin America isolates. Rivas et al. (2004) concluded that genetic drift could have been the main evolutionally factor shaping the population structure at the continental level. Earlier, Pasberg-Gauhl et al. (2000) had also associated the high levels of genetic diversity of the pathogen in Honduras and Costa Rica with the date of entry of pathogen into these countries. Molecular and chronological events of the pathogen confirm that it originated from the centre of diversity of bananas, spreading world wide at different dates. The occurrence of black Sigatoka in different parts of the world led to the initiation of research on *Musa,* especially in search of host plant resistance (Ploetz, 2000).

1.2.2 Symptoms of black Sigatoka

The first symptom of black Sigatoka is the appearance on the lower leaf surface of pale yellow (Sigatoka) or dark-brown (black leaf streak, black Sigatoka) streaks 1-2 mm long. These streaks enlarge into dark-brown to black advanced streaks, which later merge into spots with a dark-brown to black centre often surrounded by yellow halos (Stover and Simmonds, 1987). Foure (1994) identified six main stages of symptom

development as follows: yellow spots appear on the underside of the banana leaf near the apex; the spots turn rusty brown and streaking of the leaf is observed; the streaks join together and cause leaf death (necrosis); the brown streaks expand to form brown spots; oval spots turn black, surrounded by a yellow halo, and cover both sides of the leaf; and the centre of the spot dries and changes colour to grey. The banana genotypes differ in rates of disease symptom development. This variation has been used to develop black Sigatoka assessment protocols such as the youngest leaf spotted and disease development time. The understanding of symptom development in the host can be beneficial in the identification of genotypes that exhibit a hypersensitive disease reaction. Therefore knowledge of disease symptoms becomes important in the selection of black Sigatoka resistant genotypes.

1.2.3 Black Sigatoka disease epidemiology and spread

The disease Sigatoka is caused by two closely related pathogens, *Mycosphaerella musicola* Leach (the cause of yellow Sigatoka) and *Mycosphaerella fijiensis* Morelet (the cause of black leaf streak-black Sigatoka). Although the two pathogens may occur on the same host, black Sigatoka is more virulent than yellow Sigatoka (Ploetz, 1998). Black Sigatoka produces more ascospores and has more sexual cycles per year; hence it has the ability to displace yellow Sigatoka when the two pathogens co-exist (Stover, 1980). Yellow Sigatoka is a cool environment disease, and the results of a rapid rural appraisal in Uganda by Rubaihayo (1991) identified only black Sigatoka as a major disease, especially at medium and low altitudes. However, the pathogen that causes black Sigatoka has been reported to adapt to cool environments (Gauhl et al., 2000) attacking genotypes previously resistant to yellow Sigatoka (Romero, 2002). This might imply that suppressing black Sigatoka may lead to resurgence of yellow Sigatoka. The breeding approach advocated for in this investigation is to accumulate polygenic resistance against the pathogen that causes black Sigatoka. The use of proper selection strategies should be able to generate materials that are resistant to both pathogens. However, this study will give priority to controlling black Sigatoka because yellow Sigatoka is not predominant in the banana growing areas of Uganda.

Black Sigatoka is spread by both ascospores and conidia. Ascospores require high relative humidity (98-100%) for germination, while conidia germinate in a wide range of

environments (Jacome et al., 1991; Jacome and Schuh, 1992). Ascospores are smaller in size, and more abundant than conidia, therefore they are the most important in spreading disease in banana plantations (Stover, 1980). Ascospores and conidia are wind borne and the presence of water (rain or dew) facilitates the release of inoculum (Stover, 1970). According to Ploetz (2000), the minimum, optimum, and maximum temperatures for infection are 12°C, 27°C, and 36°C, respectively. The temperature conditions in banana growing areas of central Uganda are conducive for disease infection; that is why black Sigatoka is a serious problem in the region.

Apart from the environment, the genetic diversity of the pathogen and host genotype influences disease epidemiology. Fullerton and Olsen (1995) tested the pathogenic variability of different isolates of *M. fijiensis* and found that isolates from Papua New Guinea and the South Pacific were more virulent than isolates from Central America and Africa. Hayden et al. (2003) found much genotypic diversity of allele frequencies within *Mycosphaerella fijiensis* isolates among populations isolated from different regions. The diverse isolates also caused different damage levels on *Musa* genotypes. For example, a strain from Western Samoa was virulent on Calcutta 4, while strains from other regions reacted differently on this variety. Fullerton and Olsen (1995) concluded that the pathogen that causes black Sigatoka is heterogeneous in its virulence to *Musa* species. Meredith et al. (1973) established that the pathogen *M. fijiensis* has both sexual and asexual forms of reproduction. The sexual recombination of the pathogen could thus have contributed to the high levels of variability among the isolates. The sexual recombination together with the polycyclic nature of *M. fijiensis* may lead the pathogen to evolve thus overcoming the disease resistance in the host. Therefore, breeding approaches like recurrent selection that accumulate quantitative and durable resistance should be implemented.

1.2.4 Impact of the black Sigatoka disease

Black Sigatoka disease causes substantial losses in banana production. This happens because the disease attacks the leaves causing a decrease in functional leaf area. This reduction in functional leaf area results in a decline in the quality and quantity of the fruit because fruits from infected plants ripen prematurely before proper filling. In West Africa, yield losses due to black Sigatoka were reported to be 30-50% on plantains

(Mobambo et al., 1993). In Uganda, a yield loss of up to 37% was reported on bananas (Tushemereirwe, 1996). The predominant East African highland bananas are highly susceptible to the disease (Tushemereirwe, 1996; Craenen and Ortiz, 2002).

Stover and Simmonds (1987) reported that 27% of production costs in dessert banana plantations are spent on controlling black Sigatoka through spraying. In Uganda and Eastern Africa, the resource limited farmers cannot afford to spray the bananas, yet their livelihoods depend heavily on bananas. Black Sigatoka has caused severe decline in banana production to the extent that the banana consumers have shifted to alternative food sources like cassava (Rubaihayo and Gold, 1993). However, the alternative food sources are annual crops that cannot sustain food security of the subsistence farmers. For sustainable food security in Uganda, there is need to address black Sigatoka disease on bananas.

The available resistant hybrids (Rowe and Rosales, 1993; Vuylsteke et al., 1993) have failed to meet consumer requirements (Nowakunda et al., 2000; Rutherford and Gowen, 2003; Nowakunda and Tushemereirwe, 2004). Therefore there is need to breed acceptable black Sigatoka resistant banana hybrids to meet the food demands of subsistence farmers who depend on bananas in Uganda.

1.2.5 Assessment of black Sigatoka

An understanding of the host-pathogen-genotype interaction requires good disease assessment protocols. Such protocols enable the application of disease quantification in disease management when developing host plant resistance. For example, the incubation period of black Sigatoka varies with the type of banana cultivar and environmental conditions (Gauhl et al., 2000). To use such knowledge to develop banana genotypes which delay the onset of disease symptoms, irrespective of environmental conditions, requires proper understanding of the disease and the evaluation of weaknesses in different disease assessment procedures.

Vakili (1968) reported on the use of one such protocol in the assessment of the black Sigatoka resistance of different genotypes with the use of the youngest leaf spotted (YLS). This assessment is done at flowering, when the youngest leaf, with at least 10 necrotic lesions, of a recently flowered plant is counted. With this scale, the higher the number of the youngest leaf spotted, the more resistant the genotype. Meredith (1970)

developed a scale for assessing black Sigatoka resistance based on the number of functional leaves at harvest.

According to this scale, a plant with

6 or more functional leaves at harvest is very resistant,

4-6 functional leaves is resistant,

2-3 functional leaves is moderately resistant, and

0-1 functional leaves is very susceptible.

Later, the International Institute of Tropical Agriculture, IITA (1989) developed a scale for assessing resistance to black Sigatoka based on the number of functional leaves at flowering. With this assessment technique, a genotype with

8 or more functional leaves at flowering is resistant,
7 and fewer leaves at flowering is susceptible.

An increase in disease pressure may cause plants to retain fewer leaves, therefore resistant and susceptible checks are normally included in disease assessment trials as references. Youngest leaf spotted delays the selection of plants for participation in crosses to generate the next population. There is therefore a need to investigate other assessment techniques which could assess the disease response of the genotype before flowering.

A further disease assessment protocol involves measuring the percentage area of leaf damage of each leaf of a plant. Stover (1971) used a scale of 0-4 to assess black Sigatoka with this protocol. The disease damage was graded thus

0 = no spotting/necrosis,
1 = disease spots less than 5%,
2 = 5-15% necrotic tissue,
3 = 16-33% necrotic tissue,
4 = over 33% of leaf with necrotic tissue.

Burt et al. (1999) modified Stover's' scale by including 2 extra grades:

4 = 34-50% necrotic tissue, and
5 = more than 50% necrotic tissue.

The disease severity assessment by Stover (1971) was not restricted to the plant phenological stage, therefore this assessment could be implemented early (six months after planting), and then disease severities could be converted into area under disease progress curve.

Mobambo et al. (1997) investigated host response to black Sigatoka in *Musa* gemplasm of different ages under natural inoculum conditions. Black Sigatoka development on each new emerging rolled leaf (cigar leaf) was evaluated every three days. On young and mature plants records were taken of incubation time (days between emerging cigar leaf stage and appearance of symptoms); disease development time (days between emerging cigar leaf stage and appearance of spots); YLS (number of leaf bearing spots with dry centres); and life time of leaf (days between the cigar leaf stage and leaf death). Results indicated that incubation time was not useful in quantifying the host response of different *Musa* genotypes. However, there were significant correlations for disease development time, youngest leaf spotted, and life-time of leaf between young and mature plants. The research established that it was difficult to use early assessment methods such as incubation time and disease development to predict yield performance (Mobambo et al., 1997).

Twizeyimana et al. (2007) investigated a rapid screening technique of *Musa* species' susceptibility to black Sigatoka using in-vitro plantlets and detached leaves. The leaves were inoculated with mycelial fragments and conidial suspensions of *Mycosphaerella fijiensis,* the causal pathogen of black Sigatoka. Significant differences were observed among genotypes in leaf area, incubation time, and symptom evolution time. The two assays were found to be rapid and effective in space utilisation and screening *Musa* genotypes for black Sigatoka resistance. However, Lui et al. (2007) reported that detached leaf pieces did not support the development and response of symptoms as they would be developed in intact plants.

The advantage of youngest leaf spotted in predicting the yield loss in bunch weight of bananas due to black Sigatoka disease (Craenen and Ortiz, 1998) has made it a preferred disease assessment method. However, with YLS, disease response is assessed at flowering, making it expensive to maintain and manage plants up to flowering before their disease response is known. Apart from reducing maintenance

costs, an early assessment technique would identify resistant materials for participation in a recurrent selection programme. In the investigation, the reliability and effectiveness of early assessment techniques was compared with the YLS, the commonly used method in the assessment of black Sigatoka.

1.2.6 Black Sigatoka resistance in bananas

The identification of gene action controlling black Sigatoka resistance in *Musa* species stimulated further studies on disease response in banana genotypes. Ortiz and Vuylsteke (1994a) reported that resistance to black Sigatoka in *Musa* spp. is controlled by three independent alleles, a recessive allele at a major locus, and at least two minor recessive alleles (modifier genes) with additive gene effects. They further suggested that resistance genes were present in the genome of susceptible plantains, but their expressions were masked by the dominant effect of the major gene for susceptibility. Although these studies have been useful, it is not clear how the gene effects would be transmitted in tetraploid-diploid crosses.

A number of studies have been undertaken to identify the reaction of banana genotypes to black Sigatoka. Landraces, wild types, and banana genotypes generated through crosses have been evaluated against black Sigatoka. Craenen and Ortiz (1997) evaluated bananas with different ploidies for black Sigatoka resistance and concluded that the variation in response was due to individual genotypes not ploidies. In the same study, they also concluded that resistant genotypes showed more healthy leaves than susceptible genotypes and that disease onset, measured by youngest leaf spotted, was an indication of gene for gene response.

In another study, Craenen and Ortiz (1998) evaluated host response of *Musa* genotypes to black Sigatoka for incubation time, evolution time, disease development time, lifespan of the leaf, youngest leaf spotted, and youngest leaf with symptoms time. Resistant banana genotypes expressed the longest incubation time, while susceptible banana genotypes expressed shorter incubation time, evolution time, and disease development time. Incubation time in diploids was significantly correlated with fruit filling time, but black Sigatoka resistant diploids did not significantly yield better than susceptible diploids. The relationship between black Sigatoka resistance and yield in diploids therefore needs further investigation.

1.2.7 Mechanisms of resistance to black Sigatoka

An understanding of black Sigatoka resistance mechanisms will obviously improve the efficiency of selection of resistant banana genotypes. The two main forms of resistance identified in bananas are high resistance and partial resistance (Foure, 1992). With the high resistance mechanism, the host plant blocks the development of the pathogen, hence symptom expression. This high resistance is not, infact, desirable as the pathogen will always be under pressure to evolve and overcome this type of resistance in the host. There are reports that this type of resistance has been overcome, and genotypes that were initially resistant have become susceptible (Fullerton and Olsen, 1995). The Gold finger banana which has been resistant to black Sigatoka, has recently succumbed to the disease (Daniells, 2009). This process of overcoming resistance could possibly explain the unreliability of the hypersensitive reaction in protecting *Musa* genotypes against black Sigatoka. The other type of resistance reported in *Musa* is partial resistance, where there is a slow development of disease symptoms (Foure, 1992). This type of resistance is the one desired by banana breeders.

Investigations conducted on *Musa* species have identified different responses to black Sigatoka disease. The young leaves of banana plants were found to be more susceptible than the old leaves (Stover, 1973;1986). In a related study, Mobambo et al. (1997) reported slow disease development in older leaves. The deposition of epicuticular wax has been associated with leaf formation, reaching a peak at leaf number 3 or 4, counting from the youngest (Craenen et al., 1997); this could explain the variation in disease responses of leaves at different growth stages. The low stomata density on the abaxial surface of young leaves has also been associated with black Sigatoka resistance; stomata density on the abaxial surface of young leaves was negatively correlated with incubation time (Craenen et al., 1997). Japayal and Mahadevan (1968) observed the presence of chemical factors, such as phenols, that accumulated in resistant banana genotypes, suggesting that *Mycosphaerella fijiensis* infection could enhance production of phenols in resistant banana genotypes. Other factors such as reduction in the production of pseudothecia, which leads to a greater number of significant leaves at flowering in partially resistant genotypes, have also been reported (Perez et al., 2002).

There is a potential to exploit production of chemical compounds by resistant banana genotypes in selection of resistant materials, and designing control strategies against the pathogen. This potential has not been exploited. The resistance mechanism exhibited by banana genotypes of different ploidy levels and their inheritance between and within the different ploidies is not clear.

1.3 Banana breeding populations

The development of black Sigatoka resistant genotypes requires an understanding of the potential of the different *Musa* ploidies. Natural and artificial hybridisation has generated *Musa* species with a number of ploidies. Of economic importance are diploids (2n = 2x = 22), triploids (2n = 3x = 33), and tetraploids (2n = 4x = 44). Diploids and triploids occur naturally but examples of natural tetraploids are rare. Many of the available tetraploids have been synthesised through artificial hybridisation. Diploids are useful sources of resistance to pests and diseases (Rowe, 1984; Rowe and Rosales, 1993). Initially the wild diploids were used as sources of disease and pest resistance. However, when the wild diploids transferred the poor agronomic traits to their progenies the need to improve diploids for agronomic traits was realised (Rowe and Rosales, 1996).

Triploid bananas (2n = 3x = 33) are the most preferred bananas because they display the most economic combination of bunch and vegetative parts (De Langhe, 1986). However, triploid bananas are sterile and parthenocarpic, so they have no possibility of forming seeds. This sterility is disadvantageous in banana improvement. For example, triploid East African highland bananas which are susceptible to black Sigatoka (Tushemereirwe, 1996) cannot easily be improved. The process of improving the triploid bananas involves first generating tetraploids from triploids. Then the synthetic tetraploids have to be crossed with improved diploids to generate secondary triploids. This process of generating secondary triploids could lead to dilution or disappearance of some quality traits desired from East African highland bananas. There is need to understand inheritance of traits in generating secondary triploids so that appropriates measures can be taken to improve the breeding schemes.

The banana crop is a polyploid. Polyploidy refers to the state of any organism or plant in which the number of complete chromosome sets exceeds that of the diploid. Polyploidy can occur naturally by the fertilisation of an egg by more than one sperm, thus leading to a zygote with three or more sets of chromosomes; a failure of mitosis that multiplies the number of somatic chromosomes; or a failure at meiosis that produces a diploid gamete instead of a haploid. In bananas, triploids are believed to have been formed by unreduced gametes from diploids (Roboin et al., 2005). In diploids, normal meiosis is expected although unreduced gametes have been reported (Roboin et al., 2005). In triploids, meiotic paring is limited to only two homologues at a time; the third chromosome may fail to pair with either of the two, thus becoming a univalent. Also all the three homologues can pair to form a trivalent. Therefore, with their nature, triploids can form univalents, bivalents, and trivalents.

Segregation in triploids is also influenced by the genome. Triploid AAA bananas have been observed to have a higher frequency of trivalent formation at metaphase I during meiosis (Dodds, 1943), while Wilson (1946) found that the ABB and AAB groups have reduced trivalents with more bivalents. This could be explained by the degree of chromosome homology and preferential pairing of A and B chromosomes where, in AAB (the homology between the A chromosomes) and in ABB (the homology between the B chromosomes), influences bivalent formation. A higher incidence of univalent formation in both diploids, and triploids was reported to cause low pollen fertility in these species (Adeleke et al., 2004).

Autotetraploids can form univalents, bivalents, trivalents, and quadrivalents during meiotic pairing. Bivalent formation in autotetraploids is considered a normal meiotic process and because of this, in 4x by 2x crosses, the genetic analysis of triploid progenies uses principles that apply for the disomic inheritance of traits. The variation in gamete formation in tetraploids results in mixed ploidies. For example, in 4x by 2x crosses in plantains more than 90% of progenies were triploids, 3% were diploids and tetraploids were also reported in very low proportions (Vuylsteke, 2001; Oselebe et al., 2006). This suggests that before genetic analysis, ploidy analysis of progenies coming out of 4x by 2x crosses should be carried out so that valid conclusions are made.

1.4 Inheritance of traits in *Musa*

Inheritance studies have been undertaken on *Musa* by generating segregating populations to determine the number of genes involved in controlling traits and their effects. For example, Simmonds (1953) indicated that at least three dominant genes were involved in the control of parthenocarpy in crosses between wild and cultivated diploid bananas. Ortiz and Vuylsteke (1994b) generated a diploid population that segregated for albinism and reported that albinism was under the control of at least two independent recessive alleles. Ortiz and Vuylsteke (1994a) obtained tetraploids, triploids and diploids by crossing the triploid black Sigatoka susceptible plantain with the wild diploid resistant Calcutta 4. They considered these progenies as equivalent to testcrosses because Calcutta 4 selfed progenies bred true for black Sigatoka response. They analysed the offspring by grouping them into susceptible, less susceptible, and moderately resistant. From this, they developed a genetic model and reported that resistance to black Sigatoka in *Musa* spp. was controlled by three independent alleles, a recessive allele at a major locus, and at least two recessive alleles with minor modifying genes with additive gene effects. These studies seem to suggest that most traits of importance are under the control of more than one gene hence might have polygenic inheritance. The improvement of quantitative traits such as black Sigatoka in bananas will require accumulation of favourable alleles into the desired genotype. This implies that several cycles of hybridisations will have to be carried out to achieve the desired objective. It is also important to estimate the genetic gain after each cycle through genetic analysis of the traits.

1.5 Genetic analysis of traits

The analysis of quantitative and qualitative traits has established the inheritance and genetic diversity of the traits in crop plants. The use of heritability, general and specific combining ability has explored the inheritance of traits within *Musa*. On the other hand morphological and molecular qualitative traits have been used for determining variation and genetic diversity in bananas.

1.5.1 Analysis of quantitative traits

Genetic analysis of quantitative traits uses mating designs to understand relationships between individuals through estimates of variance and covariance. However, the mating design approach is difficult to use in genetic studies of bananas because of incompatibilities, difficulty in making reciprocal crosses, female sterility, limited genetic variation, and the few numbers normally generated from banana crosses.

The ratio of genotypic to phenotypic variance is known as heritability (Wrickle and Weber, 1986; Fehr, 1987). There are two types of heritability, broad sense and narrow sense heritability. Broad sense heritability, expressed as the proportion of genetic variance to the total phenotypic variance, is useful in indicating the presence of variation in a population but its predictive role in progeny performance is limited (Wrickle and Weber, 1986). Narrow sense heritability is expressed as the proportion of additive genetic variance to the total phenotypic variation. Narrow sense heritability is useful in predicting the performance of progenies based on parental performance.

In *Musa* breeding heritability estimates have been used quite often. Broad sense heritability estimates were determined in 4x by 2x plantain hybrids; the heritability estimates for disease traits ranged between 43%-69%; plant height ranged between 70-85%; bunch weight ranged between 84-94% (Ortiz and Vuylsteke, 1994c; Craenen and Ortiz, 1997). Ortiz (2000) calculated narrow sense heritability by regressing 3x parents against 4x offspring; heritability estimates for plant height, bunch weight, number of fruits, and fruit length had intermediate to low heritability estimates (0.20-0.55). From this study, it was observed that many of the traits such as short plant height and heavy bunches with many fruits in tetraploid progenies from 3x by 2x crosses could not be predicted using the phenotype of the triploid parent. It was also observed that fruit weight and circumference with heritability estimates above 0.5 could be predicted on the basis of the triploid parental phenotype. The predictive role of narrow sense heritability can be limited depending on the experimental conditions and genetic nature of parents. For example, Ortiz (2000) reported that days to flowering had a high heritability estimate of 0.8 but only 19% of the total variation was explained by the genotype of the parent, implying the heritability estimate could have been inflated by non-additive gene action.

Tenkouano et al. (1998) analysed 4x by 2x progenies and calculated general and specific combining abilities, observing little heterosis in these crosses. They concluded that 4x by 2x crosses were essential only in restoring female sterility in bananas. Compared to tetraploid females, diploid males made a relatively larger contribution to yield in 4x by 2x crosses and the conclusion was that the male phenotype was more predictive of offspring performance than the female phenotype; this suggested that greater yield gains could be achieved by increasing the frequency of these alleles in the diploid male background through recurrent selection procedures before crossing with tetraploids. However, there is still little knowledge on how black Sigatoka resistance and yield traits such as bunch weight are inherited in 2x by 2x crosses.

1.5.2 Analysis of qualitative traits

Quantitative traits have been useful in establishing the nature of inheritance of traits within crop species. On the other hand, plant taxonomists prefer qualitative traits in diversity studies because they are not affected by environment (Ortiz and Vuylsteke, 1998). These diversity studies group individuals into uniform subgroups which may be according to either the individual's origin or evolutionary development process.

Morphological and genomic differences are important in understanding the genetic diversity of crop species. Karamura (1998), after the analysis of morphological differences between East African highland bananas, was able to classify them into different banana clones. These clones were *Musakala, Nfuuka, Nakabululu, Nakitembe*, and *Mbidde*. Later, Tugume et al. (2002) used Amplified Fragment Length Polymorphic (AFLP) molecular markers to study genetic diversity among East African highland bananas and the results agreed with the morphological classification by Karamura (1998). Random Amplified Polymorphic DNA (RAPDs) markers are preferred to AFLPs, because RAPD markers require small amounts of DNA, are quick, and are not expensive. Although the reproducibility of RAPDs has been doubted (Collard et al., 2005), they have been used to study diversity among *Musa* species. For example, Jain et al. (2007) used randomly amplified DNA markers to determine the genetic diversity of materials from Indian banana germplasm. Similarly, Agoreyo et al. (2008) used RAPD markers to classify plantains into groups according to their origin. In a study by Ferreira et al. (2004), genetic diversity of banana diploids with molecular markers

established that individuals, which were closer to each other in terms of genetic distances had similar response to Sigatoka. Pillay et al. (2001) used RAPDs to analyse genetic diversity and relationships in East African highland banana germplasm. They reported a similarity range of 95.5% to 97.8% among the East African highland banana varieties. These research findings highlight the importance of molecular markers to estimate genetic diversity of banana genotypes. The genetic diversity will be important in selecting materials to constitute a population to be improved for black Sigatoka resistance and other traits.

1.6 Advances in *Musa* improvement

Several challenges are involved in the improvement of bananas against biotic and abiotic constraints. *Musa* diploids are sources of beneficial traits for *Musa* improvement, but continuous clonal propagation in diploids has led to the accumulation of structural chromosomal changes that promote female sterility (Simmonds, 1995). Female sterility is a result of human selection for parthenocarpy in edible diploids. In triploids, the preferred bananas for consumption, sterility is associated with meiotic irregularities due to an uneven number of chromosomes. Also sterility in triploids can be due to environmental factors and the genotype (Swennen and Vuylsteke, 1993). For example, in triploid East African highland bananas, seed set has been reported to be influenced by environment and cultivar (Swennen and Vuylsteke, 1993; Ssebuliba et al., 2000). A further challenge is that the long duration of the crop of two years from seed to seed, coupled with complicated seed germination, delays the selection process. However, embryo culture has improved seed germination up to 30% (Swennen and Vuylsteke, 1993). Although having mixed ploidies from crosses increases diversity, it is another challenge in the selection process. Ploidy determination has to be carried out so that the appropriate progenies are selected for further improvement.

Despite problems in generating hybrids in *Musa,* some advances have been made in producing disease and pest resistant materials with desirable agronomic and end-user traits. These advances have been the result of finding fertile female triploid bananas as well as parthenocarpic and fertile diploids. There are two approaches that have shown success in the improvement of triploid bananas for pest and disease resistance. These

approaches rely on having improved diploid male parents. In the first approach, the haploid gametes from improved diploids combine with unreduced gametes in triploids to generate tetraploids. This approach has been adopted by the Fundación Hondureña de Investigación Agrícola breeding programme in Honduras and acceptable tetraploid bananas have been generated (Rowe and Rosales, 1993; Ortiz, 1995). In Uganda, the initial tetraploids were synthesised from the East African highland bananas using a wild, seed forming, pollen fertile male parent Calcutta 4. Some of these tetraploids conserved good traits from the local bananas, but they were unsuitable for farmers because they had residual fertility and formed seeds. Because the tetraploids have inherited good traits from the triploid East African highland bananas, they are currently being used as breeding materials. The second approach that is used, involves crossing the synthetic tetraploids with improved diploids to generate secondary triploids. Dodds (1943) argued that the entire 3x set of chromosomes is transmitted to the tetraploid genome. On crossing tetraploid (4x) with diploid (2x) to generate the secondary triploids, the tetraploid genome conserved from the original triploids segregates and it might not be possible to maintain the quality traits from the original triploid (3x). However, this breeding strategy has been used to improve bananas and plantain (Pillay et al., 2004; Tomekpe et al., 2004) and successes have been recorded. However, more information is needed on the contribution of synthetic tetraploids and diploids to secondary triploids for disease resistance and agronomic traits.

The third approach was proposed by Stover and Buddenhagen (1986) who suggested recreating triploids from diploids by doubling the chromosome number of improved diploids to generate tetraploids. Then tetraploids would be crossed with improved diploids to recreate triploids. With this scheme, it might be difficult to incorporate end-user traits which are normally obtained from the local materials.

1.6.1 Black Sigatoka resistance and yield

When black Sigatoka spread to banana growing areas in the world, it was realized that control measures such as quarantine would not be effective. Plant breeding efforts were directed towards breeding high yielding, black Sigatoka resistant banana genotypes. After more than 20 years of banana breeding, Rowe and Rosales (1996) were able to improve bunch weights of diploids from 2kg to 35kg through numerous

crosses. These diploids also combined disease resistance and better bunch qualities. When one diploid was crossed with ‘Highgate’, the resulting tetraploid progenies had higher yield than ‘Highgate’. This indicated that advanced diploids could achieve improved yields in black Sigatoka susceptible triploid bananas. Research and development by the International Institute of Tropical Agriculture was able to achieve improved yield in a tetraploid hybrid. The tetraploid hybrid was black Sigatoka resistant and was 43% higher in bunch weight than the female triploid plantain parent (Mobambo et al., 1993). These two research programmes showed that it is possible to combine black Sigatoka resistance with improved bunch weights. In Uganda, the National Banana Research Programme is developing new banana hybrids using the 4x by 2x breeding scheme. Black Sigatoka resistance, bunch size, and end-user traits are some of the selection criteria in the development of new materials. From the farmer participatory evaluation of new banana hybrids, four of the hybrids that combine disease resistance, heavy bunch weights, and end-user traits have been selected by farmers (NaCRRI, 2007). It should be noted that in that study, more than 50% of the genotypes evaluated were rejected by farmers although they had black Sigatoka resistance. The understanding of the associations between black Sigatoka resistance and farmer preferred traits will accelerate selection of materials that will suit the end-user requirements.

1.6.2 Secondary traits associated with yield

Banana breeders would like to have a genotype that is high yielding, with short stature, disease and pest resistant, and drought tolerant among other qualities (Vuylsteke et al., 1993; Dantas et al., 1995). Short plants might stand strong winds better than tall plants. However, a positive correlation between dwarfism and low bunch weight has been reported, implying that reducing plant height in bananas might sacrifice banana yield. Despite the association between plant height and banana yield, short, high yielding banana genotypes that combine black Sigatoka resistance and fruit quality have been generated (Rowe, 1984; Rowe and Rosales, 1993), implying that the linkages between dwarfism and low banana bunch weights can be broken. High yield, better suckering, early flowering, shorter stature and improved fruit quality have also been bred into tetraploid plantain hybrids (Vuylsteke et al., 1993). Ortiz (1995) reported a positive

relationship between fruit size and bunch weight in diploids and a positive relationship between plant height and girth in plantain tetraploids. Ortiz and Vuylsteke (1998) reported on relationships between yield and other traits in bananas and plantains. For example, they reported that yield potential was negatively associated with days to harvest, and fruit weight was correlated positively with fruit girth in triploid (AAA) bananas. In plantains plant girth was reported to be positively correlated with bunch weight. It is not clear whether these associations are maintained from diploids and tetraploids to secondary triploids. It is therefore possible to combine other desirable traits with yield. However, there is need to understand the relationship between yield (bunch weight) and other traits so that appropriate selection and breeding strategies can be designed. This will help focus banana breeding with the possibility of selecting for multiple traits.

It has also been possible to breed for quality traits in the banana hybrids. The poor flavour introduced by the wild Calcutta 4 in dessert tetraploid hybrids was overcome by breeding apple flavoured tetraploid bananas although they had small bunches and were susceptible to *Fusarium* wilt (Ortiz, 1995). Rowe and Rosales (1996) reported a 49kg tetraploid bunch of SH3583 with black Sigatoka resistance, a long green life and excellent qualities when cooked whether green or ripe. Also SH3481, derived from 'Dwarf Prata' x SH3142, was tolerant to black Sigatoka and had a pleasant sweet acid flavour. The interactions between agronomic, disease resistance and end-user qualities affect the overall acceptability of the new banana genotypes. Information on the possible associations of traits is important in designing better banana improvement strategies.

1.6.3 Biotechnology and banana improvement in Uganda

The Uganda National Banana Research Programme is considering a holistic approach to the improvement of East African highland bananas using both conventional breeding and biotechnological approaches. It is believed that biotechnology can inject one or two genes into a banana crop that will not alter the product quality (INIBAP, 2004). Although this strategy might work for simply inherited traits, it is likely to face challenges in improving quantitative traits. Black Sigatoka resistance was reported to be under the influence of many genes (Ortiz and Vuylsteke, 1994a), therefore

biotechnology might not be appropriate for such a quantitative trait. There are also other challenges that are delaying biotechnological advances in banana transformation in Uganda. The delay in developing a biotechnology policy, limited technical and financial resources, and consumers' bias against genetically modified organisms may delay the immediate uptake of such technologies. At the moment there are very few banana genes available for use in transformation. It is not clear whether the non-banana based genes will be very useful in controlling biotic and abiotic stresses. However, biotechnology complements conventional breeding approaches in activities such as embryo culture, marker assisted selection, and disease diagnostics.

In conventional breeding DNA molecular markers are very useful. The development of molecular markers will increase the efficiency and selection process of breeding a long term crop such as bananas. However, the expression of markers in autopolyploids which have a high level of heterozygosity may be a problem.

1.6.4 Genotype-by-environment interactions

The lack of consistency of performance of genotype in different sites, years, or even seasons is termed genotype-by-environment (GxE) interaction (Wrickle and Weber, 1986). Genotype-by-environment interaction is important in the selection of new genotypes to promote in different environments. In variety selection, GxE influences decisions whether to breed for specific adaptation in unique environments or wide adaptation in a range of environments. Ortiz and Vuylsteke (1998) reported significant GxE for bunch weight, days to flowering, plant height, and girth among other traits. Although GxE studies are likely to apply to a specific set of genotypes tested in different environments, in Uganda there is limited information on GxE on banana genotypes (Baiyeri et al., 2008). As such environments have not been classified based on performance of banana genotypes to guide breeders about where to test the new banana materials. It is very important to test the new banana hybrids and establish the GxE interactions to guide future breeding activities.

1.7 Farmer preference of banana cultivars

It is important to identify traits that farmer consider important in the adoption of new banana materials. Gold et al. (2002) reported that farmers in Uganda consider

availability of planting material, maturation time, cultivar longevity, tolerance to (marginal soils, drought, pests and diseases), bunch size and marketability. However, there was wide variability in importance of the criteria depending on the region. For example, it was reported that farmers in central Uganda gave high priority to cultivar longevity and marketability (Gold et al., 2002). This is a region where banana production constraints have reduced plantation life (Rutherford and Gowen, 2003), therefore farmers would like to have banana cultivars that last longer than the ones they have lost due to banana diseases and pests. Katungi et al. (2001) reported that farmers in central Uganda would like to have bananas with big bunches, with tolerance to poor soils and drought. It should be noted that all these studies reported farmer considerations on choice of banana cultivars among the local varieties. Therefore, farmers never emphasised cooking traits because all the local cultivars meet their cooking quality requirements. Svetlana (2003) reported that farmers consider taste when choosing from exotic or new banana cultivars.

In Uganda, bananas are a major staple food, therefore cooking quality traits need to be emphasised since they might be more important in adoption of a new banana variety. Kornegay et al. (1996) reported that farmers were able to sacrifice yield over quality differences in adoption of new bean varieties. The important banana consumption qualities are taste, food colour, texture and aroma among others. However, these attributes have not been quantified to guide breeders on which one is more important than the other. The production attributes desired in new bananas are also important since the reported attributes were based on making choices within the local banana varieties. Therefore there is need to document the production attributes that farmers would desire to have in new banana materials as well as quantity the relative importance of the food quality attributes.

1.8 Conclusions from literature review

This literature review has established that, although black Sigatoka is a serious disease on bananas and plantains, it is possible to control it through breeding. The disease has a quantitative nature of resistance, therefore, disease assessment protocols should be able to detect small differences among genotypes for effective breeding and selection. The improvement of susceptible triploid bananas involves crossings with different

ploidy populations. One of the populations is the diploid population, which is considered to be the source of black Sigatoka resistance. However, the introduction of disease resistance alone in the susceptible triploids will not produce end-user banana genotypes. There is the need to establish the association of useful traits with black Sigatoka resistance and how these traits are inherited. Most of the resistance is accessed from wild clones; there is also a need to first improve these clones for other traits of importance such as bunch weight. Before this improvement can be achieved, knowledge of the inheritance of traits within the diploid and the amount of genetic variation becomes very important. The second population that becomes important is the synthetic tetraploid population because tetraploids are crossed with improved diploids to generate seed sterile triploids. The literature review has established that progress has been made, but the genetics behind black Sigatoka inheritance and associated traits is not well understood, especially from tetraploid (AAAA) and diploid (AA) bananas.

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Chapter two

Assessment of farmers' perceptions of black Sigatoka disease and preferred traits in bananas in Uganda

Abstract

The survey reported in this chapter aimed to establish farmers' knowledge of black Sigatoka disease in low and medium banana production zones, farmers' and consumer preferences for East African highland bananas, and the qualities desired in new disease resistant banana genotypes. A structured questionnaire was deployed on 59 households during October to December 2007. In June 2008, a farmers' group in Gombe subcounty, Wakiso district validated the new banana materials and their parents for farmer preferred traits. Seven percent and 3% of farmers in medium and low production zones, respectively, were aware of black Sigatoka disease. The East African highland bananas were preferred because of superior taste, softness, and yellow colour when cooked. These bananas matured early and were marketable. However, the East African highland bananas produced small bunches, lacked pest and disease resistance, and did not tolerate poor soils and drought. Farmers desired to have new banana materials with heavy bunches, resistance to pests and diseases, tolerance to drought, fast maturing, and marketable traits. Farmers indicated that before accepting new materials, the most important attributes they would consider were pleasant taste, soft texture, aroma and yellow colour of food in that order. Farmers ranked food quality aspects higher than production traits in adoption of new banana materials, because they grow bananas mainly for home consumption. The new banana clone M1311 was approved by 80% of the panellists for its taste, while its food colour was appreciated by 66% of the panellists. These findings highlighted the importance of farmer involvement in the identification of traits which have not been directly targeted for breeding.

2.1 Introduction

In Uganda, banana consumption is largely limited to highland banana cultivars that are endemic to the East Africa region (Purseglove, 1972). These bananas are popularly known as East African highland bananas. East African highland bananas[2] constitute more than 75% of the total bananas grown in Uganda (Gold et al., 1994; 2002a; Spilsbury et al., 2002; Rutherford and Gowen, 2003). Despite the popularity and uniqueness of East African highland bananas, they face production, use, and marketing constraints.

One of the major constraints affecting banana production in Uganda is black Sigatoka. Black Sigatoka has been reported to cause yield losses of up to 37% on East African highland bananas (Tushemereirwe, 1996). Cultural control measures have been recommended to farmers (Tushemereirwe et al., 2000; Rutherford and Gowen, 2003) yet the disease appears to be increasing significantly. It is not clear whether it is the deployment of unreliable control measures or a lack of knowledge of the disease by farmers that is causing this increase.

Based on the magnitude of banana constraints and production levels, banana production zones in Uganda have been classified into three major zones (Rutherford and Gowen, 2003). The East and Central zone, where banana production has severely declined and many farmers have abandoned production of the crop; the South, where banana productivity is at an intermediate level, but where there is moderate decline; and the Western, where banana productivity remains high but there has been some decline (Rutherford and Gowen, 2003). The Ugandan National Banana Research Programme (UNBRP) has demarcated three benchmark sites, at Luwero, Masaka/Ntungamo, and Mbarara/Bushenyi, to represent zones of low, medium and high banana production. It was not clear whether farmer perceptions of black Sigatoka disease in the low and medium production zones would be the same.

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[2]East African highland bananas are endemic to the great lakes region of Eastern Africa (Uganda, Kenya, Tanzania, Burundi, Rwanda, and Democratic republic of Congo; they are a major staple food and are unique to the region. In the text they are either referred to as cooking bananas or local bananas.

Conventional breeding has been conducted to improve the disease and pest resistance of East African highland bananas (Ssebuliba et al., 2000; Pillay et al., 2004; Tushemereirwe et al., 2005). In addition, disease and pest resistant hybrids have been introduced into the East African region from international *Musa* breeding programmes. However, the pest and disease resistant materials have not been acceptable to end-users because the new banana genotypes do not meet consumer requirements (Nowakunda et al., 2000; Rutherford and Gowen, 2003; Nowakunda and Tushemereirwe, 2004). This might suggest that the banana breeders' selection criteria were not entirely in agreement with farmers' requirements. Probably if the farmers were consulted, they could have suggested the traits they would desire in the new banana materials.

The consumption and production attributes are very important in decision making about adoption of a new variety. In Uganda, banana is a staple crop hence consumption attributes might be more important in adoption of a new banana variety. Kornegay et al. (1996) reported that farmers were able to sacrifice yield over quality differences in adoption of new bean varieties. The important banana consumption qualities are taste, food colour, texture and aroma among others. However, these attributes have not been quantified to guide breeders in the development of new cultivars. The production attributes are also important especially where farmers grow bananas for sale. It is also important to establish banana production attributes from the farmers' point of view. Involvement of farmers in the development of new materials can help identify major traits to be incorporated into the new materials which can accelerate their adoption.

The objectives of this study were to:

a) assess farmers' knowledge of black Sigatoka disease in central Uganda, and

b) document unique traits that give East African highland bananas their special status among banana farming communities in Uganda as a basis for the development of new black Sigatoka resistant bananas.

The following hypotheses were tested:

a) Farmers in central Uganda are aware of black Sigatoka as a banana production constraint, and

b) Farmers prefer quality and production attributes from East African highland bananas which they would like to be incorporated in the new banana materials.

2.2 Materials and methods

2.2.1 Study area

A survey was carried out in the districts of Nakaseke and Masaka which were purposively selected to represent areas with low and medium levels of banana production, respectively. Masaka district shares a border with Sembabule district in the northwest, Mpigi district in the north, Rakai district in the west and south, and Kalangala district in the east. With a land area of 6986km^2 and an average altitude of 1329m above sea level, Masaka district has generally sandy loam soils with two cropping seasons. The first season is from March to June/July, and the second from August to December. The mean annual rainfall ranges between 1100mm to 1200mm. Ethnically the district is occupied by the Baganda (majority), Banyankole, Banyarwanda and Banyoro. The main economic activities are farming and fishing. Major crops grown are bananas, maize, sweet potatoes, millet and sorghum. Administratively, the district is divided into three counties and one municipality. Each county is demarcated into sub-counties; on average three to four sub-counties constitute a county. Sub-counties are further demarcated into parishes. Ten to 15 villages (each about 120-180 households) constitute a parish.

Nakaseke district has a land area of about 1924km^2 with an average altitude of 1164m above sea level. The district shares a border with Luwero in the north, Nakasongola in the east, Kiboga in the west and Masindi in the south. Nakaseke district is demarcated into eight sub-counties. The district has mainly red sandy loam soils and receives an average annual rainfall of 1300mm with peaks in March-May and October-November. The main economic activity is agriculture with coffee, bananas and nomadic

pastoralism being the major sources of income. Ethnically the district is occupied by the Baganda (majority) and Banyankole pastoralists.

2.2.2 Sampling procedures

The survey was conducted in October and November, 2007 in the low and medium production zones, respectively. In each zone, one sub-county was selected. For each district a list of parishes in the selected sub-county was obtained. Three parishes were randomly selected for the low production zone, and four parishes for the medium production zone. Five to six villages were randomly selected in each parish.

A list of residents in each village was obtained from the local chief (Chairman LC1). With the help of field assistants and the local chief, farmers were selected randomly from each village by ballot, each household being allocated a ballot paper. The numbers selected per village were proportional to the number of households in the village. In total, 30 respondents were selected per sub-county.

2.2.3 Data collection and analysis

A structured questionnaire (Appendix 3.1) which involved open-ended questions that allowed farmers to give as much information as possible was administered. The questionnaire captured farmers' knowledge of black Sigatoka, qualities desired in East African highland bananas, constraints on the production of East African highland bananas, and qualities that farmers desired in new banana genotypes. After interviewing the farmers, the overall incidence of black Sigatoka was estimated in the farmers' banana plantations using transect walks, on a 1-10 scale where 1 represented 5% severity, 2 represented 10% severity, 3 represented 15% severity, in that order up to 10 representing 50% severity. The disease severity was estimated in all banana plantations whose owners were interviewed.

The UNBRP has identified and trained a farmers group in Gombe subcounty, Wakiso district to test new banana materials as they are developed. This group of farmers was requested to validate farmer preferred traits in 4x by 2x progenies together with their parents. Sixteen genotypes with a local cultivar 'Mbwazirume', (Table 2.1) were

selected and tested with the farmers group. The farmers group consisted of 17 panellists of which 47% were females and 53% were males.

Table 2.1 Female tetraploid parents and secondary triploids validated by farmers for quality traits

Cross	Progenies
365k-1 x Calcutta 4	
	K913
	K914
	K915
	K916
	K928
401k-1 x 8075	
	M1311
	K1313
	K1314
660k-1 x 8075	
	K1211
	K1223
	K1216
	K1224
Local check (Mbwazirume)	K1511

A group of women pealed bananas progenies shown in table 2.1 above (Plate 2.1), split them longitudinally to observe the colour of the fresh banana pulp (Plate 2.2), wrapped the bananas in banana leaves and steamed them on fire as indicated in Plate 2.3. Farmers' comments made as they prepared the bananas were recorded.

Plate 2.1. Women pealing bananas to taste

1 2 3 4 5

Plate 2.2. Banana fingers showing different colour intensities; 1 not liked while 5 most liked by banana consumers

Plate 2.2. Banana fingers with different colour intensities

1 is white, 2 is cream, 3 and 4 are yellow but 4 is more intense and 5 is orange

Plate 2.3. Preparing and steaming banana materials for testing

Farmers assessed the new banana genotypes for 'empoma' (taste), 'akawoowo' (aroma), 'obuweweevu' (mouth feel or texture) and 'langi' (food colour). The panellists were given a form written in their local language, Luganda and were requested to score each trait qualitatively following guidelines shown in Table 2.2.

Table 2.2. Description of different grades for food colour, mouth feel, aroma and taste of new banana genotypes

Score	Luganda	English
6	Kirungi nnyo, nkyagala nnyo	Like extremely
5	Kirungi nnyo, nkyagala	Like very much
4	Kirungi nkyagala	Like
3	Sikirungi	Dislike slightly
2	Kibi sikyagala	Dislike very much
1	Kibi nnyo ddala	Dislike extremely

The panellists were requested to quantify the relative importance of food colour, aroma, texture and mouth feel as it affected overall acceptability of new banana materials. Each farmer placed beans against each food attribute (Plate 2.4) as a measure of the weight attached to it.

empoma = taste, akawoowo = aroma, obuwewevu = texture langi = colour

Plate 2.4. Panellists' quantification of 'empoma', 'akawoowo', 'obuwewevu' and 'langi'

The frequencies of farmers' responses, elicited by the questionnaire in the two zones, were computed using SPSS version 15.0. The panellists' responses on the aroma, texture, colour and taste were also analysed and computed into frequencies of approval or non-approval of the trait.

2.3 Results

2.3.1 Importance and preferences of East African highland bananas

Table 2.3 shows responses of farmers on why they grew bananas. Thirty six percent of the farmers in the low production zone and 49% of the farmers in the medium production zone mentioned that they used bananas for both food and income. The low banana production zone had a higher proportion of farmers (32%) than 24% in the medium production zone who pointed out that the main reason for growing bananas was for food. A comparable proportion of 31% and 21% in low and medium production zones indicated that for them the main importance of bananas was for income. A very low proportion of farmers were using banana products such as peels, banana male buds and banana leaves for feeding animals.

Table 2.3. Responses of farmers in low and medium production zones of Uganda on the reasons for growing bananas

	Percent farmers	
Reason	Low production zone (n=30)	Medium production zone (n=29)
Food	32	24
Cash	31	22
Food and cash	36	49
Animal feed	1	5

Table 2.4 shows the farmers' reasons for preferring East African highland bananas in low and medium production zones. About 30% of farmers in the low production zone, and 37% of the farmers in the medium production zone, liked East African highland bananas because they are soft, have the preferred taste, and yellow colour after cooking. About 28% of farmers in the low production zone and 12% of farmers in the medium production zone liked East African highland bananas because they are marketable. From an agronomical perspective, 14% of the farmers in the medium production zone preferred East African highland bananas because they mature early compared to 6% who liked East African highland bananas because of early maturity in the low production zone. Seven percent of farmers in the low production zone indicated that they liked local bananas because they could plant other crops like beans, egg plants and pumpkins with the local bananas.

Table 2.4. Farmers' preferences of East African highland bananas in low and medium production zones of Uganda

Reason	Percent farmers	
	Low production zone (N=30)	Medium production zone (N=29)
Tolerates poor soil fertility	1.2	2.6
Tolerates drought	3.5	2.6
Demand less labour	4.7	3.8
Locally known	-	1.3
Good food (soft, taste)	30.3	37.1
Used to feed animals	-	1.3
Highly marketable	27.9	11.5
Accessible planting materials	-	3.8
Early maturing	5.8	14.1
Produces many suckers	3.5	2.6
Produces heavy bunches	2.3	6.4
Lives longer than 5 years	14.0	10.3
Provides shelter	-	1.3
Meets cultural norms	-	1.3
Easy to be intercropped	7.0	-

2.3.2 Problems of East African highland bananas in Uganda

Farmers noted that the local bananas had serious constraints: they were highly affected by pests and diseases, did not tolerate poor soils, were seriously affected by drought and produced small bunches (Table 2.5). About 14% of farmers in the medium production zone claimed that East African highland bananas demanded a lot of labour for agronomic practices such as weeding, mulching, and pest control such as corm removal. A low percentage of farmers (2.5%) in the medium production zone, reported that bananas were difficult to manage. Farmers in both low and medium production zones identified pests and diseases as major problems in these regions. In the medium production zone 33% of the farmers suggested that the local bananas were highly affected by the banana weevil. On the other hand about 18% of the farmers in the low banana zone indicated that the local bananas were highly affected by the banana

weevils. Although identified by a small proportion of farmers (2.5%), banana bacterial wilt was considered as a banana constraint in the low banana production zone (Table 2.5). Sixty percent of farmers in the low production zone pointed out that the local bananas produced small bunches, while 16% of farmers in the medium production zone reported the same problem of small bunches. Also 19% of farmers in the medium production zone reported that the local bananas did not tolerate poor soils compared to about 3% in the low production zone. Poor soil fertility appeared to be more of a problem in the medium production zone than in the low production zone.

Table 2.5. Problems of East African highland bananas in low and medium production zones of Uganda

	Percent farmers	
Reason	Low production zone (N=30)	Medium production zone (N=29)
Difficult to manage	2.5	13.9
Affected by weevils	17.5	33.3
Not resistant to diseases	10.0	8.3
Do not tolerate poor soils	2.5	19.4
Produce small bunches	60.0	16.7
Affected by drought	2.5	5.6
Affected by nematodes	2.5	2.8
Affected by Banana wilt	2.5	-

The farmer knowledge about the pests and diseases in the two banana production zones is presented in Table 2.6. About 44% of farmers in the low production zone were able to describe symptoms caused by banana bacterial wilt. About 21% of farmers in the medium production zone and 2% in the low production zone were able to identify and mention the symptoms of banana streak virus. Thirty six percent of farmers in the low production zone and 35% of farmers in the medium production zone were able to describe the symptoms and damage associated with the banana weevil. In both areas, very few farmers (3.1% and 6.9% in low and medium production zones, respectively) were able to describe symptoms of black Sigatoka and mentioned that it as a constraint of banana production (Table 2.6). Although farmers in the low production zone were less aware of black Sigatoka as a banana constraint, the severity of black Sigatoka

was higher (P<0.0001) in the low banana production zone than in the medium production zone. The medium production zone had more area (acres) under bananas (P<0.001) than in the low production zone (Table 2.7).

Table 2.6. Farmers' knowledge of pests and diseases on local bananas in low and medium production zones of Uganda

Constraint	Percent farmers	
Constraint	low production zone (N=30)	Medium production zone (N=29)
Banana weevils	35.9	34.5
Banana nematodes	9.4	17.2
Banana streak virus	1.6	20.7
Fusarium wilt	4.7	6.9
Banana thrips	-	6.9
Black Sigatoka	3.1	6.9
Cigar end rot	1.6	6.9
Banana bacterial wilt	43.8	-

Table 2.7. Estimated average area (acres) and mean Sigatoka incidence in low and medium production zones of Uganda

Zone	Area under banana (acres) Mean±se	Severity of black Sigatoka (%) Mean ± se
Low production	1.1±0.39	5.7 ± 1.00
Medium production	2.7±0.16	1.2 ± 0.46
T-test	4.51 (P=0.0001)	21.87 (P<0.0001)

se = standard errors of the mean

2.3.3 Awareness and preference of introduced black Sigatoka resistant bananas

As one of the interventions against black Sigatoka, the UNBRP accessed black Sigatoka resistant germplasm from other breeding programmes and gave them to farmers to evaluate for quality traits and black Sigatoka resistance. A larger proportion of farmers in low production zone (54.5%) were aware of black Sigatoka resistant materials than in the medium production zone (38.5%) (Table 2.8). However, these

farmers did not prefer the exotic bananas, because their taste was inferior to the local bananas, required more labour to manage than local bananas, produced few suckers, and took long to mature (Table 2.9). A high percentage of farmers (41.7% and 45.7%) in the low and medium zones respectively, claimed that they did not prefer exotic bananas because their taste was substandard. In the low production zone, 16% of the farmers pointed out that the black Sigatoka resistant hybrids took long to mature. In the medium production zone 20% of the respondents who had exotic bananas reported that they needed more labour to manage, while 11.4% of the respondents suggested that the black Sigatoka resistant hybrids have a low rate of sucker production (Table 2.9).

Table 2.8. Farmers' awareness of new banana hybrids in low and medium production zones of Uganda

	Percent farmers	
Awareness	low production zone (N=30)	Medium production zone (N=29)
Yes	54.5	38.5
No	45.5	61.5

Table 2.9. Reasons why farmers in low and medium banana production zones of Uganda did not like exotic bananas

	Percent farmers	
Reason	Low production zone (N=30)	Medium production zone (N=29)
No good taste	50.0	45.7
Not tolerant to drought	-	8.6
Need intensive management	-	20.0
Fail to support their bunches	8.3	5.7
Low sucker production rate	-	11.4
Take long to mature	16.7	2.9
Very thick fingers	-	2.9
Lack market	8.3	2.9
Lack resistance to banana bacterial wilt	8.3	-
Leaves big for domestic use	8.3	-

2.3.4 Farmers preferred traits in new banana genotypes

Farmers were asked to guide the breeders on what traits they should include in new materials that are being developed in order to meet their consumer needs. Farmers highlighted that the materials to be desired should have good food, heavy bunches, high market value, resistance to diseases and pests, early maturity and tolerance to drought (Table 2.10). About 28% of farmers in low production zone preferred to have new materials with good food while about 24% of farmers interviewed preferred the new materials to produce heavy bunches. About 11% of these farmers preferred bananas with high market value, while 8.6% preferred to have materials that have early maturity, 9.7% liked materials with disease resistance and only 7.5% would prefer materials that were drought tolerant (Table 2.10). The farmers from the medium production zone gave almost similar responses. About 27% preferred materials with good food, 20.6% liked to have new materials which can produce big bunches, 8.2% preferred materials that matured early and drought tolerant, and only 6.2% liked to have materials with weevil tolerance.

Table 2.10. Qualities preferred by farmers in the new banana materials in the low and medium banana production zones

	Percent of farmers	
Trait	low production zone (N=30)	medium production zone (N=29)
Tolerance to drought	7.5	8.2
Tolerance to poor soils	2.2	8.2
Heavy bunches	23.7	20.6
Good food	27.9	26.8
One which live longer	1.1	5.2
Early maturity	8.6	8.2
High sucker production	4.3	3.1
Be able to support a bunch	-	1.0
Big fingers	2.2	1,0
Medium height	-	1.0
High market value	10.8	7.2
Easy to manage	-	3.1
Tolerant to weevils	2.2	6.2
Resistant to diseases	9.7	-

The farmers ranked the preferred traits and when the rankings were computed into aggregate scores, good food (taste, soft, colour) had the highest aggregate score, followed closely by the attribute of heavy bunches. Overall, farmers attached equal importance to resistance to diseases and pests, tolerance to drought and early maturity (Table 2.11).

Table 2.11. Qualities that would be desired in new materials by farmers in Uganda

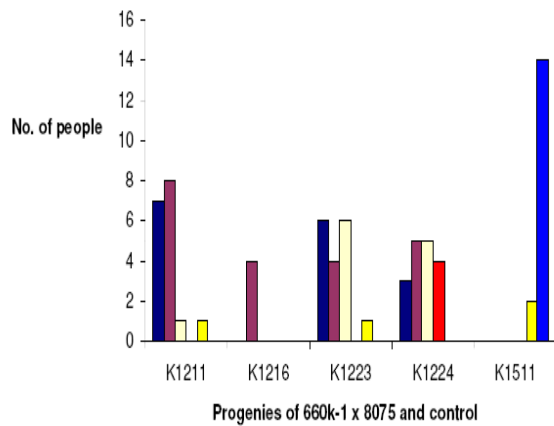
Trait	Number of farmers (N=59)				
	Rank 1	Rank 2	Rank 3	Rank 4	Aggregate
Good food (taste, soft, colour)	10	19	11	6	125
Heavy bunches	13	15	9	5	120
Resistant to pests and diseases	8	5	2	1	52
Tolerant to drought	7	6	2	0	50
Early maturing	8	3	4	1	50
Marketable	3	2	6	7	37
Live longer	3	1	1	1	18
Tolerant to poor soils	2	0	0	1	9

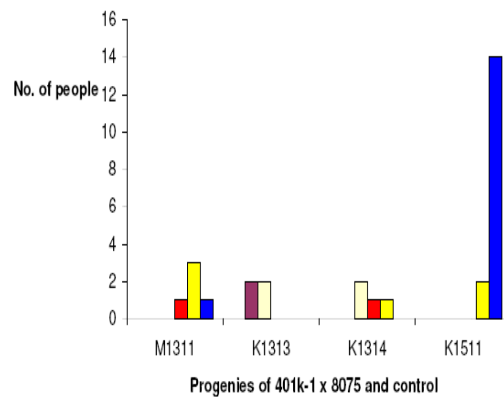
2.3.5 Verification of quality traits preferred by farmers

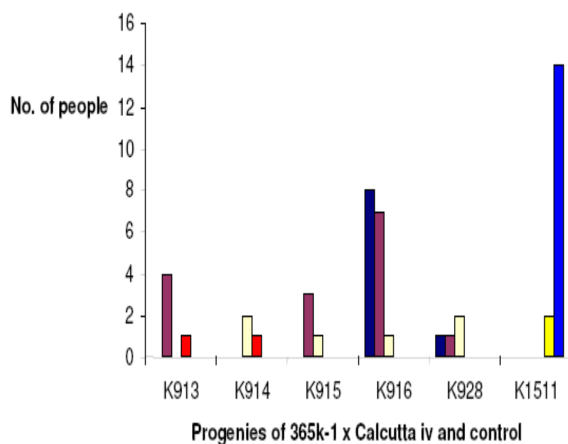
During peeling, farmers indicated that they preferred to peel bananas with big fingers and those with little banana sap. After peeling, farmers split the banana fingers longitudinally. They did not like some materials which had seeds inside. They clearly indicated that they would prefer bananas without seeds. The farmers also preferred to have bananas with yellow flesh.

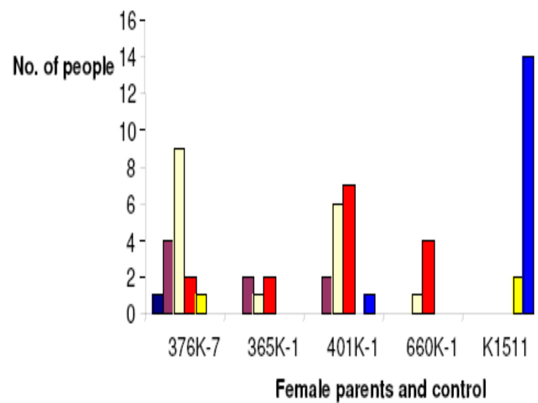
Figures 2.1 and 2.2 give panellists' responses to banana hybrids, together with their female parents, on aroma and taste. Among the progenies of 660k-1 and 8075, 12.5% of the panellists liked the aroma of K1224 and 6% appreciated the aroma of K1211. When the female parents were tested alone, 50% of the panellists preferred the aroma of 401K-1.

The progenies of 660K-1 x 8075 were tested for taste: about 25% of the farmers liked the taste of K1211 and 12% approved the taste of K1314. From the progenies of 401K-1 x 8075, 80% were satisfied with the taste of M1311, 25% liked the taste of K1313 and 25% liked the taste of K1314. The progenies of 365K-1 and Calcutta 4 did not perform well in the taste evaluation. Only 25% and 12 % appreciated the taste of K928, and K916 respectively. The taste of the female tetraploid parents was liked by the panellists. Female parents, 401K-1 and 660K-1 received the highest approval for taste among the female parents. Panellists expressed a great deal of variation in likeness of the taste of 376K-7 (Fig. 2.2).





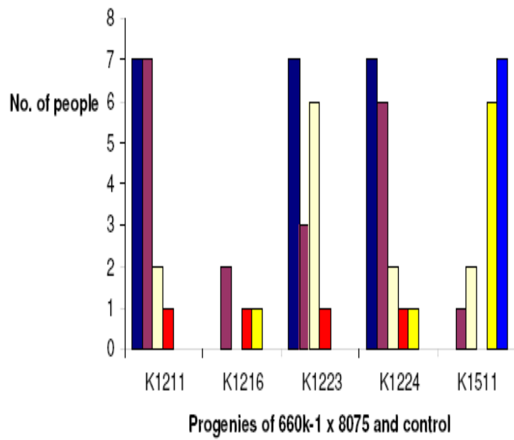


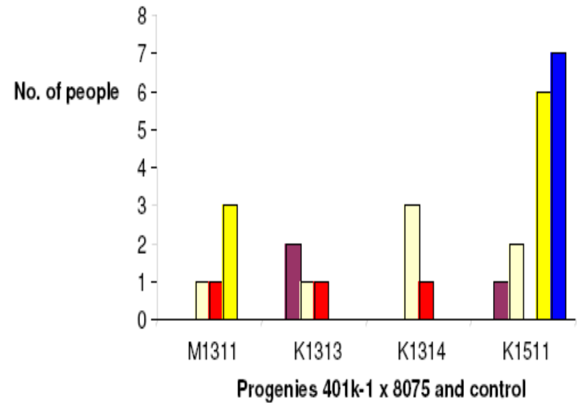


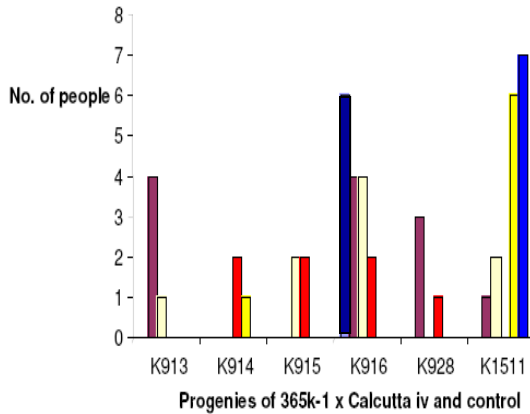
K1511 = local check ('Mbwazirume'); 367k-7, 365K-1, 401K-1 and 660K-1 are tetraploid female parents; calcutta iv (Calcutta 4) and 8075 are diploid male parents

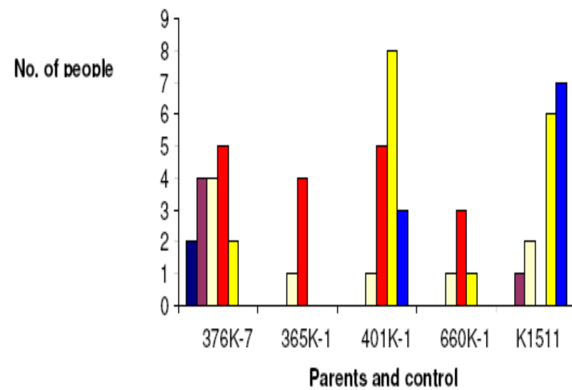
Legend: colour of the bar graph indicating panellists responses

Figure 2.1. Panellists' response on the aroma of new banana hybrids together with their female parents and local check (AAA-EA highland banana)









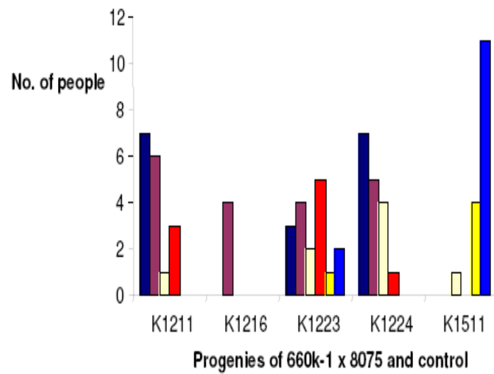
K1511 = local check ('Mbwazirume'); 367k-7, 365K-1, 401K-1 and 660K-1 are tetraploid female parents; calcutta iv (Calcutta 4) and 8075 are diploid male parents

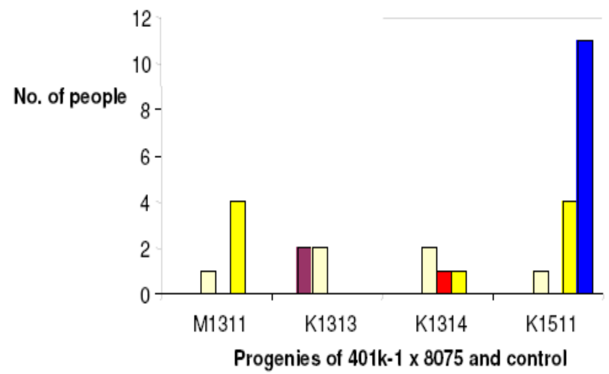
Legend: colour of the bar graph indicating panellists responses

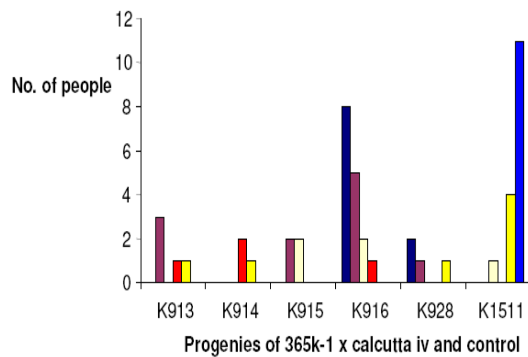
Figure 2.2. Panellists on the taste of new banana hybrids together with their female parents and local check (AAA-EA highland banana)

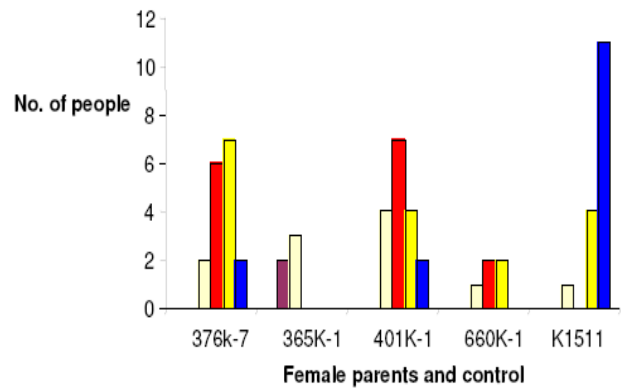
Among the progenies of 660K-1 x 8075, 19%, 50% and 6% of the panellists were satisfied with the food colour of K1211, 1223, and K1224 respectively (Fig. 2.3). For 365K-1 x Calcutta 4 progenies, panellists approved only the colour of K913 and K914. Among 401K-1 x 8075 progenies, 66% liked the food colour of M1311 and 50% approved the colour of K1314. The food colour of most of the female parents involved in this study was approved by panellists, except for 365K-1 (Fig. 2.3).

Mouth feel is another parameter that influences farmer acceptance of new materials. In this study amongst progenies of 660K-1 x 8075, the mouth feel or texture of K1223 satisfied only 6% of the panellists and 12% enjoyed mouth feel of K1224. Nineteen percent of the panellists approved the texture of K1224. The texture of the rest of the progenies was disliked. For progenies of 365K-1 x Calcutta 4, the texture of K916 was appreciated by only 6% of panellists. According to panellists, 401k-1 appeared to have the best texture (Figure 2.4).





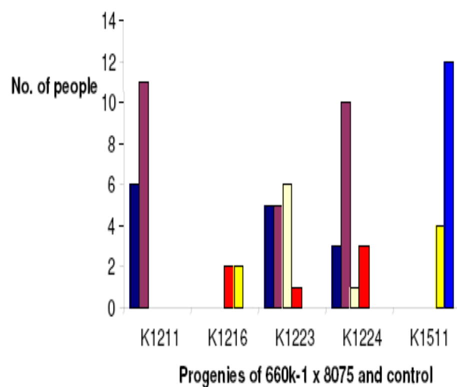


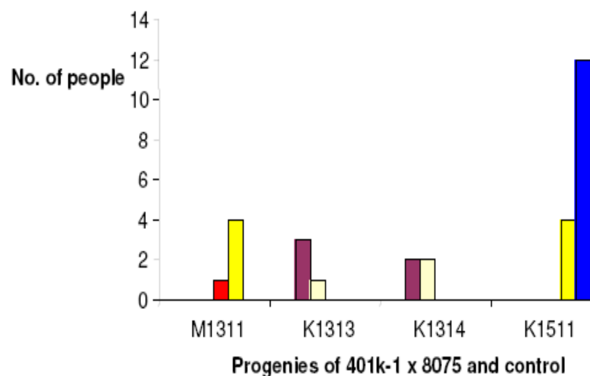


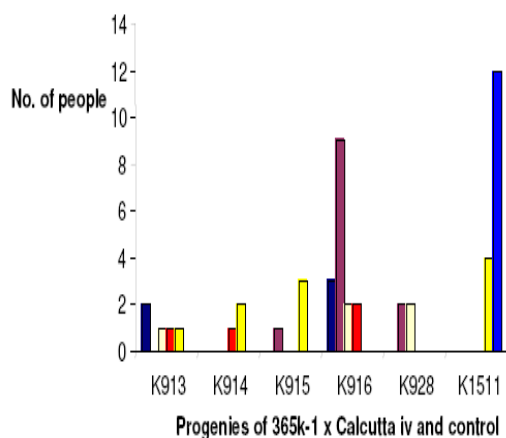
K1511 = local check ('Mwazirume'); 367k-7, 365K-1, 401K-1 and 660K-1 are tetraploid female parents; calcutta iv (Calcutta 4) and 8075 are diploid male parents

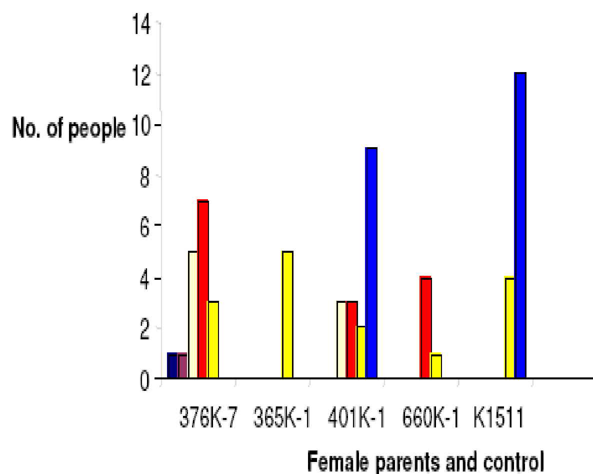
Legend: colour of the bar graph indicating panellists responses

Figure 2.3. Panellists' response on food colour of new banana hybrids together with their female parents and local check (AAA-EA highland banana)









K1511 = local check ('Mbwazirume'); 367k-7, 365K-1, 401K-1 and 660K-1 are tetraploid female parents; calcutta iv (Calcutta 4) and 8075 are diploid male parents

Legend: colour of the bar graph indicating panellists responses

Figure 2.4. Response of respondents on mouth feel of new banana hybrids together with their female parents and local check (AAA-EA highland banana)

Table 2.12 shows a summary of the comments given by farmers on the new materials they tested. Some materials satisfied farmers on a few criteria but performed poorly on other important traits. For example, K914 had the desired yellow colour and taste but it had a small bunch and small fingers.

Table 2.12. Summary of characteristics of the new banana materials as described by farmers

	Farmers comments	
Clone	What they like	what they do not like
K913	long fingers	small bunch, poor aroma, brown colour
K914	good aroma, pleasant taste, yellow colour	short fingers, small bunch
K915	medium fingers, pleasant taste	small bunch, cracked fingers, poor aroma, brown colour
K916		small bunch, poor aroma, hard texture, brown food colour
K928		small bunch, very short fingers hard texture, poor aroma
M1311	big bunch, long fingers pleasant taste, yellow colour, soft texture, good flavour	
K1313	big bunch, big and long fingers	poor taste, brown colour, hard texture, poor flavour
K1314	good flavour, yellow colour	small bunch, short fingers, hard texture
K1211	big bunch, long fingers	pink food colour, hard texture, poor aroma
K1223	long fingers	small bunch, poor taste, brown colour, hard texture
K1216	big bunch, long fingers, soft texture	brown colour, poor aroma
K1224	yellow food colour	small bunch, short fingers, poor taste, hard texture
K1511 (local check)	medium bunch, long fingers, yellow colour, pleasant taste, soft texture, good flavour	

According to farmers, 'empoma' (taste) and 'obuwewevu' (mouth feel/texture) were the most important parameters that influenced the overall acceptability of new materials. 'Langi' (food colour) and 'akawoowo' (aroma) were also equally important in their choice of new materials (Figure 2.5).

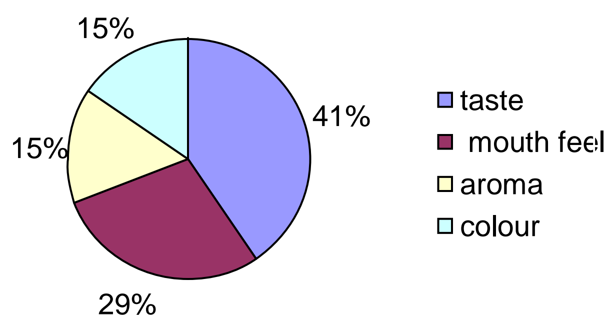


Figure 2.5. Relative importance of factors that affect overall acceptability of new banana genotypes

Plate 2.5 shows the food colour of some of the prepared bananas. The preferred yellow colour is indicated by H which was a local check. The colour of F was also preferred by farmers because it is not different from the colour of local check. The brown colour in food products E and B were not preferred by farmers.

B = K1211; E = K916; F = 401k-1; and H = Mbwazirume (local check).

Plate 2.5. Food colour of different banana materials that were tested

2.4 Discussion

2.4.1 Importance of bananas and farmer perceptions of banana constraints

The importance of bananas in the low and medium banana production zones in Uganda for food and cash was highlighted. Apart from being a traditional food security crop, Bagamba (2007) reported that in south western Uganda (an area with a relatively high banana production), banana brings more returns for labour than any other crop. Farmers also indicated that they are using banana peels, banana leaves, and male buds to feed their animals. Farmers in central Uganda have realised the need to integrate animals in bananas growing especially for manure to maintain soil fertility. Bagamba (2007) reported that the main causes of low soil fertility in the central region of Uganda were low levels of nitrogen and potassium. Similarly, farmers in the low and medium production zones identified soil fertility as a problem to production of bananas (Table 2.5). Therefore by applying animal manure to bananas, farmers will improve the levels of nitrogen in their soils.

About 60% of farmers in the low production zones indicated that the local bananas produced low bunch weights. Although a smaller proportion of farmers in the low

banana production zone reported low bunch weights, it was a general problem in the two production zones. These reduced banana yields were caused by banana weevils (according to 33% of the farmers in the low production zone and 18% in the medium production zone), low levels of banana management and banana bacterial wilt. Banana bacterial wilt was only identified in the low banana production zone, and 44% of farmers in this region could clearly describe the symptoms of banana bacterial wilt. Banana bacterial wilt had just appeared in the low production zone (Tushemereirwe et. al., 2003). By the time of the survey the disease had not spread to the medium banana production zone. Also because of the presence of this new disease outbreak, there was an intensive sensitisation campaign in areas where the disease occurred.

About 7% of the farmers in the medium production zone and 3% percent in the low production zone had knowledge of black Sigatoka as a banana production constraint. Yet there was more disease in the low production zone than in the medium banana production zone (Table 2.7). Earlier studies by Bagamba et al. (2000) had indicated that farmers in central Uganda where banana production had declined were not aware of black Sigatoka and attributed its symptoms to the banana weevil. In fact it appeared that the situation has not changed since then. About, 36% of farmers in the low production zone and 35% in the medium production zone associated low banana yields with the banana weevils. The banana breeders should work with extension workers to design programmes of educating the farmers about the banana diseases. This is important because farmer knowledge of the disease will enhance the adoption of the control strategy. The differences in production levels, crop management strategies, and the relative importance attached to the crop in the two regions could have caused the variation in responses of farmers about the banana constraints.

2.4.2 Farmer preferred traits

The consumer qualities of taste, colour, aroma and softness were the most important reasons farmers liked the East African highland bananas. For example 30% in the low banana production zone and 37% in the medium production zone preferred local bananas because of their food quality. On the other hand, 28% of farmers in the low production zone and 12% in the medium production zone liked the local banana because they were marketable. The low banana production zone is closer to the urban

centre, Kampala which provides an immediate market for the bananas. One of the reasons that led to expansion of bananas in south western Uganda was as a result of the increased access to urban markets (Bagamba, 2007). Farmers also preferred early maturing varieties as pointed out by 14% of farmers in the medium and 6% farmers in the low production zones, respectively. A higher percentage of farmers in the medium production zone preferred early maturing varieties possibly because of competition for the market in the medium production zone whereas in the low production zone, there is too much demand for bananas so the banana market is always guaranteed.

In addition to not having the preferred traits, farmers failed to adopt the black Sigatoka resistant materials because these bananas required intensive management and produced very few suckers. It emerged that quality food traits, heavy bunches, resistance to pests and diseases, tolerance to drought, and time to maturity were the most important desirable traits in that order (Table 2.11). In a study by Katungi et al. (2001) and Gold et al. (2002b), when farmers were requested to give banana selection criteria among traditional cooking banana cultivars, they chose bunch size, taste, longevity, and marketability. The most important outcome of this survey is that farmers from the two production zones surveyed expressed preference for the same traits in the local bananas. Similarly, farmers seemed to desire the same traits in the new materials. This implies that the same materials can be bred and promoted in the two banana production zones.

The Ugandan National Banana Research Programme, has been improving East African highland bananas for pest and disease resistance. Unfortunately, less than 50% of the materials exposed to farmers meet their food quality and production attributes (NaCRRI, 2007). Therefore continuous breeding efforts are required to meet end-user demands.

2.4.3 Verification of farmer preferred traits in new banana materials

An incidental discovery occurred when the panellists were preparing new banana materials for tasting, they complained of the banana sap they found in the materials while peeling them before cooking. Farmers observed that although K1211 had big fingers, it had too much sap. It thus emerged that banana sap can be a problem in the overall acceptability of new banana products. There is a need to investigate further the

role of sap in *Musa* improvement and how the sap is associated with other factors such as reaction to diseases.

Farmers tested female parents together with the new banana hybrids. The panellists approved the aroma, taste and food colour of 660k-1, 401k-1, 365k-1 and 376k-7. The progenies generated by the female parents with male parents 8075 and Calcutta 4 were also tested. The clones did not have all the quality traits required by farmers except clone M1311 (401k-1 x 8075). This clone had a peasant taste, the desired yellow colour on cooking, soft texture and good flavour. On the other hand, K916 (365k-1 x Calcutta 4) was rejected because of poor texture, poor aroma and brown food colour. It is therefore possible to breed new banana genotypes from this population that will be acceptable to farmers. Even if the female parents were liked, they cannot be promoted because they have residual fertility hence can form seeds that will cause their rejection by farmers. In fact, in the initial stages, the Ugandan National Agricultural Research Organisation (NARO) breeding programme selected tetraploids based on their taste and black Sigatoka resistance. Attempts were made to promote them with banana farmers, but when they developed seeds these materials were rejected (S. Mpiira Personal communication). This explains why NARO has adopted the 4x by 2x (Pillay et al., 2004) breeding strategy to restore sterility in secondary triploids which are later promoted to farmers. Therefore, the important outcome of this study is that desirable traits can be obtained from the female tetraploids and incorporated into breeding new materials but there is lack of knowledge behind the inheritance of desirable traits from *Musa* breeding populations.

What farmers consider as acceptable food (with the desired yellow colour, soft texture, and aroma) is an important factor that will affect the adoption of new banana varieties. In the present investigation, farmers were challenged to quantify the consumer traits they considered important. The pleasant taste, soft texture, yellow food colour, and aroma in that order were identified as the most important consumer traits in the choice of new banana materials. Although one finds different tastes within East African highland bananas, Svetlana (2003) found that taste as a consumption attribute was only significant when farmers were making cultivar choices amongst exotic or new banana cultivars. In a study carried out in Luwero, one of the areas under the low banana production zone, farmers indicated to Rutherford and Gowen (2003) that taste

and soft texture were among the traits that constituted acceptable food. Recently, Batte et al. (2008) reported that the soft texture and yellow colour of the cooked product were the most important sensory parameters determining the acceptability of new banana hybrids to farmers. On the other hand, Akankwasa et al. (2008) reported that taste was an important attribute for accepting a new product, while other studies suggest that in addition to sensory aspects, the nutritional value of the product is important (Ayinde and Adewumi, 2008). For most crops consumer preferences affect the over-all acceptability of new varieties (IRRI, 1985; Janick, 2005). However, consumer qualities are complex traits to breed for (Spillane and Thro, 2000), and success in securing acceptability is not guaranteed. For instance, the NARO banana breeding programme has come up with better yielding, pest and disease resistant materials that have not been accepted by farmers (Nowakunda, personal communication). With the current knowledge of biotechnology and physiology advancing over time, future work should investigate the biosynthetic pathways of important consumer traits such as food colour, aroma, and texture and their relationships; the aim would be to possibly identify molecular markers for such traits to speed up and perfect selection and the breeding process. There is also need to understand the inheritance of consumer quality traits like colour, taste, texture and aroma within *Musa* species.

2.5 Conclusion

In conclusion this study was able to show that banana is an important food and cash crop in Uganda. Although its production is affected by a number of constraints like black Sigatoka, farmers were not aware of black Sigatoka as a banana constraint. The survey also established that farmers desired to have new banana materials which maintain the consumer acceptable traits (taste, aroma, texture) and early maturity of local bananas. Also the breeders should aim to select new materials with heavy bunches, resistance to pests and diseases, and tolerance to drought and early maturity if they are to be adopted by the farmers. The verification of the food quality traits indicated that the desired taste, soft texture, the yellow colour and the aroma of the new materials influence overall acceptability of the new product. For example the pleasant taste, yellow colour, appealing flavour and soft texture of the clone M1311 were approved by the panellists while clone K916 was rejected because of its hard

texture, brown food colour, and poor taste. These findings highlight the importance of farmer desired traits in new banana materials. Therefore banana breeders should aim to incorporate the end-user preferred traits if the new materials are to be adopted.

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Appendix 2.1. Farmers role in the development of black Sigatoka resistant bananas in Uganda

1. Date of interview …………………………………………………………………
2. Name of household head …………………………………………………………..
3. Name of respondent ………………………………………………………………..
4. Gender of respondent ……………..
5. Age ………………………………….
6. Village ………………………………
7. sub-county ………………………....
8. Educational level

a) none
b) primary
c) secondary
d) tertiary

9. Reasons for growing bananas

Reason	Rank
a) Food	…………………………………………..
b) Cash	…………………………………………..
c) Both	…………………………………………..
d) Other (specify)	…………………………………………..

10. How many acres of bananas do you have? ………………..
11. Mention the major banana constraints

Constraint Rank

a) …………………………………………………………………………………
b) …………………………………………………………………………………
c) …………………………………………………………………………………
d) …………………………………………………………………………………
e) ………………………………………………………………………………....
f) ………………………………………………………………………………….
g) …………………………………………………………………………………
h) …………………………………………………………………………………
i) ………………………………………………………………………………….

12. What do you like about local bananas (agronomic, marketing, utilisation, taste etc)

a) …………………………………………………………………………......
b) ……………………………………………………………………………..
c) ……………………………………………………………………………..
d) ……………………………………………………………………………..
e) ……………………………………………………………………………..
f) ……………………………………………………………………………...
g) ……………………………………………………………………………..
h) ……………………………………………………………………………..
i) ………………………………………………………………………………

13. What don't you like about local bananas

a) ……………………………………………………………………………….
b) ……………………………………………………………………………….
c) ……………………………………………………………………………….
d) ……………………………………………………………………………….

e) ………………………………………………………………………………………….
f) …………………………………………………………………………………………..

14. Do you experience any diseases in your banana plantation? Yes/no
15. Mention the diseases and their symptoms
Disease symptoms

a) …………………………………………………………………………………………

b) ………………………………………………………………………………………….

c) ………………………………………………………………………………………….

d) ………………………………………………………………………………………….

e) ………………………………………………………………………………………….

16. What is the magnitude of Black sigatoka on a scale of 1-10 (I less important and 10 most important)?
17. Mention the different ways on how you are trying to control the disease
Control measure
a) …………………………………………………………………………………………..

b) …………………………………………………………………………………………..

c) …………………………………………………………………………………………..

d) …………………………………………………………………………………………..

e) ……………………………………………………………………………………

18. Do you have exotic/improved varieties? Yes/no
19. Where did you get them from? …………………………………………………………..
20. Which types do you have?
………………………………………………………………………………………………
………………………………………………………………………………………………
………………………………………………………………………………………………
………………………………………………………………………………………………
…………………………………………………………
21. Farmers use of resistant materials (1-5 scale) 1 less used, 5 mostly used
22. Mention the ways in which the improved varieties are superior to the local ones
………………………………………………………………………………………………
………………………………………………………………………………………………
………………………………………………………………………………………………
………………………………………………………………………………………………
…………………………………………………………
23. If the farmer does not have improved banana varieties, is he/she aware of them? Yes/No ……………………………………………..
24. What are the problems associated with improved varieties?

a) …………………………………………………………..
b) ………………………………………………………….
c) ………………………………………………………….
d) ………………………………………………………….
e) …………………………………………………………..
f) …………………………………………………………..

25. If we are to get new varieties of bananas, what qualities would you want them to have?

Quality Rank

a) ……………………………………………………………………………………….

b) ………………………………………………………………………………………..

c) ……………………………………………………………………………………….

d) ……………………………………………………………………………………….

f) ……………………………………………………………………………………..

Thank you

Chapter three

Appraisal of methods for assessing black Sigatoka resistance in diploid banana populations

Abstract

The effective improvement of banana diploids for black Sigatoka resistance requires identification of a stable, reliable and efficient technique to assess disease damage and predict yield loss due to the disease. Three disease assessment techniques were appraised. These comprised: (i) assessing disease severity 6 months after planting, (ii) estimating disease development over time in the different accessions, and (iii) assessing the youngest leaf spotted (YLS) at flowering. The assessment was implemented on 18 diploid accessions together with susceptible and resistant checks. The accessions were planted in a 4 x 5 rectangular lattice design with two replicates at Kawanda Agricultural Research Institute in Uganda during 2005 to 2007. Natural disease inoculum was used with experimental plots planted in between the rows of a susceptible local cultivar that acted as a spreader. All the three assessment techniques used were able to classify the *Musa* accessions into resistant and susceptible classes. However, the rankings of the clones into resistant and susceptible by the different assessment techniques were not consistent. The rankings of YLS correlated positively with those of area under disease progress curve (AUDPC) ($P<0.05$). The AUDPC rankings correlated strongly with the rankings of disease development time ($P<0.001$). The YLS and AUDPC predicted significantly total leaves at flowering ($R^2=0.53$). Also AUDPC and YLS significantly predicted bunch weight although the coefficient of determination was low. Overall AUDPC resulted in the highest coefficient of determination ($R^2=0.84$) in detecting black Sigatoka response among the diploid *Musa* clones. Taking into consideration the time involved in maintaining the banana plants up to flowering before disease assessment using YLS, it is recommended that the disease resistance be assessed six months after planting and the disease severity data converted into AUDPC data.

3.1 Introduction

Black Sigatoka is one of the major diseases reducing banana yields in Uganda. The subsistence farmers are at risk of food insecurity since the disease can cause yield loss of 30-50% on bananas and plantains (Mobambo et al., 1993; Tushemereirwe, 1996). The most viable and sustainable approach of controlling black Sigatoka for resource limited farmers is by use of host plant resistance (Stover and Buddenhagen, 1986; Swennen and Vuylsteke, 1993). Host plant resistance involves evaluating materials in order to select sources of resistance for use in generating new hybrids. Reliable methods of assessing disease resistance of banana genotypes are essential in order to identify black Sigatoka resistant materials to involve as parents in the breeding programme in Uganda.

Banana breeding has relied on diploids because they are male fertile and are resistant to pests and diseases (Swennen and Vuylsteke, 1993; Vuylsteke, 2001; Tushemereirwe et al., 2005). The initial wild diploids used transmitted the poor agronomic traits to their progenies (Rowe and Rosales, 1996). It therefore became important to improve banana diploids for both agronomic and disease resistance traits. The best way to improve multiple traits (agronomic and disease traits) at the same time would be to use recurrent selection procedures. The use of recurrent selection procedures will help accumulate alleles for disease resistance as well as other important agronomic traits in the population. Black Sigatoka resistance has been reported to have quantitative resistance (Ortiz and Vuylsteke, 1994), therefore there is need for an assessment technique which can detect small differences among the accessions within the population.

One of the methods, the youngest leaf spotted (YLS) Vakili (1968), can be used to differentiate response of *Musa* genotypes to black Sigatoka resistance. Youngest leaf spotted was also reported to predict yield loss in terms of bunch weights in kilogrammes (Craenen and Ortiz, 1998). Because YLS has been reliable in disease assessment and determining reduced banana yields, it has been widely used to assess disease damage in *Musa* species. Youngest leaf spotted, normally done at flowering, involves recording the youngest leaf with at least 10 necrotic lesions by counting from

the top-most leaf. It takes bananas about 9 to 12 months from planting to flowering, hence making it expensive to maintain and manage banana plants whose disease response is unknown. Besides, breeding materials need to be selected early so that they participate in crosses at flowering. Youngest leaf spotted is a good method of assessing the disease but it delays the selection process and makes conventional breeding an expensive process.

Investigations have been made on identifying early assessment techniques to distinguish response of *Musa* genotypes to black Sigatoka resistance. Mobambo et al. (1997) investigated host response of different ages of *Musa* germplasm to black Sigatoka resistance under natural infestation. Disease incubation time, disease development time, youngest leaf spotted and life time of leaf of different banana genotypes of young and old plants were recorded and the disease responses correlated. There were significant correlations for disease development time, youngest leaf spotted and life time of leaf between young and mature plants. However, it was noted that early evaluation for disease response using disease development time could not predict agronomic traits like yield performance (Mobambo et al., 1997). Craenen and Ortiz (1998) investigated influence of black Sigatoka on the growth and yield of diploid and tetraploid hybrid plantains. In diploids disease incubation time correlated significantly with days to fruit filling. The relationship was not significant for tetraploid hybrids. These findings suggested that disease development is unable to predict yield loss due to black Sigatoka in bananas, although it can detect disease response among different *Musa* accessions.

Recently Twizeyimana et al. (2007) investigated a rapid screening technique of *Musa* species to black Sigatoka using in-vitro plantlets, and detached leaves. The plantlets and detached leaves were evaluated in-vitro for their response to black Sigatoka resistance. The investigation concluded that the two assays were rapid and effective in space utilisation and screened *Musa* genotypes to black Sigatoka resistance. However, Liu et al. (2007) doubted whether detached leaf pieces support development of disease symptoms and plant responses that would compare to those that would be observed using intact plants with attached leaves. This is because in intact plants with attached leaves the relationship between the root system (mineral and organic nutrition, water uptake, soil micro-organism) and the foliar system (photosynthesis, transpiration, foliar

emission) is maintained (Mauricio Guzman Personal Communication, 2008). Also laboratory experiments can be expensive since it involves buying consumables for raising inoculums, and it takes a lot of time to produce enough inoculum.

The deficiencies in all these early assessment techniques suggest that there is need to identify a method of quantifying black Sigatoka resistance that is stable, reliable and able to predict yield. Disease severity using Area Under Disease Progress Curve (AUDPC) has been used to quantify rusts (Holland and Munkvold 2001; Kushwaha et al., 2007), mildews (Lipps et al., 1989; Danielsen and Munk, 2004) and leaf spots (Jeger and Viljanen-Rollinson, 2001; Asea et al., 2002) in cereals, legumes and potatoes. In potatoes, AUDPC based on three-leaf method showed the highest negative correlation with yield and is regarded as the best method to predict yield loss caused by downy mildew (Danielsen and Munk, 2004). In bananas and plantains AUDPC was used to differentiate banana and plantain genotypes' response to black Sigatoka (Vera, 2008). However, usefulness of AUDPC in yield prediction in bananas and plantains has not been reported. Unlike YLS, AUDPC is not limited to a standard stage of growth of a banana plant. From information available, it appeared possible to differentiate banana genotypes response to black Sigatoka using youngest leaf spotted, area under disease development curve and disease development over time (DDT) but the efficiency and reliability of AUDPC and DDT have not been established.

3.1.1 Objectives

The objectives of the study were:

a) to compare the efficiency of youngest leaf spotted, disease development time and area under disease progress curve for black Sigatoka assessment in a diploid population, and

b) to investigate the relationship between area under disease progress curve and disease development over time with bunch weight in diploid bananas.

3.1.2 Hypotheses

The hypotheses of the study were:

a) youngest leaf spotted, area under disease progress curve and disease development over time are equally effective in discriminating diploid banana genotypes for their resistance to black Sigatoka disease, and

b) youngest leaf spotted, area under disease progress curve and disease development over time has a significant relationship with bunch weight (kg) of banana diploids.

3.2 Materials and methods

3.2.1 Germplasm

The materials evaluated for black Sigatoka resistance were obtained from the International Institute of Tropical Agriculture (IITA), Fundación Hondureña de Investigación Agrícola (FHIA) and International Network for the Improvement of Bananas and Plantains (INIBAP). Others were developed from Kawanda Agricultural Research Institute (KARI) in Uganda through inter-diploid crosses. Germplasm characteristics are shown in Table 3.1.

Table 3.1. Sources and characteristics of diploid materials used in black Sigatoka resistance assessment

Clone	Ploidy	Source	Principal selection criteria
Yangambi Km 5	3x	INIBAP	Resistant to black Sigatoka
SH 3142	2x	FHIA	Test material
Morong Princesa	2x	INIBAP/IITA	Resistant to black Sigatoka
Wambo	2x	INIBAP/IITA	Test material
Opp 861	2x	IITA	Test material
9719	2x	IITA	Resistant to black Sigatoka
Pagtou	3x	INIBAP	Test material
8615-1	2x	KARI	Test material
8075	2x	IITA	Resistant to black Sigatoka
Calcutta 4	2x	FHIA	Resistant to black Sigatoka
1535K-1	2x	KARI	Test material
5365	2x	KARI	Test material
202SH	2x	KARI	Susceptible to black Sigatoka
Pisang lilin	2x	INIBAP	Resistant to black Sigatoka
8532	2x	KARI	Test material
Pitu	2x	IITA	Test material
3202	2x	KARI	Test material
Galeo	2x	INIBAP	Test material
Musa acuminata subsp malaccencis	2x	FHIA/IITA	Resistant to black Sigatoka
Grand Naine	3x	INIBAP	Susceptible to black Sigatoka

IITA = International Institute of Tropical Agriculture
FHIA = Fundación Hondureña de Investigación Agrícola
INIBAP = International Network for the Improvement of Bananas and Plantains
KARI = Kawanda Agricultural Research Institute.

3.2.2 Experimental design and management

The diploid accessions were planted at KARI on 2nd December 2005. Kawanda Agricultural Research Institute is located at 00’ 25’’N, 00’ 32’’E at an altitude of 1210m above sea level and experiences a bimodal type of rainfall with “short” rains starting in March/April to June and the “long” rains start in August to November/December.

The land was ploughed using a tractor, thereafter it was marked, holes of 45cm diameter by 45cm in depth were dug. Soil was mixed with about 5kg of kraal manure in the hole before planting. The planting materials were obtained from the banana fields at KARI. Before planting, the banana corms were pared and dipped in chlorpyrifos

(15ml per 20l of water) for about one hour to disinfect the corms against nematodes and banana weevils.

Apart from 18 diploids (AA) two other clones (one black Sigatoka susceptible check, Grand Naine, AAA and the resistant check Yangambi Km 5, AAA) were included to make a total of 20 accessions which were planted in a 4 x 5 rectangular lattice design. Each plot had six plants per block replicated two times. The natural disease pressure which is high at KARI was the source of inoculum. However, to ensure that each plant had high exposure to the disease, the experimental plots were planted between rows of a local cultivar 'Mbwazirume', AAA-EA that was used as a spreader for black Sigatoka.

Weed control was implemented regularly by spraying with glyphosate applied at least four times in a year. Detrushing was practised minimally because there was need to maintain the disease inoculum around the test plants. Desuckering, removal of excess plants to maintain at most three plants per mat was also implemented two times a year.

Black Sigatoka was assessed and compared using three disease assessment protocols. These were assessing disease severity six months after planting, estimating disease development over time in the different accessions and assessing the Youngest Leaf Spotted (YLS).

Six months after planting black Sigatoka was assessed using the modified Stover (1971) scale. The proportion of the diseased leaf was estimated out of 100%. Leaves which had zero percent infection, 12% infection, 25% infection and 50% infection were photographed, and kept in the folder for reference every time disease was being assessed (Plate 3.1). Infected leaves between the severity infections indicated in Plate 3.1 were also estimated accordingly. Each leaf was assessed individually and the overall disease damage per plant was computed. This assessment was repeated four times at intervals of 14 days. Disease damage was converted into the area under disease progress curve (AUDPC) using the formula given below as suggested by Shaner and Finney (1977):

$AUDPC = \sum_{i}^{n=1} [(X_{i+1} + X_i)/2][t_{i+1} - t_i]$

Where,

X_i= proportion of the host tissue damaged at i^{th} day

t_i = the time in days after appearance of the disease at i^{th} day, and

n = the total number of observations

Plate 3.1. Diseased banana leaves illustrating the scale used to quantify reaction of genotypes to disease

Assessing disease development over time started six months after planting. It is presumed that an emerging leaf (cigar leaf), picks the disease spores from the atmosphere. The date of leaf emergence was recorded. This leaf was observed continuously through when the disease symptoms appeared, when the leaf was 12% dead, 25% dead, 50% dead up to when the leaf was completely dead. These data sets were used to compute days from leaf emergence to symptom appearance, days from symptom appearance to 12% leaf damage, days from symptom appearance 25% leaf damage, days from symptom appearance to 50% leaf death and days from symptom appearance to complete leaf death.

At flowering the youngest leaf spotted (the first leaf showing at least 10 necrotic spots) was recorded. Also, the total number of leaves both at flowering and harvest were recorded.

3.2.3 Data analysis

Data sets were analysed using Statistical Analysis Software (SAS) version 9.1 (SAS Inc., 2002) using the model $\gamma_{ij}=\mu + \beta_i + g_j + \epsilon_{ij}$

where,

γ_{ij} = black Sigatoka response

μ = overall mean

β_i = block effect (nested in replication)

g_j = genotype response

ϵ_{ij} = experimental error

The generalised linear model was used to perform the analysis of variance. For those parameters that were significant, the least square means (lsmeans) for each accession were computed. To compare how the three assessment protocols ranked the *Musa* accessions, means generated using the different assessment techniques were used to rank the accessions in reference to the susceptible and resistant checks. These were separated using the least significant difference at a probability level P=0.05. Phenotypic correlations were computed in order to compare the relationships between the disease assessment protocols and agronomic parameters and the correlation coefficients were tested at a probability level of P=0.05 using Pearson method.

Multi-colinearity among the associated traits was eliminated using variance inflation ratio (VIR). The regression analysis was carried out between bunch weight, and number of functional leaves at flowering, and disease parameters using a stepwise regression approach, in order to find out if the coefficients of the parameters had a genuine effect on the dependent variable (yield and total leaves at flowering). In order to find out the efficiency of the assessment techniques, their coefficients of variation and determination were compared.

3.3 Results

3.3.1 Ranking of genotypes by the three methods

Table 3.2 shows the means of youngest leaf spotted (YLS), area under disease progress curve (AUDPC), and days from symptom appearance to 25% leaf damage (DTQ). All the three methods ranked genotypes into resistant and susceptible clones. According to YLS, Yangambi Km 5 was the most resistant genotype, Grande Naine the most susceptible genotype and Calcutta 4 was ranked as having moderate resistance. On the other hand, AUDPC ranked Calcutta 4 as the most resistant and 202SH as the most susceptible clone. Days from symptom appearance to 25% leaf damage ranked *Morong princesa* as the most resistant and Grande Naine as the most susceptible. Generally, DTQ and YLS ranked Grande Naine as the most susceptible genotype. On the contrary, YLS ranked 3202 as a susceptible genotype but DTQ ranked it as one of the resistant genotypes.

The AUDPC had the highest R^2 of 0.84 followed by DTQ with R^2 of 0.76. The youngest leaf spotted had the least R^2 of 0.59. However, AUDPC had the highest coefficient of variation of 44%. When the AUDPC data were transformed (log(x+1), the coefficient of variation reduced to 17%. Although the R^2 slightly reduced to 80%, it was still higher than the R^2 of YLS and DTQ (Appendix 3.2). The YLS and DTQ had relatively low coefficients of variation of 19% and 23%, respectively (Table 3.2).

From analysis of variance (Appendices 3.1 and 3.2) days from leaf emergence to symptom appearance (incubation time) and days from leaf emergence to 12% leaf damage were also able to discriminate the accessions into resistant and susceptible clones. However incubation time had a lower coefficient of determination (R^2=0.34) than the number of days it took from leaf infection to 12% and 25% leaf damage. The number of days it took from leaf infection to 12% leaf damage had a lower R^2 than the number of days it took to develop disease symptoms up to 25% leaf damage. Therefore the number of days it took a leaf to 25% leaf damage was selected for comparison with the other two methods because, it had and a higher R^2=0.76 than the other two methods (Appendix 3.1).

Table 3.2. Means of Youngest Leaf spotted (YLS), Area Under disease Progress Curve (AUDPC) and days from symptom appearance to 25% leaf damage (DTQ) of *Musa* accessions at KARI

Accession	YLS		AUDPC		DTQ	
	Mean±se	Rank	Mean±se	Rank	Mean±se	Rank
Yangambi Km 5**	9.5±0.59	1	157±91.5	5	38.2±3.06	8
SH 3142	8.8±0.88	2	531±92.1	12	43.5±4.17	4
Morong Princesa	7.9±0.62	3	77±98.3	3	49.9±3.42	1
Wambo	7.3±0.88	4	621±120.7	15	26.3±3.28	15
Opp 861	7.2±0.67	5	124±94.1	4	39.3±3.17	5
9719	7.2±0.59	6	67±119.1	2	31.3±3.60	14
Pagtou	6.1±0.61	7	924±95.2	16	20.5±3.14	17
8615-1	6.0±0.55	8	596±93.4	14	32.2±2.75	12
8075	5.9±0.59	9	278±91.6	9	45.9±3.17	3
Calcutta 4	5.9±0.59	10	46±110.3	1	38.8±3.25	6
1535K-1	5.7±0.59	11	311±89.9	10	33.2±2.84	11
5365	5.6±0.59	12	576±99.3	13	31.5±2.97	13
202SH	5.5±0.72	13	1789±100.7	20	15.2±3.37	18
Pisang lilin	5.1±0.57	14	344±92.7	11	33.4±2.77	10
8532	5.0±0.57	15	164±91.2	6	38.4±2.97	7
Pitu	4.8±0.58	16	1027±87.5	19	15.1±2.97	19
3202	4.6±0.57	17	230±92.8	8	48.2±3.10	2
Galeo	4.6±0.74	18	1020±103.1	18	23.6±2.98	16
Musa acuminata subsp malaccencis	4.5±0.69	19	202±104.9	7	33.7±3.17	9
Grand Naine*	3.7±0.67	20	924±114.5	17	9.9±3.16	20
$LSD_{(0.05)}$	1.6		226		7.3	
R^2	0.59		0.84		0.76	
CV (%)	23		44		19	

** Resistant check; * susceptible check; se standard errors of the means

3.3.2 Relationships among disease rating methods

The different assessment protocols ranked the diploid accessions (1 most resistant and 20 most susceptible). The rankings were correlated and results are shown in Table 3.3. The ranks of genotypes using area under disease progress curve and days from leaf emergence to 25% leaf damage were positively correlated ($P<0.001$). The rank correlations of AUDPC and YLS were positively correlated and significant ($P<0.05$). The correlation coefficients of ranks of DTQ and YLS were not significant although they had a positive relationship. The ranks of total leaves at flowering correlated positively ($P<0.001$) with the ranks of YLS.

Table 3.3. Spearman rank correlations of different assessment methods for black Sigatoka in banana diploids at Kawanda Agricultural Research Institute

	Youngest leaf spotted	Area under disease progress curve	Days from leaf emergence to 25% leaf damage	Total leaves at flowering
Youngest leaf spotted	1			
Area under disease progress curve	0.37143*	1		
Days from leaf emergence to 25% leaf damage	0.35789	0.73835**	1	
Total leaves at flowering	0.66466**	0.31729	0.32632	1

* Data significant at P< 0.05;** Data significant at P<0.001

Table 3.4 shows Pearson correlation coefficients of different assessment protocols in *Musa* accessions with bunch weight and total leaves at flowering at Kawanda Agricultural Research Institute. Youngest leaf spotted had a strong but negative correlation (P<0.01) with AUDPC. The YLS had a weak and positive correlation (P<0.05) with days from leaf emergence to 25% leaf damage (DTQ). The area under disease progress curve had a negative but strong correlation (P<0.001) with total leaves at flowering.

In addition to disease assessment protocols, YLS had a positive and strong correlation with total leaves at flowering (P<0.001) and a weak but significant (P<0.05) positive correlation with bunch weight (P<0.05). Also AUDPC had a negative and strong correlation (P<0.01) with total leaves at flowering and a negative strong correlation (P<0.001) with bunch weight. The DTQ had a strong positive correlation (P<0.01) with total leaves at flowering and a positive correlation with bunch weight (P<0.01) (Table 3.4).

Table 3.4. Pearson correlation coefficients of different assessment protocols in *Musa* accessions with bunch weight (kg) and total leaves at flowering at KARI

	YLS	TLF	AUDPC	DAA	DQQ	DTQ	DH	DFF
Youngest leaf spotted (YLS)								
Total leaves flowering(TLF)	0.7061***							
Area und. disease (AUDPC)	-0.3139**	-0.4096**						
Dys disease symptom(DAA)	0.1447ns	0.1692ns	-0.4109***					
Dys leaf infection 12%(DQQ)	0.2142*	0.2922**	-0.6560***	0.5742***				
Dys 25% leaf damage(DTQ)	0.1958*	0.3219**	-0.7074***	0.5195***	0.8680***			
Dys 50% leaf damage (DH)	0.1371ns	0.2994*	-0.6645***	0.4294***	0.7731***	0.9009***		
DFF (100% damage-DAA)	0.0872ns	0.1685ns	-0.5257***	0.2395*	0.6398***	0.7789***	0.8829***	
Bunch weight (BWT)	0.287*	0.1749ns	-0.3958***	0.3958***	0.3828***	0.3370**	0.3646**	0.3093*

ns data non significant, *, **, *** data significant at P=0.05, P=0.01 and P=0.0001, respectively

When a stepwise regression was carried out between total leaves at flowering and disease parameters, AUDPC, DAA and YLS were involved in the regression equation (TLF=5.97-0.0008AUDPC + 0.005DAA + 0.82YLS; R^2=53.8) where TLF is total number of functional leaves at flowering. The AUDPC was significant (P=0.039) and YLS was highly significant (P<0.001). Although days from leaf infection to when symptoms appeared (DAA) was included in the equation, they were not significant (P=0.894) (Table 3.5).

To find out which of the disease parameters could be used to predict yield in the *Musa* accessions, a stepwise regression was carried out between bunch weight and disease parameters. Area under disease progress curve, and YLS were significant in yield prediction with probabilities of (P<0.001 and P=0.017, respectively). Total leaves at flowering, and days from flowering to harvest were not significant (P>0.05) although they were included in the regression equation (Table 3.6).

Table 3.5. Stepwise regression of total leaves at flowering (TLF) on disease assessment parameters

Variable	Parameter estimate	Standard error	t-value	P-value
Constant	5.97	1.37	4.35	<.001
Area under disease progress curve	-0.000979	0.00047	-2.09	0.039
Days to symptom appearance	0.0047	0.0349	0.13	0.894
Youngest leaf spotted	0.8166	0.0939	8.7	<.001

TLF=5.97-0.0008AUDPC + 0.005DAA + 0.82YLS; R^2=53.8

Table 3.6. Stepwise regression of bunch weight (BWT) on disease parameters and days from flowering to harvest

Variable	Parameter estimate	Standard error	t-value	P-value
Constant	-5.00	2.75	-1.82	0.073
Area under disease progress curve	0.004317	0.000992	4.35	<.001
Days to harvest (DTH)	0.00562	0.00855	0.66	0.512
Total leaves at flowering (TLF)	0.286	0.242	1.18	0.24
Youngest leaf spotted (YLS)	0.713	0.294	2.43	0.017

BWT = -5 + 0.004AUDPC + 0.006DTH + 0.29TLF + 0.713YLS; R^2 = 0.24

3.4 Discussion

3.4.1 Ranking of genotypes by the three methods

Generally the assessment protocols classified the *Musa* accessions into resistant and susceptible clones. However, the rankings of the diploid genotypes were not consistent across the three assessment methods. For example YLS ranked Yangambi Km 5 as the most resistant while AUDPC ranked *Morong princesa* as the most resistant. Also genotype, 3202 was ranked by YLS among the susceptible genotypes but DTQ indicated the same genotype was resistant to black Sigatoka. The ranking of Yangambi km 5 as the most resistant genotype by YLS was expected because this genotype was used as a resistant check and it was chosen based on its YLS (IITA, 1989; Orjeda, 1998).

The high variation of black Sigatoka resistance among the diploid accessions evaluated using AUDPC could have caused a high coefficient of variation of AUDPC. However transforming AUDPC data with log(x+1) reduced the coefficient of variation. Youngest leaf spotted and days from leaf infection to 25% leaf damage had reasonably low coefficients of variation. The AUDPC had the highest coefficient of determination R^2 (0.84) among all the assessment techniques. This indicated that AUDPC accounted for most of the variation in disease resistance among the diploid clones. Area under disease progress curve also correlated significantly (r^2 = -0.3139) with YLS which has been used to assess black Sigatoka resistance among *Musa* species. In the bunch weight prediction model, AUDPC was also significant implying that it might also be used to predict yield losses in banana diploids due to black Sigatoka. Days from leaf infection up to 25% leaf damage due to black Sigatoka also had a better R^2 (0.76) than YLS (0.59), better coefficient of variation (19%) than AUDPC. Yet the days it took a leaf up to 25% leaf damage to black Sigatoka could not be used either to predict bunch weight or total leaves at flowering. Also a lot of time had to be committed in the field to follow up a leaf from emergence up to 25% leaf damage. Therefore, AUDPC seemed to be able to assess black Sigatoka damage before flowering, and appeared reliable in discriminating diploid *Musa* clones into resistant and susceptible ones. Days from leaf infection to symptom appearance (incubation time) was not significantly correlated with youngest leaf spotted and did not significantly affect total leaves at flowering. In

previous studies, Jones (2000) reported that incubation time did not correlate with black Sigatoka resistance. Results from this study also suggest that it might not be feasible to use incubation time to assess black Sigatoka resistance among *Musa* genotypes because it had a low $R^2 = 0.34$ (Appendix 3.1).

3.4.2. Relationship among disease rating methods

The number of functional leaves at flowering is an indication of disease resistance. The number of total leaves at flowering is also important in fruit filling. The negative correlation between total leaves at flowering with AUDPC suggested that in this diploid population plants with high disease severity assessed by AUDPC were expected to have reduced number of leaves at flowering. This is true since the disease causes leaf death through necrosis.

Youngest leaf spotted and AUDPC had a significant linear relationship with total leaves at flowering (TLF) and bunch weight. This suggested that YLS at flowering and AUDPC six months after planting could be used to predict TLF and BWT. Prediction of BWT using AUDPC will help in selection of materials that combine high yield with disease resistance. In a study by Craenen and Ortiz (1998) disease resistance based on YLS helped in selection of *Musa* genotypes that combined higher yields with disease resistance. The positive correlation in rankings of YLS and AUDPC also suggest that AUDPC might be as efficient as YLS in discriminating the clones according to their disease resistance. However, YLS is done at flowering when a lot of resources could have been spent to maintain materials whose disease response is unknown. Besides, it might also be late to design/plan crosses to use to improve susceptible banana plants especially in a recurrent selection improvement programme. At the moment, there are no molecular markers that have been applied in selection of black Sigatoka resistant *Musa* genotypes. There is need to select an appropriate early and reliable assessment technique that can predict yield in the selected genotypes. The AUDPC might be used to select resistant banana materials earlier than the YLS method.

Table 3.2 shows Calcutta 4 as the most resistant accession to black Sigatoka disease using AUDPC. On the other hand, YLS indicated that Calcutta 4 has moderate resistance. The high level of resistance identified by AUDPC could imply a hypersensitive disease reaction of Calcutta 4. In other studies Calcutta 4 was reported

to exhibit a hypersensitive disease reaction to black Sigatoka (Craenen and Ortiz, 1998). This type of resistance is not good for quantitative resistance breeding since it can easily break down. The breakdown of resistance in Calcutta 4 has been reported elsewhere (Jones, 2000). It is therefore necessary to use an assessment technique that can detect hypersensitive reaction so that we avoid selecting such materials in a population improvement programme. It appears that AUDPC can identify such type of disease reaction.

Normally, banana plants do not flower at the same time due to genotype and environmental differences. This implies that using YLS method plants will be assessed over a period of time. The fluctuations in environmental conditions may introduce differences even among the same genotypes. Similarly, following disease development in a leaf from emergence up to when leaf is 25% damaged takes a minimum of 50 days in resistant genotypes (Table 3.2). Therefore environmental changes may also influence disease expression even among the same genotype thus introducing errors in the data. On the contrary, AUDPC is not restricted to a stage of either plant growth or leaf damage. Therefore AUDPC assessment can be implemented at any time. However, at least three assessments are required to compute AUDPC (Shaner and Finney, 1977). Assessing the disease severity at an interval of 14 days all the AUDPC assessments can be conducted within one month. From the present study, because of its high R^2=0.84, and its ease of use, this investigation recommends using AUDPC to assess black Sigatoka resistance in *Musa* genotypes.

3.5 Conclusions

In conclusion there was a positive relationship between youngest leaf spotted, area under disease progress curve, and disease development time in assessing diploid clones for black Sigatoka resistance. However, youngest leaf spotted and area under disease progress curve were the only methods found in the present study to predict bunch weight and total leaves at flowering. Area under disease progress curve was considered to be the best method among the three because it had the best coefficient of determination (R^2=0.84) and required less time than disease development time to assess disease damage. Therefore, AUDPC would be recommended to assess disease resistance of *Musa* genotypes to black Sigatoka.

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Appendix 3.1. Analysis of variance of disease development on diploid accessions at Kawanda Agricultural Research Institute during 2005 to 2007

Dependent Variable: Days from leaf emergence to symptom appearance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	28	1409.759334	50.348548	1.85	0.0147
Error	98	2673.075311	27.276279		
Corrected Total	126	4082.834646			

R-Square	Coeff Var	Root MSE	DAA Mean
0.345289	16.22900	5.222670	32.18110

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block(Rep)	9	150.1770696	16.6863411	0.61	0.7844
Geno	19	944.1677997	49.6930421	1.82	0.0304

Dependent Variable: Days from leaf emergence to 12% leaf damage

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	28	13645.67763	487.34563	7.84	<.0001
Error	98	6090.18064	62.14470		
Corrected Total	126	19735.85827			

R-Square	Coeff Var	Root MSE	DQQ Mean
0.691415	14.23525	7.883191	55.37795

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block(Rep)	9	439.28761	48.80973	0.79	0.6303
Geno	19	10852.83576	571.20188	9.19	<.0001

Dependent Variable: Days from leaf emergence to 25% leaf damage

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	28	17822.44852	636.51602	14.29	<.0001
Error	97	4319.42450	44.53015		
Corrected Total	125	22141.87302			

R-Square	Coeff Var	Root MSE	DQ Mean
0.7604921	19.42154	6.673091	64.03175

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block(Rep)	9	584.15011	64.90557	1.46	0.1748
Geno	19	13883.98534	730.73607	16.41	<.0001

Dependent Variable: Days from leaf emergence to 50% leaf damage

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	28	14260.91243	509.31830	10.92	<.0001
Error	94	4383.59164	46.63395		
Corrected Total	122	18644.50407			

R-Square	Coeff Var	Root MSE	DH Mean
0.764886	9.646898	6.828906	70.78862

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block(Rep)	9	1016.82900	112.98100	2.42	0.0160
Geno	19	10749.64205	565.77063	12.13	<.0001

Appendix 3.2. Mean of incubation time and days from leaf emergence to 12% lead damage of diploid accessions at Kawanda Agricultural research Institute during 2005 to 2007.

Accession	Incubation time Mean±se	Days from leaf emergence to 12% leaf damage Mean±se	Transformed area under disease progress curve log (x+1) Mean±se
Yangambi Km 5	32.4± 2.96	53.8±4.46	1.1 ± 0.20
9719	30.5± 2.44	55.3±3.68	2.2 ± 0.16
8532	29.4± 2.60	61.5±3.93	2.5 ± 0.16
8075	29.7± 2.44	54.6±3.67	2.6 ± 0.17
5365	33.8± 2.54	70.3±3.84	2.3 ± 0.17
3202	39.7±2.67	70.2±4.03	0.8 ± 0.20
Calcutta 4	26.5±2.77	39.1±4.18	3.2 ± 0.18
202SH	31.6±2.33	56.8±3.52	2.3 ± 0.16
1535k-1	30.0±2.25	53.5±3.40	2.7 ± 0.17
8615-1	28.1±2.58	42.8±3.89	2.9 ± 0.17
Pagtou	40.1±2.81	80.3±4.24	1.3 ± 0.18
Morong Princesa	37.1±2.27	63.1±3.43	2.5 ± 0.17
Pisang lilin	30.6±3.42	57.2±5.17	2.6 ± 0.17
SH3142	31.5±2.69	50.8±4.06	2.7 ± 0.22
Wambo	28.8±2.44	37.9±3.68	3.0 ± 0.16
Pitu	31.0±2.66	60.6±3.93	2.0 ± 0.17
Opp861	33.9±2.61	61.6±3.93	2.2 ± 0.19
Musa acuminata subsp malaccencis	32.5±2.45	44.6±3.69	3.0 ± 0.19
Yangambi km 5	33.1±2.51	59.4±3.79	1.7 ± 0.16
Grande Naine	32.1±2.59	38.2±3.92	2.9 ± 0.20
LSD	6.4	9.1	0.5
CV(%)	16	14	17
R^2	0.35	0.69	0.80

Chapter four

Variation in diploid (AA) and tetraploid (AAAA) banana populations for black Sigatoka resistance and agronomic traits

Abstract

Breeders require variation and high frequency of the desired alleles in the diploid and tetraploid populations for banana improvement to be successful. There is limited information on genetic variation of diploids and tetraploids currently used in the improvement of bananas in Uganda. The objective of this investigation was to determine the amount of phenotypic and genetic variability for black Sigatoka resistance and agronomic traits in the diploid (AA) and tetraploid (AAAA) populations in Uganda. Phenotypic analysis was conducted on the two populations, and molecular analysis using RAPD markers on the tetraploid population on field banana genotypes planted at Kawanda Agricultural Research Institute during 2005 to 2007. The diploid population showed significant ($P<0.001$) variation for plant height, plant girth, days from flowering to harvest, bunch weight and number of suckers. The diploid population also showed significant ($P<0.001$) differences for youngest leaf spotted, total leaves at flowering, area under disease progress curve, and number of functional leaves at harvest. Principal component analysis, with 85% of total variation accounted for in the diploid data indicated that agronomic traits, plant height and girth explained most of the variation observed in the diploid population. Linear regression analysis showed that youngest leaf spotted, plant girth, and number of clusters predicted up to 42% of variation in bunch weight of diploids. In the tetraploid population significant differences were observed for plant height, girth, and number of suckers ($P<0.05$). Youngest leaf spotted and total leaves at flowering had higher loadings on principal component one. When linear regression analysis was carried out, girth, number of clusters and days from flowering to harvest accounted for 59% of the variation in bunch weight in the tetraploid population. Genetic distances computed from RAPD markers ranged from 0.04 to 0.39 indicating limited genetic variability in the tetraploid population. Therefore there is need to increase the diversity in the tetraploid population.

4.1 Introduction

Genetic variation is important in the improvement of the banana diploid and tetraploid populations for black Sigatoka resistance and other traits such as yield. Although, wild and domesticated banana diploids have been used as sources of disease resistance (Rowe, 1984; Rowe and Rosales, 1996; Swennen and Vuylsteke, 1993; Pillay et al., 2004; Tushemereirwe et al., 2005), little attention has been given to the improvement of agronomic traits in the tetraploid population. The improvement of traits requires genetic variation among the parents to achieve response to selection. For instance, Rowe and Rosales (1993) and Ortiz (1995) reported that the Fundación Hondureña de Investigación Agrícola (FHIA) programme spent more than 20 years to breed an improved diploid with agronomic and disease resistance traits. It could have been possible that these breeders were working with a population which had limited genetic variation for agronomic traits like bunch weight and plant girth. It is possible to make quick genetic gains if there is enough genetic variation in the population for the traits of interest (Fehr, 1987).

Natural variation exists in wild relatives of bananas which breeders can exploit. For example, characterisation of wild *Musa acuminata* from Papua New Guinea using PCR and primers derived from highly repetitive sequences were found to be genetically diverse (Jarret et al., 1993). The diploids being used to improve bananas in Uganda are a mixture of both wild and synthetic diploids whose level of variation is unknown. The synthetic tetraploids that were derived from East African highland bananas are used to recreate seed sterile triploids when crossed with improved diploids (Pillay et al., 2004). These synthetic tetraploids were generated from a sub-group of bananas which are similar, by using a few male parents (Karamura, 1998; Ssebuliba et al., 2006). Although these tetraploids are used in restoring female sterility in secondary triploids, the variation of these tetraploids is unknown. Besides, it could be possible to make 4x by 4x crosses hence improving the tetraploid population. However, to achieve progress, it is essential to work with parents that show genetic variation for the traits of interest.

Statistical analysis using variance, range, and coefficient of variation of phenotypic traits can be used to investigate variability and genetic diversity among plant species.

Differences in DNA[3] structure of plant species are also used to assess diversity and establish the genetic relationships among plants. Tugume et al. (2002) used Amplified Fragment Length Polymorphic (AFLP) molecular markers to study genetic diversity among East African highland bananas and the results agreed with morphological classification by Karamura (1998). The Random Amplified Polymorphic DNA (RAPDs) markers are preferred to AFLPs, because RAPD markers require small amounts of DNA, are quick, and are not expensive. Although the reproducibility of RAPDs has been doubted (Collard et al., 2005), they have been used to study diversity among *Musa* species. For example, Jain et al. (2007) used randomly amplified DNA markers to determine genetic diversity of materials from Indian banana germplasm. Similarly, Agoreyo et al. (2008) used RAPD markers to classify plantains into groups according to their origin. Ferreira et al. (2004) also used molecular markers to determine the genetic diversity of banana diploids.

4.1.1 Objectives

The objectives of the study were as follows:

a) to determine the phenotypic and genotypic variation for black Sigatoka resistance and agronomic traits in the diploid (AA) and tetraploid (AAAA) populations in Uganda,

b) to determine phenotypic correlations between agronomic and disease resistance traits in diploid (AA) and tetraploid (AAAA) bananas in Uganda, and

c) to determine the genetic diversity of tetraploid (AAAA) bananas by using RAPD markers.

[3] DNA is Deoxyribonucleic acid. It is the hereditary material in organisms

4.1.2 Hypotheses

The following hypotheses were tested:

a) there is high genotypic variation for black Sigatoka resistance and agronomic traits in the diploid (AA) and tetraploid (AAAA) populations of bananas in Uganda,

b) there is a strong relationship between black Sigatoka resistance and agronomic traits in diploid (AA) and tetraploid (AAAA) bananas in Uganda, and

c) there is a high level of genetic diversity in tetraploid bananas (AAAA) to be detected by RAPD markers.

4.2 Materials and methods

4.2.1 Diploids and tetraploids germplasm

The diploid and tetraploid materials evaluated for variation for black Sigatoka resistance and other traits are shown in Table 4.1.

Table 4.1. Sources and ploidy levels of diploids and tetraploids evaluated for variation in black Sigatoka resistance and agronomic traits

Diploids	Ploidy	Source	Synthetic tetraploids	Pedigree
Yangambi Km 5**	3x	INIBAP	1201k-1	Nakawere x Calcutta 4
SH 3142	2x	FHIA	1377k-1	Enzirabahima x Calcutta 4
Morong Princesa	2x	INIBAP/IITA	1411k-1	Tereza x Calcutta 4
Wambo	2x	INIBAP/IITA	1438k-1	Entukura x Calcutta 4
Opp 861	2x	IITA	1747k-1	Entukura x Calcutta 4
9719	2x	IITA	1154k-1	Nakayonga x Calcutta 4
Pagtou	3x	INIBAP	199k-4	Tereza x Calcutta 4
202SH	2x	KARI	365k-1	Kabucuragye x Calcutta 4
8075	2x	IITA	376k-7	Nante x Calcutta 4
Calcutta 4	2x	FHIA	401k-1	Entukura x Calcutta 4
1535K-1	2x	KARI	660k-1	Enzirabahima x Calcutta 4
5365	2x	KARI	199k-1	Tereza x Calcutta 4
202SH	2x	KARI	199k-3	Tereza x Calcutta 4
Pisang lilin	2x	INIBAP	222k-1	Nfuuka x Calcutta 4
8532	2x	KARI	246k-1	Kabucuragye x *Musa Balbisiana*
Pitu	2x	IITA	917k-1	Enzirabahima x *M. Burmanica*
3202	2x	KARI		
Galeo	2x	INIBAP		
Musa acuminata subsp malaccencis	2x	FHIA/IITA		
Grand Naine*	3x	INIBAP		

* Susceptible check

** Resistant check

IITA = International Institute of Tropical Agriculture

FHIA = Fundación Hondureña de Investigación Agrícola

INIBAP = International Network for the Improvement of Bananas and Plantains

KARI = Kawanda Agricultural Research Institute

Synthetic tetraploids were sourced from KARI

4.2.2 Experimental design and management

The experiment was planted at Kawanda Agricultural Research Institute, which is located at 00' 25''N, 00' 32''E at an altitude of 1210m above sea level. Kawanda

Agricultural Research Institute has a bimodal type of rainfall with “short” rains starting in March/April to June and the “long” rains starts in August to November/December. The experimental field was ploughed with a tractor, marked and holes of 45cm in diameter by 45cm in depth prepared at a spacing of 3m by 3m. Kraal manure was applied in the prepared holes before planting at a rate of 10kg per plant. Before planting, the banana corms were pared and dipped in chlorpyrifos (15ml per 20l of water) for one hour to disinfect them against nematodes and banana weevils. The diploid clones were planted in a 4 x 5 rectangular lattice design. Each plot had six plants planted between rows of a local cultivar ‘Mbwazirume’, AAA-EA used as a spreader for black Sigatoka. The accessions were replicated two times. It was not possible to get enough planting materials from all the 16 synthetic tetraploids for evaluation, therefore nine tetraploids were planted in a randomised complete block design with two replicates where each plot had five plants per genotype. In both trials natural infestation of black Sigatoka was used. However, the natural infestation was enhanced by a spreader. The experimental plots were planted between rows of the spreader cultivar, ‘Mbwazirume”. The trials were planted on 2nd December 2005 which was at the beginning of the dry season. There was an effort to provide water, once every seven days. Some plants did not sprout, gap-filling was organised six months after planting. Plants that never established after gap-filling were considered as missing plots.

Weeds were managed by spraying with a systemic herbicide, glyphosate. Six months after planting, data were collected on number of suckers and disease severity where each plant in the plot was assessed. The severity was estimated as the proportion (percent) of the plant that was infected. The assessment was repeated three times at an interval of 14 days. The disease severities were converted into area under disease progress curve Shaner and Finney (1977).

$$AUDPC = \sum_{i=1}^{n} [(X_{i+1} + X_i)/2][t_{i+1} - t_i]$$

Where,

X_i = proportion of the host tissue damaged at ith day

t_i = the time in days after appearance of the disease at ith day, and

n = the total number of observations

At flowering, data were collected on, the number of functional leaves, plant height (cm), plant girth at 100cm from the ground, and youngest leaf spotted. At harvest data were collected on the number of functional leaves, bunch weight (kg) and the number of hands.

4.2.3 Laboratory evaluation of tetraploids

Tetraploid banana leaf tissues were collected from the germplasm collection plots at Kawanda Agricultural Research Institute. The DNA was extracted according to Gawal and Jarret (1991). Banana leaf samples were ground in liquid nitrogen with a mortar and pestle. The powdered tissue was added to 20ml pre-heated extraction buffer (CTAB 2%). The reagents were incubated at 60°C for 40 minutes after which 60µl of mercaptoethanol were added. Fifteen millilitres of methylchloroform were added, and mixed by inversion for 15 minutes. The mixture was centrifuged for 5 minutes at 5000xg at room temperature. Equal volume of ice-cold isopropanol was mixed with the sample by inversion until DNA precipitated. The DNA precipitate was rinsed using 70% ethanol. DNA was blotted to dry on a paper towel. The DNA was dissolved in 250µl TE buffer and stored at 4°C.

The amplification reaction contained 1 x PCR mix, 1.5mM $MgCl_2$, 0.2mM dNTP, 20ng Primer, I U Taq plus polymerase and 20ng template DNA. Amplifications were done in a Biometra gradient thermocycler with the cycling profile as follows: an initial denaturation at 94°C at 4 minutes, followed by 32 cycles of I minute at 94°C, I minute at 62°C, and 45 seconds at 72°C with a final extension of 6 minutes at 72°C. The PCR products were mixed with a 1/5 volume of loading buffer and separated on a 1.8% (w/v) agarose gel, in a 0.5TBE at 130V for 45 minutes. The gels were then stained in a 0.4µg/mL ethidium bromide dye. The DNA fragments were then visualised under UV light. To select primers that could amplify RAPD fragments, PCR was carried out to screen 14 random primers of arbitrary sequence (Operon technologies Limited) and eight primers were selected based on their level of polymorphism with DNA extracted from the banana tissues. These primers were used to estimate genetic diversity among the tetraploid genotypes. The primers used were:

OPC 19 - 5'– GTTGCCAGCC-3'; OPC18- 5' – TGAGTGGGTG-3'

OPD 15– 5' – CATCCGTGCT-3'; OPD 02– 5'–GGACCCAACC-3'

OPA 15 – 5' – TTCCGAACCC-3'; OPD 05 - 5'- TGAGCGGACA-3'

OPAX 14- 5' – ACAGGTGCTG-3'; OPC 11- 5'– AAAGCTGCGG-3'

4.2.4 Data analysis

Analysis of variance was performed using generalised linear model of SAS version 9.1 (SAS, 2002) to test the genetic variance among the diploid and tetraploid populations for agronomic and disease traits. The model used for analysis of variance was:

$\gamma_{ij}=\mu + \beta_i + g_j + \epsilon_{ij}$ (Hill et al., 1998),

where,

γ_{ij} = response of the trait,

μ = overall mean,

β_i = block effect,

g_j = genotype response, and

ϵ_{ij} = experimental error.

The broad sense heritability estimates were computed as follow:

$H^2 = (\delta^2_g /(\delta^2_g + \delta^2_e)$

where,

(H^2) is broad sense heritability,

(δ^2_g) is genetic variance, and

(δ^2_e) residual or environmental variance.

Standard error of heritability was calculated as suggested by Hallauer and Miranda (1988):

$SE(H^2) = 2SE(\delta^2_g)/ (\delta^2_g + \delta^2_e)$

where,

$SE(\delta^2_g)$ is the square root of genetic variance.

Minimum and maximum values for each trait in the population were estimated. The agronomic and disease traits in each population were subjected to principal component analysis using correlation matrix-method to explore the relative contribution of each trait in the overall variation in each population. To estimate the relationship between agronomic and disease parameters within each population, Pearson correlation coefficients were generated and tested at P = 0.05. To find out how yield (bunch weight) in (kg) was affected by agronomic and disease parameters, a stepwise regression analysis was carried out for the tetraploid and the diploid populations.

Amplified fragments were scored for either present (1) or absent (0). Jaccard's similarity coefficients were computed as follows:

$GS = a/(a + b + c)$ (Nei and Li, 1979)
where,

a is the number of fragments present in both samples,

b and c are the number of fragments present only in sample 1 and in sample 2, respectively.

The similarity coefficients were then converted into distance coefficients using the formula, distance matrices = 1– GS (Nei and Li, 1979).

Principal component analysis using average distance method was carried out to group the tetraploids into different clusters.

4.3 Results

4.3.1 Phenotypic variation in disease and agronomic traits

The diploid accessions were significantly different ($P<0.001$) for the youngest leaf spotted, total leaves at flowering, plant height, plant girth, number of functional leaves at harvest, number of clusters per bunch, days from flowering to harvest, area under disease progress curve, and number of suckers (Table 4.2). The tetraploid clones differed significantly ($P<0.05$) in plant height, girth, and number of suckers (Table 4.3).

Table 4.2. Mean squares of analysis of variance of agronomic and disease traits of diploid bananas evaluated at Kawanda Agricultural Research Institute during 2005-2007

Source of variation	df	Youngest leaf spotted	Total leaves at flowering	Height (cm)	Girth (cm)	No. of leaves at harvest	Bunch weight (kg)	No. of hands
Block(replication)	9	5.5	11.7	900	20.6	3.4	11.2	2.1
Genotype	17	13.2	24.1	19669	324	36.7	69.4	11.3
Error	116-170	5.3	3	667	8.9	1.9	6.4	1.2
CV(%)		23	16	13	9	55	61	16
R^2(%)		54	59	82	85	77	69	66

Table 4.2 continued

Source of variation	df	Days to harvest	Area under disease progress curve	No. of suckers
Block(replication)	9	2899	58365	4.1
Genotype	17	8886	1632411	16.9
Error	116-170	1005	50325	3.4
CV(%)		19	55	53
R^2(%)		71	77	22

All variances for genotypes were highly significant at P=0.0001; Presence of missing plots caused variation in error degrees of freedom

Table 4.3. Mean squares of analysis of variance of agronomic and disease traits of tetraploid bananas evaluated at Kawanda Agricultural Research Institute during 2005-2007

Source of variation	df	Youngest leaf spotted	Total leaves at flowering	Height (cm)	Girth (cm)	Bunch weight (kg)	No. of hands	Days to harvest	Area under disease progress curve	No. of suckers
Replication	1	0.8	1.5	1009	9	1.2	0.2	370	53715	1.1
Genotype	8	1.4ns	4.1ns	4600*	40*	2.6ns	0.8ns	311ns	44869ns	4.2*
Plant(genotype)	25	1.2ns	3.0ns	1203ns	23ns	1.6ns	0.5ns	172ns	33513ns	3.0ns
Error	15	1.7	2.0	1965	18	3.3	1.2	228	23879	1.3
CV(%)		26	16	16	11	31	21	33	24	22
R^2(%)		0.68	0.82	0.73	0.80	0.73	0.71	0.81	0.84	0.71

* Significant at P= 0.05; ns = non significant

The diploid accession had higher maximum values for youngest leaf spotted, total leaves at flowering, plant height, number of functional leaves at harvest, bunch weight, days to harvest, and area under disease progress curve than the tetraploid clones. The range (maximum-minimum) values were higher in diploids than in the tetraploids (Table 4.4).

Table 4.4. Maximum and minimum values of different traits in diploid and tetraploid populations evaluated at Kawanda Agricultural Research Institute during 2005-2007

	Diploids			Tetraploids		
Trait	Mean	Minimum	Maximum	Mean	Minimum	Maximum
Youngest leaf spotted	6.6	2.0	11	4.9	2	7
Total leaves at flowering	10.4	6.0	20	8.7	4	11
Plant height (cm)	253.0	113.0	360	227.0	117	308
Plant girth (cm)	37.0	18.0	51	39.0	30	56
NSL at harvest	2.2	0.0	14	0.4	0	3
Bunch weight (kg)	5.4	0.3	20	5.8	2	9
Number of hands	7.3	4.0	12	5.0	3	8
Days to harvest	158.0	55.0	328	112.0	79	142
AUDPC	478.0	0.0	2439	588.0	0	1111
Number of suckers	3.4	0.0	10	1.8	0	6

The broad sense heritability estimates were higher in the diploids than in the tetraploid accessions (Table 4.5). The heritability estimates ranged between 0.16-0.82 in the diploid population. The area under disease progress curve had the highest broad sense heritability estimate of 0.82 in the diploids while youngest leaf spotted had the least estimate of 0.16. The heritability estimates in the tetraploid population were very low. The highest broad sense heritability estimate was 0.22 for the number of suckers in the tetraploid population.

Table 4.5. Broad sense heritability estimates of different traits in diploid and tetraploid populations evaluated at Kawanda Agricultural Research Institute during 2005-2007

Trait	Broad sense heritability (H^2) diploids	Broad sense heritability (H^2) tetraploids
Youngest leaf spotted	0.16(0.31)	-
Total leaves at flowering	0.47(0.58)	0.12(0.45)
Plant height (cm)	0.78(0.03)	0.14(0.02)
Plant girth (cm)	0.82(0.26)	0.13(0.16)
Number of leaves at harvest	0.73(0.70)	-
Bunch weight (kg)	0.55(0.39)	-
Number of hands	0.51(0.71)	-
Days to harvest	0.50(0.03)	0.04(0.03)
Area under disease curve	0.80(0.03)	0.09(0.04)
Number of suckers	0.33(0.51)	0.22(0.32)

- the estimates were very close to zero; Values in parentheses are standard errors of heritability

After principal component analysis of agronomic and disease parameters in the diploid and tetraploid populations four principal components were retained in each population. These principal components explained 85% and 78% of the total variation in the data for diploids and tetraploids, respectively (Tables 4.6 and 4.7). The agronomic parameters (plant height, girth, number of clusters and bunch weight) contributed higher to principal component 1 than disease resistance parameters in the diploid population. In the diploid population disease resistance traits (total leaves at flowering, number of functional leaves at harvest, and youngest leaf spotted) had high loadings on principal component 2 (Table 4.7). In the tetraploid population, apart from bunch weight, disease parameters accounted for most of the variation under principal component 1 and agronomic parameters accounted for most variation in the principal component 2. Generally, bunch weight and AUDPC had high loadings on principal component 1 for both populations (Tables 4.6 and 4.7).

Table 4.6. Latent vector loadings for agronomic and disease traits in the diploid population evaluated at Kawanda Agricultural Research institute during 2005-2007

	Prin1	Prin2	Prin3	Prin4
Area under disease progress curve	0.43931	-0.1645	-0.27393	-0.1978
Bunch weight (kg)	0.42537	0.10873	0.12071	-0.1166
Number of clusters	0.3621	-0.1327	0.48597	0.37473
Days to harvest	-0.0833	-0.3075	0.71079	-0.0362
Plant girth (cm)	0.48333	0.15041	-0.00127	0.31973
Plant height (cm)	0.49009	-0.0009	-0.17156	-0.1225
No. functional leaves at harvest	-0.07074	0.53053	-0.10003	0.50405
Total leaves at flowering	0.02374	0.55777	0.25244	0.00928
Youngest leaf spotted	0.09296	0.47502	0.25657	-0.6589

Table 4.7. Latent vector loadings for agronomic and disease traits in the tetraploid population evaluated at Kawanda Agricultural Research institute during 2005-2007

	Prin1	Prin2	Prin3	Prin4
Area under disease progress curve	-0.32197	0.30575	0.24531	0.47896
Bunch weight (kg)	0.41206	0.38147	-0.21248	0.15221
Number of clusters	0.37528	-0.1879	-0.34588	-0.3191
Days to harvest	0.20846	0.21052	-0.49105	0.5533
Plant girth (cm)	-0.00449	0.63959	0.35359	-0.0074
Plant height (cm)	-0.24769	0.51259	-0.04239	-0.4861
No. functional leaves at harvest	0.39471	-0.0174	0.10493	-0.2812
Total leaves at flowering	0.39123	0.00846	0.52902	0.0802
Youngest leaf spotted	0.41477	-0.0958	0.34092	0.1328

4.3.2 Relationship among disease and agronomic traits

Pearson correlation coefficients among agronomic and disease traits in diploid and tetraploid populations are shown in Table 4.8. In the diploid population youngest leaf spotted (YLS) had a strong and positive correlation with total leaves at flowering (TLF), and number of functional leaves at harvest (NFH). Youngest leaf spotted had a negative but weak correlation with area under disease progress curve (AUDPC). The correlation coefficient between YLS and bunch weight (BWT) was weak (0.223) but significant at P=0.05. Total leaves at flowering were strongly correlated with NFH. However, there was a negative but weak correlation between TLF and AUDPC. Plant

height was strongly correlated with girth, AUDPC, BWT and number of clusters. Days from flowering to harvest were negatively correlated with NFH. Area under disease progress curve was negatively correlated with BWT, while BWT was also strongly correlated with number of clusters.

In the tetraploid population, YLS was correlated strongly and positively with TLF. Youngest leaf spotted was correlated significantly with NFH but had a negative but significant correlation with AUDPC. Total leaves at flowering was correlated significantly with NFH and BWT, plant height had a positive correlation with girth. Number of functional leaves at harvest had a significant positive correlation with BWT but had a significant and negative correlation with AUDPC. There was a strong positive correlation between bunch weight and number of clusters. Also BWT had a significant positive correlation with days from flowering to harvest (Table 4.8).

Table 4.8. Pearson correlation coefficients among agronomic and disease traits in diploid and tetraploid banana populations

	YLS	TLF	HT	GR	DYTH	NFL	AUDPC	BWT	CLST
Diploids									
Youngest leaf spotted (YLS)	1								
Total leaves at flowering (TLF)	0.667***	1							
Height (HT) cm	0.032	-0.104	1						
Girth (GR) cm	0.131	0.119	0.809***	1					
Days to harvest (DYTH)	-0.185	-0.176	-0.129	-0.152	1				
Leaves at harvest (NFL)	0.371**	0.501***	-0.172	0.106	-0.423***	1			
Area under disease (AUDPC)	-0.216*	-0.327*	0.683***	0.578***	-0.113	-0.286*	1		
Bunch weight (BWT) kg	0.223*	0.161	0.564***	0.634***	-0.136	-0.04	-0.536***	1	
No. Clusters (CLST)	-0.044	0.05	0.463***	0.512***	0.15	-0.133	0.337*	0.478***	1
Tetraploids									
Youngest leaf spotted (YLS)	1								
Total leaves at flowering (TLF)	0.573***	1							
Height (HT) cm	-0.234	-0.148	1						
Girth (GR) cm	0.043	0.07	0.414**	1					
Days to harvest (DYTH)	0.099	0.019	-0.142	0.017	1				
Leaves at harvest (NFL)	0.320*	0.351*	-0.225	0.016	0.071	1			
Area under disease (AUDPC)	-0.283*	-0.127	0.05	0.201	-0.1	-0.340*			
Bunch weight (BWT) kg	0.265	0.307*	-0.078	0.282	0.478**	0.360*	-0.113	1	
No. Clusters (CLST)	-0.21	0.194	0.029	0.006	0.243	0.244	-0.358*	0.586**	1

* Significant at P=0.05; ** Significant at P=0.01; *** Significant at P=0.001.

Table 4.9 shows results of a stepwise regression of bunch weights (kg) against agronomic and disease parameters. In diploids, girth, youngest leaf spotted and number of clusters per bunch accounted for 42% of the variation in bunch weight. Plant girth, numbers of clusters per bunch, and days to harvest were important in predicting bunch weight and they accounted for 59% of the variation in bunch weight in the tetraploid population.

Table 4.9. Stepwise regression of bunch weight against agronomic and disease parameters in diploid and tetraploid populations

Variable	Parameter Estimate	Standard Error	Type II SS	F Value	Pr > F
Diploids					
Intercept	-12.82	2.03	435.89	39.92	<.0001
Youngest leaf spotted	0.56	0.175	113.563	10.4	0.0018
Girth (cm)	0.33	0.059	342.238	31.35	<.0001
No. clusters per bunch	0.56	0.287	44.953	4.12	0.0456
Tetraploids					
Intercept	-5.08	1.835	7.994	7.68	0.0089
Girth (cm)	0.08	0.031	6.658	6.39	0.0161
No. fingers per bunch	0.47	0.21	5.176	4.97	0.0323
No. clusters per bunch	0.7	0.19	14.029	13.47	0.0008
Days to harvest	0.04	0.011	10.151	9.75	0.0036

Diploids: bwt (kg) = -12.82 + 0.56yls + 0.34gr + 0.56clst; R^2 =0.42.

Tetraploids: bwt (kg) = -5.08 + 0.07gr + 0.47 nfh + 0.069clst + 0.037dyth; R^2=0.59.

4.3.3 Genetic distances

The genetic distances ranged from 0.04 to 0.39. The lowest distance of 0.04 was between 199k-3 and 199k-4, 199k-3 and 660k-1, 199k-4 and 660k-1. The highest distance was 0.39 and this was between 401k-1 and 1201k-1. Generally 401k-1 and 1747k-1 were slightly diverse from the rest of the genotypes. Most of the genotypes had distances less than 0.15 (Table 4.10).

Figure 4.1 shows a dendrogram of clusters analysis computed using the average distance method in SAS (2002). This analysis groups similar individuals together. For example 199k-3, 199k-4 and 660k-1, 1747k-1 and 401k-1, 1438k-1 and 1754k-1, were grouped together.

Table 4.10.Matrix of pair-wise genetic distance between the 16 banana tetraploids based on RAPD data generated with 8 primers

	1201k-1	1377k-1	1411k-1	1438k-1	1747k-1	1154k-1	199k-1	199k-3	199k-4	222k-1	246k-1	365k-1	376k-7	401k-1	660k-1	917k-
1201k-1	0.00															
1377k-1	0.17	0.00														
1411k-1	0.14	0.09	0.00													
1438k-1	0.21	0.19	0.15	0.00												
1747k-1	0.38	0.32	0.33	0.28	0.00											
1154k-1	0.17	0.20	0.15	0.09	0.28	0.00										
199k-1	0.13	0.11	0.10	0.22	0.33	0.18	0.00									
199k-3	0.14	0.12	0.06	0.15	0.32	0.14	0.08	0.00								
199k-4	0.13	0.10	0.06	0.14	0.32	0.14	0.06	0.04	0.00							
222k-1	0.20	0.12	0.12	0.20	0.28	0.12	0.13	0.12	0.08	0.00						
246k-1	0.25	0.14	0.12	0.14	0.29	0.15	0.10	0.10	0.06	0.08	0.00					
365k-1	0.12	0.10	0.08	0.18	0.33	0.18	0.08	0.12	0.08	0.13	0.13	0.00				
376k-7	0.23	0.22	0.19	0.13	0.25	0.12	0.21	0.17	0.16	0.14	0.15	0.21	0.00			
401k-1	0.39	0.36	0.34	0.31	0.28	0.29	0.36	0.34	0.34	0.32	0.32	0.36	0.25	0.00		
660k-1	0.12	0.09	0.06	0.16	0.32	0.15	0.08	0.04	0.04	0.12	0.10	0.10	0.19	0.33	0.00	
917k-	0.16	0.11	0.08	0.17	0.33	0.19	0.08	0.11	0.08	0.12	0.14	0.08	0.22	0.34	0.10	0.0

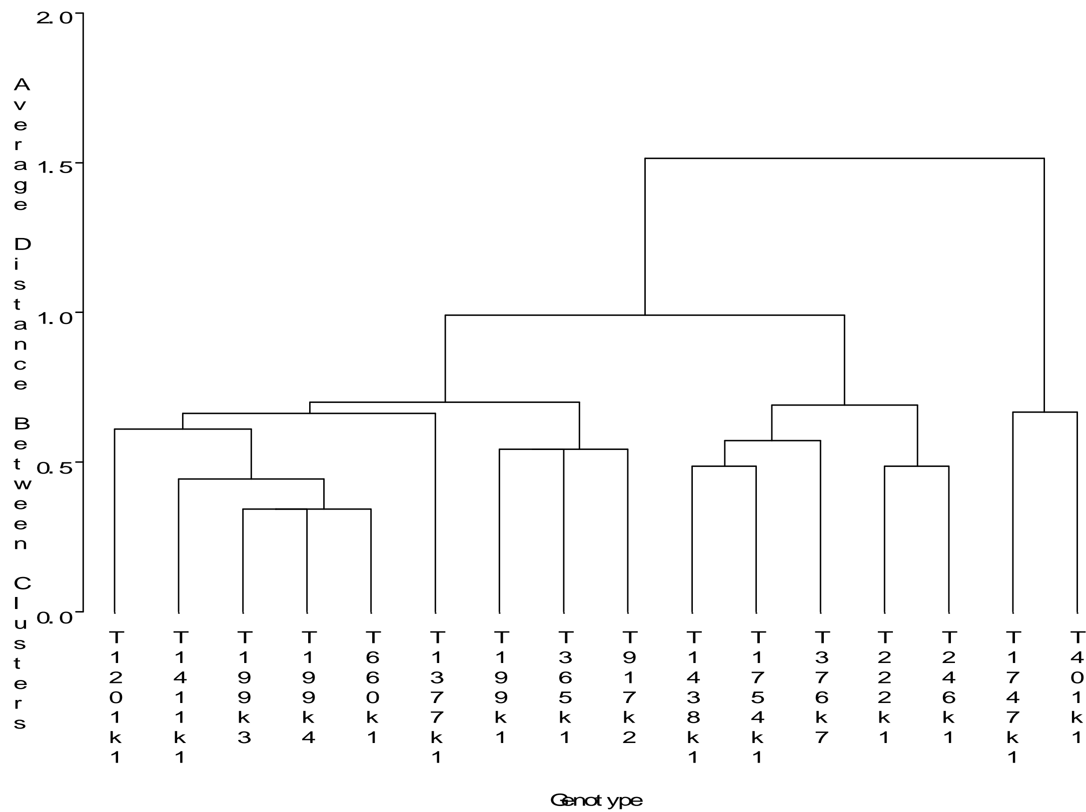

Figure 4.1. A dendrogram showing synthetic banana tetraploids clustered by average distance method

4.4 Discussion

4.4.1 Variation in diploids for disease and agronomic traits

Significant variation was observed for both black Sigatoka resistance and agronomic traits in the diploid population. In banana improvement, diploids have been used as sources of resistance (Rowe and Rosales, 1996; Swennen and Vuylsteke, 1993; Vuylsteke et al., 1993), although the genetic variation has not been quantified. The present study showed that there is a potential to improve diploids for both agronomic and black Sigatoka resistance traits. Extremely low values for traits like bunch weight (Table 4.4) observed in diploids indicate that a lot of time and resources will be spent to improve such traits to desirable levels. These results implied that genetic gain is

expected for those traits that showed variability. However challenges related to incompatibilities and, pollen sterility in some banana diploids will need to be addressed.

Principal component analysis showed that the diploid population varied more in agronomic traits (plant height and girth) which might suggest that since the selection of diploids was based on disease resistance (Rowe and Rosales, 1996; Swennen and Vuylsteke, 1993; Vuylsteke et al., 1993), the diploid population has received little improvement in the agronomic traits. Therefore there is a potential to improve agronomic traits in the diploid population.

4.4.2 Variation in tetraploids for disease and agronomic traits

Significant differences in the tetraploid population were observed for plant height, plant girth, and the number of suckers indicating that these traits showed variability in the tetraploid population. The broad sense heritability estimates for plant height (0.14) and plant girth (0.13) were rather low suggesting limited genetic variability.

Although tetraploid improvement has not been carried out in *Musa* species by making 4x by 4x crosses, Tenkouano et al. (1998) pointed out it might be possible to improve suckering behaviour in the tetraploid background. It might also be possible to improve plant height, number of suckers, and plant girth in the tetraploid background if tetraploid progenies can be recovered from 4x by 4x crosses.

The tetraploid population evaluated was not significant for disease traits, the black Sigatoka resistance traits, youngest leaf spotted, total leaves at flowering and the number of leaves retained at harvest could explain a large proportion of the observed variation in the tetraploid population as indicated by results of principal component analysis.

Molecular analysis using RAPD markers on the banana tetraploids indicated that most of the clones were closely related. Genotypes, (199k-3, 199k-4 and 660k-1), (1747k-1 and 401k-1), and (1438k-1 and 1754k-1) were clustered together. There were only two clones (1747k-1 and 401k-1) whose dissimilarity ranged between 25%-39% from the rest of the clones. The dendrogram clusters were in agreement with the pedigree data of the genotypes. The pedigree data show that the tetraploids were generated by crossing the East Africa highland bananas with a common male parent Calcutta 4.

Moreover, the limited variation among the synthetic tetraploids was not surprising as these materials were generated from the same clone set of East African highland bananas (Karamura, 1998; Ssebuliba et al., 2006). The synthetic tetraploids are very important breeding population in restoring female sterility (Pillay et al., 2004), but had not been characterised for either variation in black Sigatoka resistance or agronomic traits. Therefore, it was found necessary to investigate the variation of the tetraploids (AAAA), because of the role they play in the improvement of East African highland bananas. There is need to increase diversity in this crucial tetraploid population.

4.4.3 Relationship among disease and agronomic traits

Based on phenotypic correlations and regression analysis, the most important traits that influenced bunch weight in the diploids were girth, youngest leaf spotted, and the number of clusters; while girths, the number of functional leaves at harvest, the number of clusters, and days from flowering to harvest were important in tetraploids. The influence of girth on banana bunch weight was also reported by Kumar et al. (2007) hence plant girth should be considered when selecting for better yields in bananas. Plant height had a strong relationship with bunch weight in diploids. Similarly, Ortiz and Vuylsteke (1995) reported a positive relationship between dwarfism with low bunch weight in plantain. However, short plants are desired because they reduce losses due to breakage by wind. It is believed that selecting from large segregating populations may break the possible linkages between dwarfism and low bunch weight. Rowe and Rosales (1996) were able to breed banana diploids that combine short stature and high bunch weight (35kg).

Youngest leaf spotted had a positive relationship with the total leaves at flowering, the number of functional leaves at harvest and bunch weight. The negative relationship of YLS with AUDPC means that with an increase in YLS, the disease severity will be reduced. It is therefore possible to improve yields in diploids by improving resistance to black Sigatoka. The number of clusters per bunch had a positive relationship with bunch weight in the diploids and tetraploid populations (Table 4.9). Therefore, in both diploids and tetraploids, selection for increased number of clusters might be possible since the number of clusters was variable within the two populations using diverse parents.

4.5 Conclusion

In conclusion the diploid population showed large variation for disease resistance and agronomic traits. However, the tetraploid population showed low variability in plant height, girth and the number of suckers. Strong relationships were observed among agronomic traits, and disease traits. Girth and number of clusters were very important in determining bunch weight in the two populations. There was limited genetic diversity in the available tetraploid populations as indicated by the distance matrices computed using RAPD markers. The present study is the first to characterise the synthetic tetraploid (AAAA) bananas for their phenotypic and genotypic variation, and it is recommended that diversity should be increased in this population by generating more tetraploids.

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Chapter five

Genetic studies of black Sigatoka resistance and associated traits in tetraploid-diploid banana crosses

Abstract

The generation of banana triploids from tetraploid-diploid crosses requires knowledge on the influence of the parents on black Sigatoka resistance and agronomic traits to the triploid progenies. The mating scheme was designed as a 4x5 North Carolina II mating design. Due to problems in seed set and germination, progenies from 2 male parents with 4 female parents were evaluated at two sites in Uganda. The results showed that the diploid parents were more resistant to black Sigatoka than the tetraploid parents. The male-parent triploid progeny heritability estimate for the number of leaves at harvest was greater than the female parent estimate. The diploid parents had higher correlation coefficients for the total leaves at harvest with the triploid progenies than tetraploid parents with triploid progenies. Disease development over time took more days in diploid parents than in the tetraploid parents with the triploid progenies as intermediates. These results suggested that diploids transferred black Sigatoka resistance to the triploid progenies as measured by the number of standing leaves and disease development overtime. The tetraploid parents were taller with heavier bunches than the diploid parents. There was a positive correlation ($P<0.05$) between tetraploid female parents and triploid progenies for plant height and bunch weight. The triploid progeny-tetraploid female parent heritability estimates for plant height (0.92) and bunch weight (0.72) were highly significant. The better parent heterosis of 128% was from the female of the family 401k-1 x 8075 at Kamenyamigo site. These results indicated that the female synthetic tetraploids influenced plant height and bunch weight in the triploid progenies. Therefore, it is important to select the tetraploids with heavy bunches to effectively improve yield in triploid progenies generated by tetraploid-diploid crosses. Plant girth, finger length and finger diameter had a strong linear relationship with bunch weight in triploid progenies. However, the tetraploid-diploid progenies had a significant ($P<0.05$) family-by-site interaction for bunch weight indicating that new banana genotypes need to be tested across different environments to select stable genotypes to promote to end-users.

5.1 Introduction

The yield losses caused by black Sigatoka on bananas in Uganda have resulted in food insecurity problems especially in the subsistence sector. Black Sigatoka causes severe yield decline on bananas through reducing the photosynthetic area and thus causing poor bunch filling. All the East African highland banana cultivars are susceptible to black Sigatoka with a yield loss estimated at 37% (Tushemereirwe, 1996). Cultural and chemical management strategies for black Sigatoka have not proved effective as observed from available reports. Banana production in large commercial plantations depends greatly on fungicide sprays to control black Sigatoka. However, resistance of *Mycosphaerella fijiensis* to benomyl in Honduras was reported (Stover, 1977; Romero and Sutton, 1997). Resource limited farmers who cannot afford chemicals use cultural control methods like deleafing to control black Sigatoka. Although, deleafing reduces disease inoculum (Carlier et al., 2000) reduction of leaves reduces the photosynthetic area available for fruit filling. In irrigated banana fields, efficient irrigation methods combined with optimum plant densities have been practised to reduce relative humidity (Wilemaker, 1990; Carlier et al., 2000) without stopping germination of ascospores. The use of host plant resistance appears to be the most economical and sustainable way of managing black Sigatoka on bananas and plantains.

Despite sterility in triploids, some cultivars among the East African highland bananas are fertile thus making it possible to improve the triploid bananas through hybridisation. The female fertile East African highland varieties were crossed with a diploid male parent Calcutta 4 to generate synthetic tetraploids (Ssebuliba et al., 2000). The synthetic tetraploids were resistant to black Sigatoka and conserved desirable traits from the original East African highland bananas. Other banana breeding programmes through this triploid x diploid breeding scheme have generated tetraploids that acquired resistance to black Sigatoka resistance (Vuylsteke et al., 1993a; Rowe and Rosales, 1996). However, with exposure to viable pollen, the synthetic tetraploids would form seeds. The seed formation would reduce the fruit quality hence making them unacceptable to farmers.

Pillay et al. (2004) and Tomekpe et al. (2004) proposed a scheme to produce sterile triploid bananas by crossing tetraploids with diploids to generate secondary triploids.

The Ugandan National Banana Research Programme has adopted this scheme in the improvement of East African highland bananas to black Sigatoka and other biotic constraints.

The meiotic behaviour of autotetraploids complicates genetic studies between the tetraploid-diploid crosses. During meiosis, tetraploid chromosomes can form univalents, bivalents, trivalents, and quadrivalents in different proportions. For instance, Vuylsteke (2001) and Oselebe et al. (2006) observed more than 90% triploids progenies, 3% diploids and less than 1% tetraploids and pentaploids progenies from tetraploid plantain with diploid crosses. This implies that ploidy analysis has to be carried out to confirm progeny outcomes. The presence of dominant and recessive alleles at a locus is another factor that makes genetic studies in tetraploids difficult. The nulliplex, simplex, duplex, triplex and quadruplex genotypes will form different gamete frequencies depending on whether chromosomes form univalents, bivalents, trivalents and quadrivalents (Singh, 1993). The poor seed set in bananas, self incompatibilities and low numbers generated from banana crosses makes it difficult to relate progeny outcomes with expected gamete ratios thus making it difficult to undertake genetic studies in autotetraploids.

Despite the challenges some inferences have been made on the outcomes of crosses involving tetraploid bananas. Tenkouano et al. (1998) reported that plantains female tetraploids parents were influential in determining yield (t $ha^{-1}yr^{-1}$), leaf retention index and time to flowering in the secondary triploids generated through tetraploid-diploid crosses. After analysing the contribution of tetraploids to triploid progenies, Ortiz (1995) and Tenkouano et al. (1998) suggested that some traits like days to flowering and suckering behaviour can be improved in the tetraploid background. One approach can be by carrying out 4x by 4x crosses. This approach could help accumulate quality traits derived from the East African highland bananas in the current synthetic AAAA tetraploids, provided there would not be self-incompatibility and inbreeding problems within tetraploid progenies. The studies reported have been carried out on plantains. There is no information reported on the influence of synthetic tetraploid bananas on the triploid bananas generated from tetraploid-diploid crosses.

Despite reports that some traits could be improved in the tetraploid background, banana improvement has concentrated on improving traits in the diploid background. Apart from traits like disease resistance which has been confirmed to be inherited from the diploids (Vuylsteke et al., 1993a; Rowe and Rosales, 1996), the information on inheritance of other beneficial traits from diploids through tetraploid-diploid crosses to triploids is not available. This information will help breeders to identify which traits to improve in the parental background.

It is also important to understand the relationship between black Sigatoka resistance and other traits of importance if black Sigatoka resistant materials are to be acceptable to end-users. Ortiz (1995) reported a positive relationship between fruit size and bunch weight in diploids and a positive relationship between plant height and plant girth in plantain tetraploids. Later Ortiz and Vuylsteke (1998) reported that yield potential was negatively associated with days to harvest, and fruit weight was correlated positively with fruit girth in (AAA) bananas. It is not clear how these traits are inherited in triploid progenies from tetraploid-diploid crosses. Information on the possible associations of traits is important in designing better banana improvement strategies.

5.1.1 Objectives

The objectives of the study were as follows:

1. to determine the influence of tetraploid and diploid parents on black Sigatoka resistance and agronomic traits in the triploid progenies generated from tetraploid-diploid crosses,
2. to determine phenotypic correlations between black Sigatoka resistance traits and agronomic traits in triploids generated from tetraploid by diploid crosses, and
3. to determine yield in triploid progenies from the tetraploid-diploid crosses at two sites in Uganda.

5.1.2 Hypotheses

The following hypotheses were tested:

1. The diploids and synthetic tetraploids are equally important in determining agronomic and black Sigatoka resistance traits in triploid progenies generated from tetraploid-diploid crosses,
2. There are strong and positive phenotypic correlations between black Sigatoka resistance parameters and agronomic traits in triploid progenies generated from AAAA by AA crosses, and
3. The triploid progenies from the tetraploid-diploid crosses have the same yield across the two sites in Uganda.

5.2 Materials and methods

5.2.1 Progenies for genetic studies

The male parents (AA) were selected based on their pollen fertility, bunch weights and black Sigatoka resistance while the selection of female parents (AAAA) was based on their acceptability and yield in terms of bunch weight. Female and male flowers were bagged to prevent contamination with unwanted pollen. Crosses were designed using a 4x5 North Carolina II mating design. The females that were selected were 199k-4, 365k-1, 376k-7, 401k-1 and 660k-1. The selected males were Calcutta 4, 8075, 9719 and Pitu (Table 5.1). Hand pollinations were performed between 6.30am and 7.30am according to Shepherd (1960). Clusters of anthers with pollen from the male parent were rubbed on the stigmas of designated female parents. The female flowers are arranged in clusters. The clusters open in succession on several days. The pollinations were carried out as flowers opened. On average it took 3-4 days to pollinate a 5-6 cluster bunch. The plastic bag was removed a day after pollination of the last cluster. The pollinated bunches were labelled with tags indicating the cross number, parents and the date when pollination was started. The same information was recorded in a field book. In addition, the field book had information on the day each cluster was

pollinated. At physiological[4] maturity the pollinated bunches were harvested, ripened in an enclosed room and seeds extracted. Immediately after extraction, seeds were taken to the laboratory and germinated in-vitro using the embryo culture method (Vuylsteke and Swennen, 1992). Some crosses were unable to generate seeds; others generated very few seeds and some seeds never germinated. Progenies were obtained from Calcutta 4 (376k-7, 365k-1, and 401k-1), 8075 (376k-7, 401k-1, and 660k-1) and 9719 x 401k-1 (Table 5.1).

Table 5.1. Number of plants generated from tetraploid (AAAA) by diploid (AA) crosses in a 4 x 5 North Carolina II design carried out December 2005 to February 2006

	Females				
Males	376k-7	365k-1	401k-1	660k-1	199k-4
Calcutta 4	20	22	26	-	-
8075	32	-	18	20	-
9719	-	-	16	-	-
Pitu	-	-	-	-	-

- = no plants generated

5.2.2 Experimental design and management

The triploid progenies together with their parents were planted in a randomised complete block with two replicates at each site. Each replicate had six to eight plants of each cross from Table 5.1. The test families were exposed to natural black Sigatoka infestation. To ensure that each test plant had an equal exposure to the disease, each family was planted between rows of a black Sigatoka susceptible local check 'Mbwazirume' which served as a spreader for the disease. Plant spacing was at 3m by 3m.

The two sites were Kawanda Agricultural Research Institute (KARI) and Masaka District Farm Institute, Kamenyamigo. Kawanda Agricultural Research Institute is located at 00' 22''N, 00' 32''E at an altitude of 1193m above sea level. Rainfall and temperature for the two sites are shown in Appendix 5.1. The strip where the experiment was laid out at Kawanda site has a sandy clay loam texture with a pH range of 5.4-5.9 (determined in water). Kamenyamigo is located at 00' 18''S, 00' 33''E at an altitude of 1240m above

[4] Physiological maturity is the stage when the banana fingers have completely filled

sea level. The soil has a sandy clay texture. The soil pH was about 5.1 (determined in water). The rainfall and weather conditions for this site are given in Appendix 5.1. The trials were planted on 26th April 2007 and 3rd May 2007 at KARI and Kamenyamigo sites, respectively. All the experimental plots both in Kamenyamigo and KARI had low levels of Nitrogen (critical level 0.2mg/kg). About 10kg of kraal manure (containing 9.2% organic matter, 0.42%N, 1093mg/kg of phosphorus, 4134mg/kg of potassium, 4860mg/kg of calcium, and 1476mg/kg of magnesium, and pH of 9.7) was applied per hole at planting and 6 months after planting.

Two and six months after planting the trials were mulched with swamp grass to a thickness of about 10cm. A mulch thickness of 10cm was maintained by applying more mulch 9 months after the initial application. Five months after planting kraal manure was applied again. In the KARI site, because of high banana weevil infestation, furadan was applied at a rate of 30g per mat[5] in October 2007. Desuckering to maintain 2-4 plants per mat was carried out at 4 and 7 months after planting in the two trials.

5.2.3 Data collection and analysis

Parents and their F1 progenies were studied for agronomic and disease traits. Ploidy levels of the F1 progenies were determined using flow cytometry, and genetic analysis was carried out on triploids. Six months after planting, number of suckers produced and disease severity were recorded. Disease severity was assessed on individual plant basis according to the modified Stover (1971) scale. The proportion of affected leaves was estimated in percentage. This was repeated three times after every 14 days. The disease severity was later converted into Area Under Disease Progress Curve (AUDPC) using a formula described by Shaner and Finney (1977):

$$AUDPC = \sum_{i}^{n-1} [(X_{i+1} + X_i)/2][t_{i+1} - t_i]$$

Where,

X_i = proportion of the host tissue damaged at i^{th} day,

t_i = the time in days after appearance of the disease at i^{th} day, and

n = the total number of observations

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[5] the initial banana planted, produced new plants (suckers), the whole set of bananas emerging from the initial plant make up a mat or stool

Disease development among the parents and their progenies was also assessed by recording the date of leaf emergence and following the process of black Sigatoka development from symptom appearance up to when leaf was 100% dead. At flowering, plant height and plant girth at 100cm from ground level, the number of youngest leaf spotted and the total number of functional leaves were recorded. At physiological maturity, banana bunches were harvested, number of functional leaves and bunch weight (kg) recorded. The total numbers of clusters/hands per bunch, number of fingers per cluster, finger length and finger diameter of two randomly selected fingers from the second hand were recorded.

Due to failure in some crosses, the analysis was not carried out as a North Carolina II design. The analysis was conducted on triploids using the generalised linear model (SAS, 2002),

$Y_{ijk} = u + R_{es} + S_i + f_j + P_{ef} + Sf_{ij} + E_{ijk}$

where,

Y_{ijk} is the observed response,

u is the general mean,

R_{es} is the replication effect nested in site,

S_i is the site effect,

f_j is family effect,

P_{ef} is progeny effect nested in the family,

Sf_{ij} is the interaction between site and family, and

E_{ijk} is the residual/error effect.

The generalised linear model (glm) procedure was used to test effect of site, family, progeny within family effect, and site-by-family interaction. To compare the effect of males in crosses, orthogonal contrasts were generated with generalised linear model between progenies with Calcutta 4 as a male parent and progenies with 8075 as a male parent. Also orthogonal contrasts of offsprings of two female parents that shared a male parent Calcutta 4 were generated.

Phenotypic correlations among traits of the progenies were calculated using the SAS procedure. Regression analysis using a stepwise selection method was carried out to model bunch weight against agronomic and disease parameters in the triploid

progenies. The condition for entry of a trait in the model was set at P=0.15 and for stay was set at P=0.05. Therefore all the parameters that were retained in the regression model were significant (P=0.05). Mid-parent and better parent heterosis was computed for plant girth and bunch weight.

Heterosis was calculated at a progeny level as:

$$MPH = \frac{(P-MP)*100}{MP}$$

$$BPH = \frac{(P-BP)*100}{BP}$$

Where,

MPH = mid-parent heterosis

P = performance of progeny

MP = average performance of parents

BP = performance of the better parent

BPH = better parent heterosis

To test the contribution of diploids and tetraploids to the triploid progenies, the triploid progenies were correlated with either parent for agronomic and disease resistance traits. Also parent offspring heritability estimates were computed to assess the influence of the parents on their progenies. Offsprings were regressed against both male and female parents using the regression model:

$Yi = a + bX_i + e_i$

where,

Y_i is mean measurement of offspring,

X_i is measurement of the parent,

b is the regression of Y_i on X_i,

e_i is the error associated with the Y_i, and

a is the intercept/overall mean.

The parent-offspring regression coefficients were tested with hypothesis H_o: b=0, using the formula,

$$t = \frac{b-0}{Seb}$$

where,

t is the t-test

b is the regression of Y_i on X_i, and

Seb is the standard error of b.

There were missing plots due to different reasons (Figure 5.1) therefore in the analysis the treatments/genotypes were unbalanced.

Abnormal growth

Plant broken before maturity

Diseased plant: Banana bacterial wilt

Bunch failed to fill normally

Plate 5.1. Causes of missing plots in AAAA by AA evaluation trials

5.3 Results

5.3.1 Parental performance for black Sigatoka resistance and other traits

Table 5.2 shows the mean squares of parents partitioned for site, genotype and site-by-genotype interaction. The performance of parents between sites differed significantly for youngest leaf spotted (YLS) ($P<0.05$), girth ($P<0.01$), transformed (square root) number

of functional leaves at harvest (P<0.05), bunch weight (P<0.05), total fingers, and finger length (P<0.05). Overall, there were significant differences (P<0.01) between the parental genotypes for (YLS), total leaves at flowering (TLF), and transformed number of functional leaves at harvest (NFL) (Table 5.2). The agronomic traits plant height (HT), girth (GR), bunch weight (BWT), total fingers per bunch, finger length and finger diameter were highly significant (P<0.01) among the parents. Finger diameter and functional leaves at harvest had significant site-by-genotype interactions (P<0.05). In total there were seven parents; three diploids (Calcutta 4, 8075 and 9719) and four tetraploids (365k-1, 376k-7, 401k-1 and 660k-1).

Table 5.3 shows the parental means and their standard errors. Among the diploids, 9719 had significantly (P<0.05) higher YLS, TLF, and number of functional leaves at harvest than Calcutta 4. The genotype 8075 also had significantly higher YLS and TLF than Calcutta 4. On the other hand, Calcutta 4 retained more leaves (P<0.05) at harvest than 8075. Calcutta 4 also had the least (P<0.05) area under disease progress curve (AUDPC) than 8075 and 9719. For the tetraploid female parents, 660k-1 had a higher (P<0.05) YLS than 401k-1. The rest of the genotypes 376k-7, 365k-1 and 660k-1 were not significantly different from each other (P>0.05) for YLS. The genotype 660k-1 had significantly more TLF than 376k-7 and retained more leaves at harvest than 401k-1 (P<0.05). The genotype 401k-1 had significantly higher (P<0.05) AUDPC than 365k-1 and 660k-1. Overall the diploid parents had significantly higher YLS and lower area under disease progress curve than the tetraploid parents.

The diploid Calcutta 4 was the shortest genotype, had the least bunch weight, and the least finger diameter (P<0.05). The bunch weight of 8075 was significantly higher (P<0.05) than that of 9719. However, the bunch weight of 8075 was not significantly different (P>0.05) from that of 376k-7, 365k-1, and 401k-1. The tetraploid genotype 660k-1 had the heaviest (P<0.05) bunch weight among the parents evaluated (Table 5.3). Tetraploids parents had larger finger diameter, and were taller than diploid parents (Table 5.3).

Table 5.2. Mean squares of analysis of variance of disease and agronomic traits of diploid and tetraploid parents evaluated at Kamenyamigo and Kawanda in Uganda during 2006 to 2008

Source of variation	DF	Youngest leaf spotted	Total leaves at flowering	Plant height (cm)	Plant girth (cm)	Transformed functional leaves at harvest	Bunch weight (kg)	No. clusters	Total fingers	Finger length (cm)	Finger diameter (cm)
Rep(Site)	2	2.3	4.4	2455	108	0.7	34.7	4.1	5684	0.5	2.4
Site	1	11.2*	2.7	1374	284**	1.5*	15.1*	10.4*	4955*	22.3*	0.1
Genotype	6	51.8.1**	50.0**	35207**	483**	4.7**	93.8**	31.2*	4150.0**	34.3**	29.9**
Site*genotype	6	1.1	2.7	646	32	1.7**	4.7	0.8	847.7	4.9	3.5*
Error	110-189	1.7	3.0	845	24	1.7	3.5	2.0	602.7	2.7	0.9
CV(%)		18	18	12	13	33	28	19	22	14	9
R^2		0.52	0.37	0.60	0.47	0.46	0.6	0.45	0.36	0.51	0.76

* significant at P=0.05; ** significant at P=0.01. The error degrees of freedom varied due to the missing plot effect

Table 5.3. Parental means of diploid and tetraploid parents that generated triploid progenies

Parent	Youngest leaf spotted	Total leaves at flowering	Plant height (cm)	girth (cm)	No. of leaves harvest	Bunch weight (kg)	Finger diameter (cm)	Area under disease progress curve	No. of suckers	No. of clusters	No. of fingers	Finger length (cm)
Calcutta 4	8.0±0.45	9.6±0.52	152.7±8.52	28.0±1.98	2.4±0.47	0.8±0.2	6.7±0.72	4±121.9	1.5±0.93	6.7±0.52	102.6±11.52	8.6±1.01
8075	8.8±0.40	11.0±0.43	227.8±7.07	36.8±1.80	1.2±0.27	7.5±0.88	8.6±0.47	157±112.8	2.5±0.65	9.7±0.34	132.6±8.03	14.5±0.44
9719	9.1±0.39	11.3±0.43	202.3±7.04	36.2±1.80	2.7±0.27	4.5±0.89	9.5±0.49	142±114.3	0.5±0.63	6.9±0.34	103.9±8.04	10.6±0.52
376k-7	6.3±0.39	8.6±0.43	265.3±7.13	41.7±1.81	0.5±0.30	7.3±0.90	11.1±0.48	330±112.9	0.3±0.63	6.3±0.35	93.5±8.25	11.2±0.49
365k-1	6.7±0.39	9.3±0.42	239.4±7.18	39.4±1.82	1.0±0.27	9.0±0.93	11.7±0.48	291±112.4	0.8±0.63	7.7±0.34	112.3±8.00	12.0±0.48
401k-1	5.9±0.39	8.1±0.42	264.3±6.90	40.9±1.79	0.3±0.28	7.4±0.90	11.6±0.47	441±113.0	0.9±0.64	6.4±0.34	97.2±8.09	11.7±0.46
660k-1	6.9±0.40	9.6±0.44	256.3±7.63	42.3±1.87	1.3±0.33	9.5±0.93	11.6±0.49	240±113.1	0.9±0.65	7.3±0.39	110.2±8.80	12.6±0.52
$LSD_{(0.05)}$	0.8	1.1	24.9	2.6	0.8	1.5	0.67	149	1.3	0.96	17.8	1.2

± are standard errors of the mean

Table 5.4 shows the finger diameter of parents at the two sites in Uganda. Genotype 365k-1 had the biggest fingers of 12.2cm in diameter at Kawanda but did not maintain its high performance at Kamenyamigo. Its performance in finger diameter at Kamenyamigo ranked third thus indicating genotype-by-site interaction. Overall, the diploid and tetraploid genotypes performed better at Kawanda site than Kamenyamigo site although there were no significant differences (P>0.05) in finger diameter between the two sites.

Table 5.4. Finger diameter (cm) of male and female parents planted at Kawanda and Kamenyamigo sites in Uganda during 2006 to 2008

Genotype	Finger diameter (cm)	
	Kawanda Mean±se	Kamenyamigo Mean±se
Calcutta 4	5.2±0.67	8.2±0.30
8075	9.0±0.30	8.2±0.27
9719	9.8±0.40	9.2±0.94
376k-7	11.4±0.30	10.6±0.35
365k-1	12.2±0.26	10.9±0.38
401k-1	11.8±0.31	11.2±0.31
660k-1	12.0+0.31	11.2±0.38
LSD $_{(0.05)}$	1.2	1.0
Site mean	10.2	9.9

±se are standard errors of the mean

5.3.2 Progeny performance for black Sigatoka performance and other traits

The tetraploids-diploid half-sib family progenies were significantly (P<0.01) different from each other for the area under disease progress curve (AUDPC) (Table 5.5). There were significant differences in AUDPC (P<0.01) of the family progenies among the two sites. Orthogonal contrasts of progenies generated by Calcutta 4 and 8075 as male parents did not show any significant differences (P>0.05) for disease parameter traits such as youngest leaf spotted, total leaves at flowering, number of functional leaves at harvest and area under disease progress curve (Appendix 5.2). However, the progenies generated by Calcutta 4 and 8075 as male parents showed differences among

themselves in the days from flowering to harvest (P=0.04) and plant girth (P=0.04) (Appendix 5.2).

Parents 365k-1 and 376k-7 did not show any significant differences in black Sigatoka resistance traits (Table 5.3). The half-sib progenies generated from these two female parents with a common male parent Calcutta 4 significantly differed in plant height (P<0.05), number of functional leaves at harvest (P<0.05), total fingers per bunch (P<0.05), and area under disease progress curve (P<0.001) (Appendix 5.2).

The families were significantly different (P<0.05) among sites for girth, bunch weight, and AUDPC. However, there was a significant (P<0.05) family-by-site interaction for the days from flowering to harvest and bunch weight (P<0.05). The progenies within the families differed significantly for girth (P<0.01), bunch weight and total fingers (P<0.05) (Table 5.5). The within family variation for girth and AUDPC was greater than between the family variation.

Table 5.5. Mean squares of analysis of variance of disease and agronomic parameters of AAAA by AA families evaluated at Kamenyamigo and Kawanda in Uganda during 2006 to 2008

Source of variation	DF	Girth (cm)	Days to harvest	Transformed bunch weight (kg)	No. clusters	Total fingers	Area under disease curve
Rep(Site)	2	81.2	2440	0.39	1.1	154	3827
Site	1	105.9*	323	0.09	7.0*	363	129850**
Family	6	33.3*	1480*	1.97**	2.7	1212	124591**
Progenies/family	49	57.3**	1036	0.82*	2.1	1192*	27069
Site*Family	6	51.2	2021*	1.38*	1.9	1033	22525
Error	92-120	25.5	802	0.39	1.5	701	25260
R^2		0.44	0.47	0.65	0.47	0.50	0.75
CV(%)		13	16	27	17	25	42

Error degrees of freedom were variable because of missing plot effect
* data significant at P=0.05; ** data significant at P=0.01

Table 5.6 shows progeny means of bunch weights at the Kawanda and Kamenyamigo sites in Uganda during 2006 to 2008. The families had heavier bunches in Kawada than Kamenyamigo. The progenies of 660k-1 x 8075 had the heaviest bunches at Kawanda, but they did not maintain the heavy bunch weights at Kamenyamigo site. Then the cross 401k-1 x 8075 had the heaviest bunches at Kamenyamigo site but it did not maintain the weights at Kawanda site. This lack of consistency in performance showed that there was site-by-family interaction.

Table 5.6. Mean bunch weight (kg) of progeny means at Kawanda Agricultural Research Institute and Kamenyamigo during 2006 to 2008

Pedigree	Kawanda	Kamenyamigo
	Mean±se	Mean±se
376k-7 x Calcutta 4	6.2±1.15	6.2±1.30
365k-1 x Calcutta 4	8.3±0.91	5.1±1.30
401k-1 x Calcutta 4	5.2±1.26	4.4±0.98
376k-7 x 8075	5.2±0.91	4.2±0.90
660k-1 x 8075	12.1±1.13	6.5±1.40
401k-1 x 8075	6.9±1.21	10.5±1.40
401k-1 x 9719	3.7±1.22	4.0±1.16
$LSD_{(0.05)}$	2.4	4.3
Site mean	6.9	5.3

±se = standard errors of the mean

Table 5.7 shows progeny means of days from flowering to harvest at the two sites. Although, there was a significant site-by-family interaction for days from flowering to harvest, the two sites never influenced significantly the days from flowering to harvest of the family progenies. The family progenies of 401k-1 x Calcutta 4 took a significantly shorter time ($P<0.05$) from flowering to harvest than the family progenies of 376k-7 x Calcutta 4 at Kawanda site. The progenies of the cross 660k-1 x 8075 took the shortest time from flowering to harvest at Kamenyamigo site (Table 5.7).

Table 5.7. Mean number of days from flowering to harvest of family progenies at Kawanda Agricultural Research Institute and Kamenyamigo during 2006 to 2008

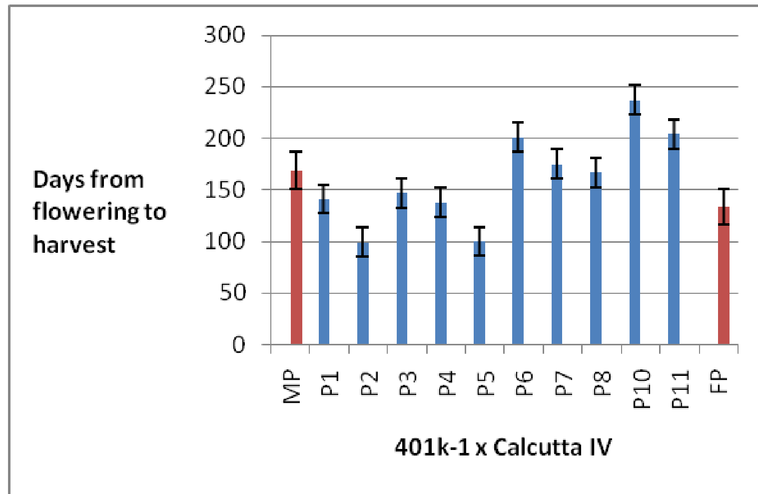
Pedigree	Days to harvest	
	Kawanda Mean ± se	Kamenyamigo Mean ± se
376k-7 x Calcutta 4	174 ± 9.6	154 ±12.0
365k-1 x Calcutta 4	173 ± 7.9	192± 12.0
401k-1 x Calcutta 4	145±10.0	186 ± 9.7
376k-7 x 8075	158 ± 7.8	166 ± 7.9
660k-1 x 8075	163 ± 9.3	144 ±13.3
401k-1 x 8075	170±11.1	149 ±15.6
401k-1 x 9719	164 ± 9.9	184 ±11.6
LSD	28	40
Site mean	163.9	167.9

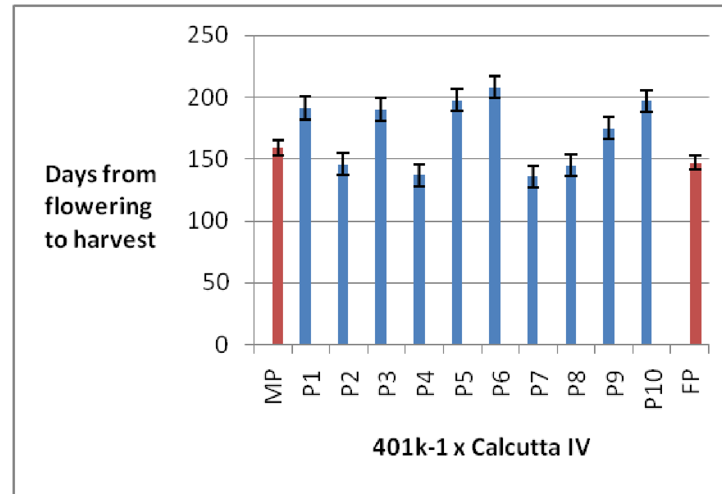
Figure 5.1 shows the days from flowering to harvest of individual clones/progenies within the families at the two sites. The family 401k-1 x Calcutta 4 at Kawanda had two progenies that took the shortest time from flowering to harvest of 100d. These two progenies took fewer days from flowering to harvest than the diploid and tetraploid parents. The same family had a progeny that took about 230d from flowering to harvest. At Kamenyamigo, the same family had progenies that took less days from flowering to harvest than the parents although this was not significant (P>0.05).

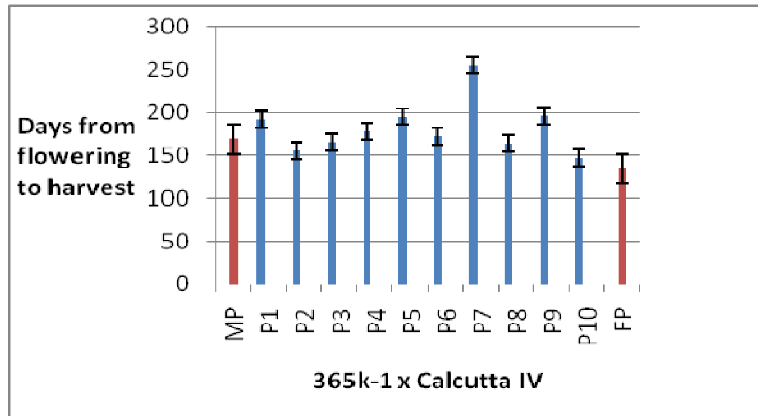
All the progenies of the family 365k-1 x Calcutta 4 at Kawanda took more days from flowering to harvest than the female parent. Apart from progeny 7 which took about 250 days from flowering to harvest, the other progenies took almost the same number of days from flowering to harvest as the male parent. On the other hand, at Kamenyamigo, the progenies of 365k-1 x Calcutta 4 took more days from flowering to harvest than the male and female parents except the two progenies that took almost same number of days as the female parent.

At Kawanda, the family 376k-7 x 8075 had only one progeny that took fewer days from flowering to harvest than those of the parents. Otherwise most of the progenies for this cross had intermediate days between the parental values. Similarly at Kamenyamigo, the 376k-7 x 8075 family had progenies that took about 110 days from flowering to harvest which was less than the number of days taken by the male and female parents (Figure 5.1).

Kawanda Kamenyamigo







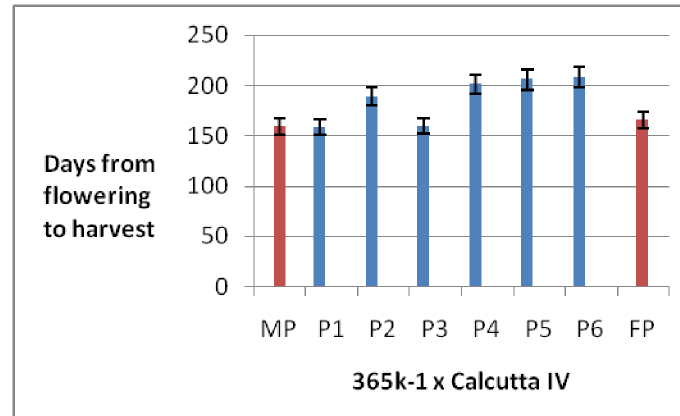
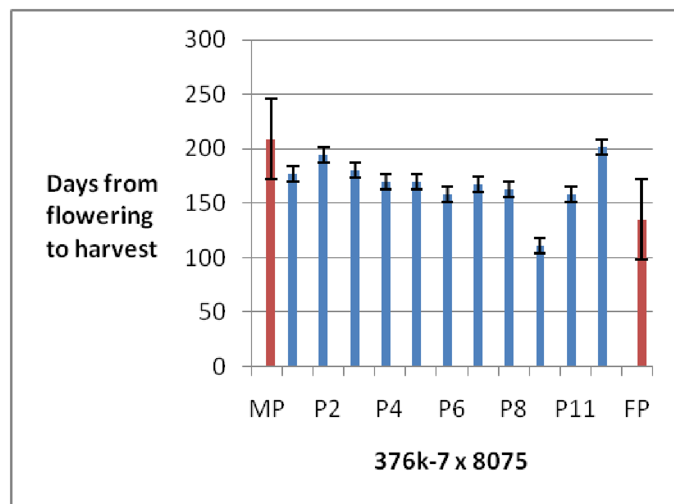
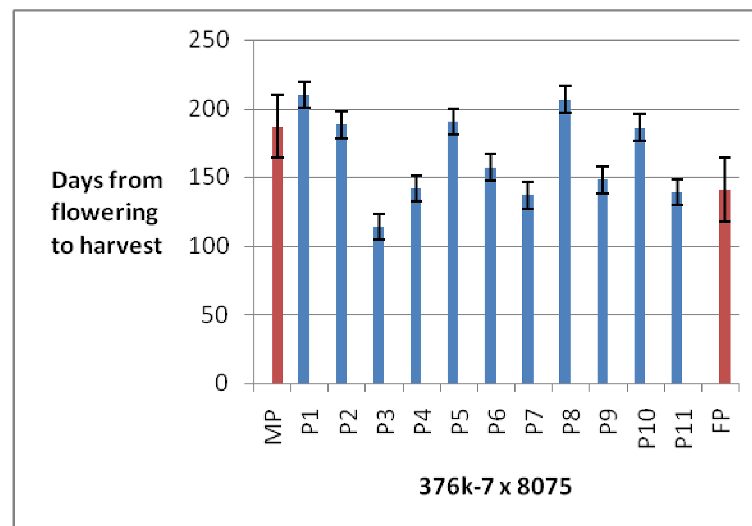


Figure 5.1. Days from flowering to harvest of triploid progenies from tetraploid-diploid crosses in Uganda 2006-2008





MP = male parent; FP = female parent; P1 ..PN = individual clones/progenies within the family.

Figure 5.2.continued

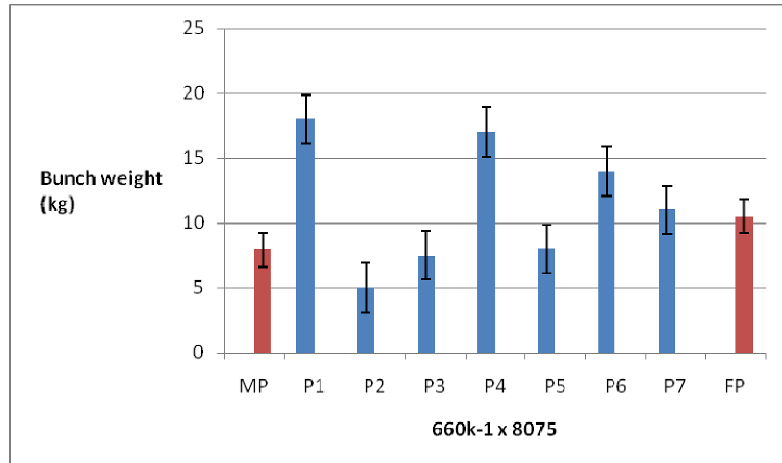
Figure 5.2 shows the bunch weight of the triploid progenies together with their diploid and tetraploid female parents. The family 660k-1 x 8075 had progenies with the highest bunch weight at Kawanda. At this site, two clones from the 660k-1 x 8075 family had bunch weights over 15kg while at Kamenyamigo the best progeny from this cross had a bunch weight of about 11kg. At both sites the family had progenies with low bunch weights of less than 5kg.

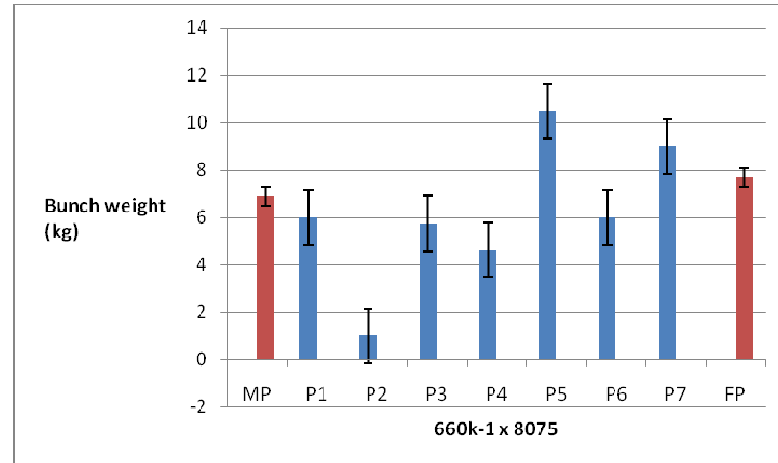
Apart from one progeny at Kamenyamigo which had a heavier bunch weight than the parental values, the 376k-7 x 8075 family had progenies with bunch weights between the parental values. From this family, a number of progenies recorded bunch weights of less than 5Kg which was extremely low.

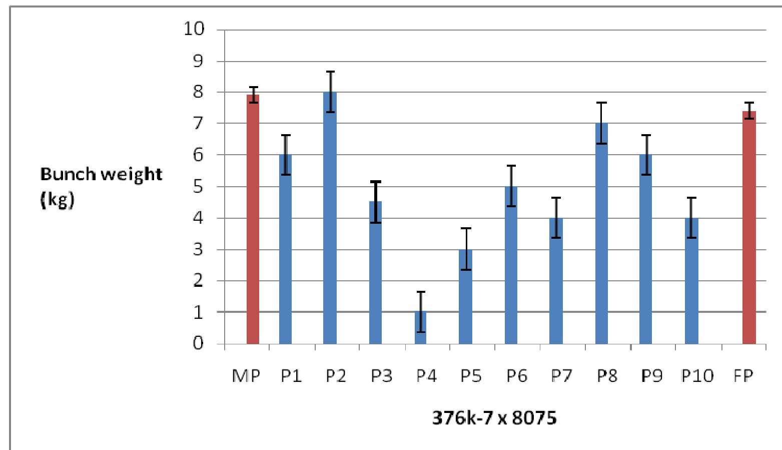
The male parent Calcutta 4 had the least bunch weights among the male parents. However, at Kawanda, the family 365k-1 x Calcutta 4 had progenies which were heavier than the female parent. However at Kamenyamigo, the progenies had bunch weights either equal to or lower than the female parent but higher than the male parent (Figure 5.2).

Kawanda

Kamenyamigo







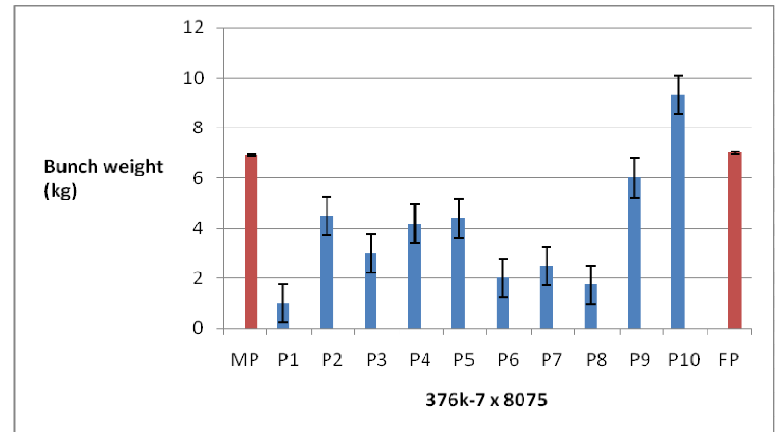
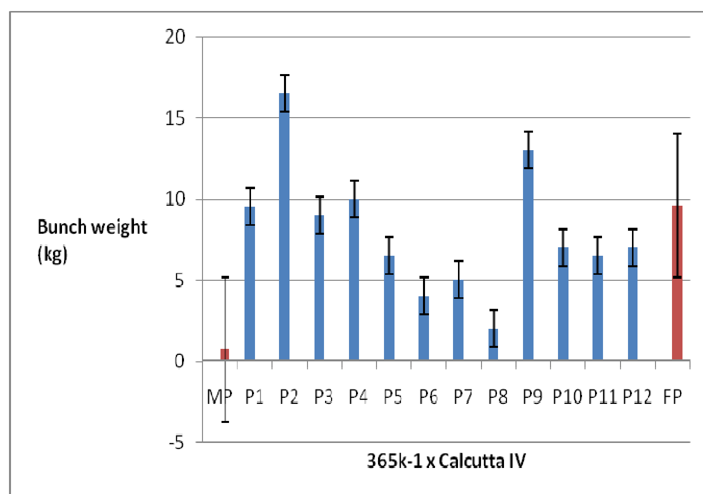
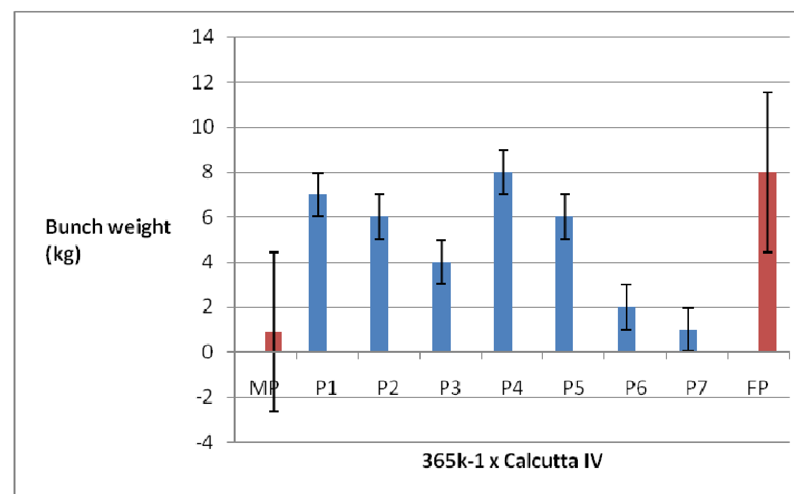


Figure 5.3. Bunch weight of triploid progenies from tetraploid-diploid crosses in Uganda 2006-2008





MP = male parent; FP = female parent; P1 ..PN = individual clones/progenies within the family, Calcutta IV = Calcutta 4.

Figure 5.4. Continued

The results from area under disease progress curve showed that progenies of 365k-1 x Calcutta 4 were the most resistant to black Sigatoka. Also progenies of 660k-1 x 8075 were more resistant than progenies of the cross 376k-7 x Calcutta 4 (Tables 5.8). The progenies of 660k-1 x 8075 had significantly larger plant girth measured at 100cm from ground level than the rest of the progenies.

Table 5.8. Progeny means of plant height and area under disease progress curve in triploid AAA hybrids

Pedigree	Plant girth (cm) Mean ± se	Area under disease progress curve Mean ± se
376k-7 x Calcutta 4	39.7±1.31	519±171.0
365k-1 x Calcutta 4	38.7±1.24	239±170.2
401k-1 x Calcutta 4	38.8±1.21	383±169.8
376k-7 x 8075	38.5+1.10	375±169.8
660k-1 x 8075	42.1±1.39	360±171.4
401k-1 x 8075	41.4±1.47	402±172.2
401k-1 x 9719	36.8±1.25	425±170.0
LSD(0.05)	2.9	109

se = standard errors of the mean

5.3.3 Phenotypic correlation and regression analysis

Table 5.9 shows phenotypic correlations among traits in AAAA by AA progenies. There were significant correlations between disease traits and agronomic traits. Youngest leaf spotted correlated strongly, positively with total leaves at flowering ($P<0.001$), and days to flowering but negatively with area under disease progress curve. Total leaves at flowering had a negative relationship with AUDPC and a positive and significant relationship with number of functional leaves at harvest. Plant height was positively correlated with plant girth, bunch weight, number of fruits and finger diameter. Plant girth was highly correlated with bunch weight (r^2=0.5138) number of fingers (r^2 =0.5928) and finger diameter (r^2= 0.3271). The days from flowering to harvest were negatively correlated with number of functional leaves at harvest, while bunch weight was positively correlated with number of fruits, number of clusters and finger diameter. However, when the traits of triploid parent progenies were correlated with the same traits of their female and male parents, plant height and bunch weight of the triploid progenies correlated positively ($P<0.05$) with the same traits in tetraploid female parents. Also triploid progenies had a positive relationship with the male parents for the youngest leaf spotted and bunch weight although the relationship was not significant ($P>0.05$) (Table 5.10).

Table 5.9. Phenotypic correlations among black Sigatoka resistance traits and yield in 4x by 2x autotriploid progenies

	Youngest leaf spotted	Total leaves at flowering	Plant height (cm)	Plant girth (cm)	Days to flowering	Area under disease progress curve	Days to harvest	No. of leaves at harvest	Bunch weight (kg)	No. of clusters	Total fingers	Finger length (cm)
Youngest leaf spotted	1											
Total leaves flowering	0.76***	1										
Plant height (cm)	0.16	0.07	1									
Plant girth (cm)	0.13	0.02	0.65***	1								
Days to flowering	0.32***	0.18	0.44***	0.01	1							
Area un.disease curve	-0.37**	0.42**	0.23*	0.23*	-0.03	1						
Days to harvest	-0.01	0.05	-0.01	-0.07	-0.03	-0.11	1					
No. of leaves at harvest	0.17	0.22*	-0.10	0.09	-0.29**	-0.14	-0.40***	1				
Bunch weight (kg)	-0.08	-0.17	0.31**	0.51***	-0.08	0.23*	-0.04	0.02	1			
No. of clusters	0.04	0.06	0.05	0.13	0.14	0.1	0.09	0.02	0.17	1		
Total fingers	0.03	0.08	0.37***	0.59***	-0.23*	0.03	0.10	0.18	0.46***	0.42***	1	
Finger length (cm)	0.09	0.07	0.14	0.05	0.07	0.06	0.04	-0.01	0.13	-0.06	0.07	1
Finger diameter (cm)	-0.01	-0.13	0.26*	0.33**	0.01	0.09	-0.09	0.07	0.77***	0.05	0.19	0.19

*** data significant at $P<0.001$; ** data significant at $P<0.01$; * data significant at $P<0.05$

Table 5.10. Pearson correlation coefficients between plant height, bunch weight, number of functional leaves at harvest and youngest leaf spotted between tetraploids, diploid, and their triploid progenies

	Triploid progenies			
	Height (cm)	Bunch weight (kg)	No. functional leaves at harvest	Youngest leaf spotted
Diploid parents	0.07	0.20	0.17	0.12
Tetraploid parents	0.40*	0.32*	-0.02	0.09

* Data significant at P = 0.05

Table 5.11 shows a stepwise regression of bunch weight against agronomic traits in triploid progenies. Plant girth, total fingers per bunch, and finger diameter had a linear relationship with bunch weight (R^2 = 0.69). Total fingers per bunch and finger diameter were highly significant (P<0.0001) in influencing bunch weight in the triploid progenies. Plant girth was also significant (P<0.05).

Table 5.11. Stepwise regression of bunch weight against agronomic traits in 4x by 2x triploid progenies

Variable	Parameter estimate	Standard error	Type II SS	F Value	Pr > F
Intercept	-13.96204	1.61431	437.543	74.8	<.0001
Girth (cm)	0.10184	0.04883	25.4453	4.35	0.0391
Total fingers	0.05096	0.00849	210.66	36.01	<.0001
Finger diameter (cm)	1.12164	0.10526	664.171	113.55	<.0001

Regression model bwt = -13.7 + 0.1gr + 0.05 fingers + 1.12 finger diameter R^2=0.69

5.3.4 Heritability estimates between tetraploid (AAAA) and diploid (AA)

Table 5.12 shows trait heritability estimates for AAAA and AA crosses estimated by male and female parent offspring regression. The heritability estimates ranged from 0-0.92. The progeny-female tetraploid parent heritability estimate for plant height was 0.92 and it was significant (P<0.01). The heritability of bunch weight estimated by progeny-tetraploid parent regression method was also significant (0.78) (P<0.05). The

progeny-male and progeny-female heritability estimates of the rest of the traits were not significant (P>0.05). However, the progeny-diploid parent heritability estimate of the number of leaves at harvest was higher than the progeny-female parent estimate. The progeny-female parent heritability estimates for finger length and finger diameter were higher than the progeny-male parent heritability estimates, although they had high standard errors. The heritability estimates of the male parent Calcutta 4 (progeny-male parent) youngest leaf spotted and plant girth were higher than the heritability estimates of the male parent 8075 (progeny-male parent).

Table 5.12. Heritability estimates of parent offspring regression for black Sigatoka resistance traits and agronomic traits in tetraploid-diploid progenies (triploid) in Uganda during 2006–2008

Trait	Male parent regression	Female parent regression
Youngest leaf spotted	0.16(0.15)	0.17(0.20)
Number of leaves at flowering	-	0.14(0.13)
Plant height (cm)	0.06(0.09)	0.92*(0.23)
Plant girth (cm)	0.07(0.12)	0.28(0.22)
Number of leaves at harvest	0.17(0.12)	-
Bunch weight (kg)	0.27(0.17)	0.78*(0.28)
Number of clusters	0.08(0.10)	0.02(0.15)
Total fingers	0.05(0.15)	-
Finger length	-	0.43(0.45)
Finger diameter	0.04(0.14)	0.39(0.25)
Calcutta 4		
Youngest leaf spotted	0.44(0.26)	0.32(0.31)
Number of leaves at harvest	0.26(0.18)	0.08(0.30)
Days from flowering to harvest	-	0.27(0.29)
Plant girth (cm)	0.56(0.52)	0.36(0.30)
8075		
Youngest leaf spotted	-	0.16(0.27)
Number of leaves at harvest	0.42(0.23)	-
Days from flowering to harvest	0.10(0.19)	-
Plant girth (cm)	0.19(0.31)	0.20(0.37)

T test Ho: b=0 was non significant except for * which was significant at $P<0.05$. – values were negative so are not reported; values in parentheses are standard errors of the regression coefficients

5.3.5. Heterosis for bunch weight and girth in 4x by 2x crosses

Heterosis was computed based on individual progenies within a family for the best 15 progenies for plant girth and bunch weight. Table 5.13 shows the mid-parent and better parent heterosis values for plant girth at Kawanda and Kamenyamigo. The mid-parent heterosis values at Kawanda site ranged from –0.1% to 41.7%. The progenies with Calcutta 4 as a male parent had higher mid-parent heterosis than the progenies with 8075 as a male parent. The better parent heterosis values for girth were the female

tetraploid parents. The cross 401k-1 x 8075 had the highest better parent heterosis of 25% at Kawanda. The mid-parent heterosis for Kamenyamigo also reflected that progenies with Calcutta 4 had higher mid-parent heterosis values than progenies with 8075 as a male parent. The male parent progenies of 9719 also had higher heterosis values than those of 8075 for plant girth at Kamenyamigo. The cross 660k-1 x 8075 had the highest better parent heterosis for girth (39%) at Kamenyamigo.

The mid-parent and better parent heterosis values for bunch weight at Kawanda and Kamenyamigo are presented in Table 5.14. At Kawanda site some progenies had mid-parent heterosis values greater than 100% implying the progeny produced heavier bunches than the mid-parent. The family 401k-1 x Calcutta 4 had a progeny with a mid-parent heterosis value of 130% and 401 x 9719 had a mid-parent heterosis of 159%. At Kamenyamigo site the mid parent heterosis for bunch weight ranged from -12.6% to 138%. The better parent heterosis was observed from the 401k-1 x 8075 family with the best parent heterosis of 128% (Table 5.14).

Table 5.13. Mid-parent and better parent heterosis (%) for plant girth in 4x by 2x crosses at Kawanda and Kamenyamigo sites during 2006 to 2009

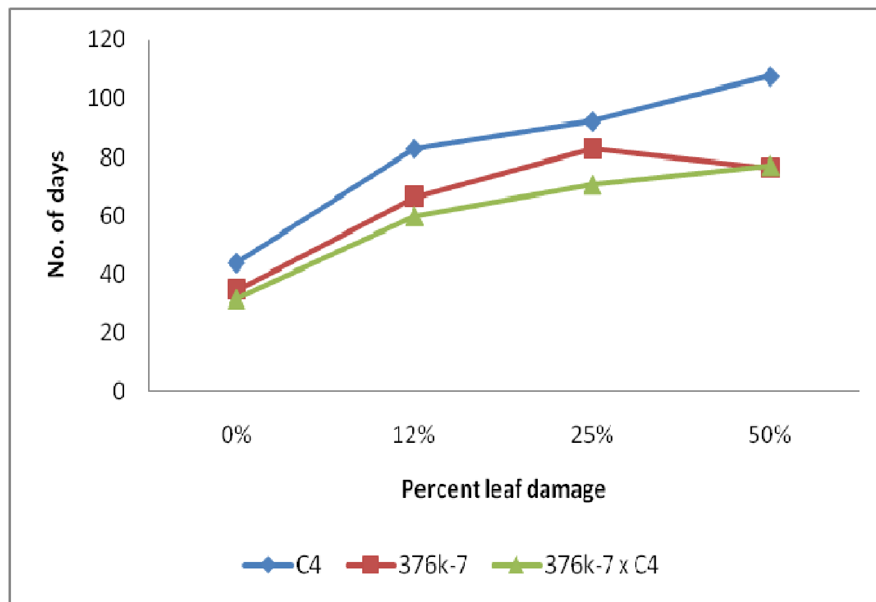
	Kawanda site					Kamenyamigo site					
Pedigree	Mean male parents	Mean female parents	F1 Girth (cm)	Mid parent heterosis	Better parent heterosis	Pedigree	Mean girth male parents	Mean girth female parents	F1 girth (cm)	Mid parent heterosis	Better parent heterosis
376k-7 x Calcutta 4	30.0	42.4	43.5	20.3	2.7	376k-7 x Calcutta 4	26.3	40.7	39.0	16.5	-4.1
376k-7 x Calcutta 4	30.0	42.4	42.5	17.3	0.1	376k-7 x Calcutta 4	26.3	40.7	51.6	53.9	26.7
365k-1 x Calcutta 4	30.0	39.2	44.8	29.3	14.2	365k-1 x Calcutta 4	26.3	39.4	42.1	28.1	6.8
365k-1 x Calcutta 4	30.0	39.2	42.0	21.4	7.1	365k-1 x Calcutta 4	26.3	39.4	39.7	20.8	0.7
401k-1 x Calcutta 4	30.0	41.9	51.0	41.7	21.6	365k-1 x Calcutta 4	26.3	39.4	39.0	18.7	-1.1
401k-1 x Calcutta 4	30.0	41.9	44.0	22.3	4.9	401k-1 x Calcutta 4	26.3	39.4	40.0	21.8	1.5
376k-7 x 8075	38.7	42.4	41.7	2.8	-1.7	401k-1 x Calcutta 4	26.3	39.4	45.4	38.3	15.3
660k-1 x 8075	38.7	45.3	46.0	9.4	1.4	376k-7 x 8075	36.5	40.7	41.3	7.0	1.5
660k-1 x 8075	38.7	45.3	45.0	7.1	-0.7	376k-7 x 8075	36.5	40.7	41.3	6.9	1.4
660k-1 x 8075	38.7	45.3	45.0	7.0	-0.8	376k-7 x 8075	36.5	40.7	39.4	2.0	-3.3
660k-1 x 8075	38.7	45.3	44.5	6.0	-1.8	660k-1 x 8075	36.5	39.4	41.4	9.0	5.0
660k-1 x 8075	38.7	45.3	44.0	4.7	-3.0	660k-1 x 8075	36.5	39.4	54.9	44.8	39.4
660k-1 x 8075	38.7	45.3	42.0	-0.1	-7.4	401k-1 x 9719	34.3	39.4	45.1	22.3	14.4
401k-1 x 8075	38.7	41.9	52.5	30.2	25.2	401k-1 x 9719	34.3	39.4	43.1	16.9	9.3
401k-1 x 8075	38.7	41.9	45.5	12.8	8.5	401k-1 x 97193	34.3	39.4	47.1	27.7	19.5

Table 5.14. Mid-parent and better parent heterosis (%) for bunch weight in 4x by 2x crosses at Kawanda and Kamenyamigo sites during 2006 to 2009

	Kawanda site					Kamenyamigo site					
Pedigree	Mean bunch weight males	Mean bunch weight females	F1 bunch weight	Mid parent heterosis	Better parent heterosis	Pedigree	Mean bunch weight males	Mean bunch weight females	F1 bunch weight	Mid parent heterosis	Better parent heterosis
376k-7 x Calcutta 4	0.7	7.4	10.4	156.2	40.2	376k-7 x Calcutta 4	0.9	7.0	7.5	89.9	7.1
376k-7 x Calcutta 4	0.7	7.4	8.9	119.1	19.9	376k-7 x Calcutta 4	0.9	7.0	7.6	92.9	8.8
365k-1 x Calcutta 4	0.7	9.6	9.4	82.0	-2.3	376k-7 x Calcutta 4	0.9	7.0	6.3	58.2	-10.7
365k-1 x Calcutta 4	0.7	9.6	9.9	91.7	2.9	376k-7 x Calcutta 4	0.9	7.0	9.4	137.5	34.0
365k-1 x Calcutta 4	0.7	9.6	11.6	125.7	21.1	365k-1 x Calcutta 4	0.9	8.0	7.6	71.2	-4.8
365k-1 x Calcutta 4	0.7	9.6	11.0	113.6	14.6	365k-1 x Calcutta 4	0.9	8.0	6.4	43.4	-20.2
401k-1 x Calcutta 4	0.7	7.0	8.9	130.5	26.8	365k-1 x Calcutta 4	0.9	8.0	7.4	65.9	-7.7
376k-7 x 8075	7.9	7.4	7.9	2.9	0.0	401k-1 x Calcutta 4	0.9	7.2	7.4	82.3	2.5
660k-1 x 8075	7.9	10.4	16.9	84.4	62.3	401k-1 x Calcutta 4	0.9	7.2	7.6	88.1	5.8
660k-1 x 8075	7.9	10.4	7.9	-13.9	-24.3	376k-7 x 8075	6.9	7.0	9.0	28.8	27.9
660k-1 x 8075	7.9	10.4	14.1	54.4	35.8	660k-1 x 8075	6.9	7.7	6.4	-12.6	-17.1
660k-1 x 8075	7.9	10.4	9.3	1.1	-11.1	660k-1 x 8075	6.9	7.7	10.1	38.6	31.4
660k-1 x 8075	7.9	10.4	17.9	95.4	71.9	401k-1 x 9719	4.3	7.2	6.4	11.0	-11.4
401k-1 x 9719	4.5	7.0	8.9	54.3	26.8	401k-1 x 8075	6.9	7.2	13.4	90.0	85.9
401k-1 x 9719	4.5	7.0	14.9	158.7	112.5	401k-1 x 8075	6.9	7.2	16.4	135.0	127.5

5.3.6. Disease development in AAAA by AA crosses

The number of days from leaf emergence up to when lead damage due to black Sigatoka among Calcutta 4, 8075, 365k-1, 376k-1, 401k-1, 660k-1, and their progenies was 50% were plotted against percent leaf damage. Results are shown in Figures 5.1 and 5.2. Disease inoculation time varied between 28 days and 43 days overall. All male parents (Calcutta 4 and 8075) took more days than the female parents to attain the percent leaf damage of 12%, 25% and 50% leaf damage. However, the hybrids were intermediates in disease development trends followed by their parents.



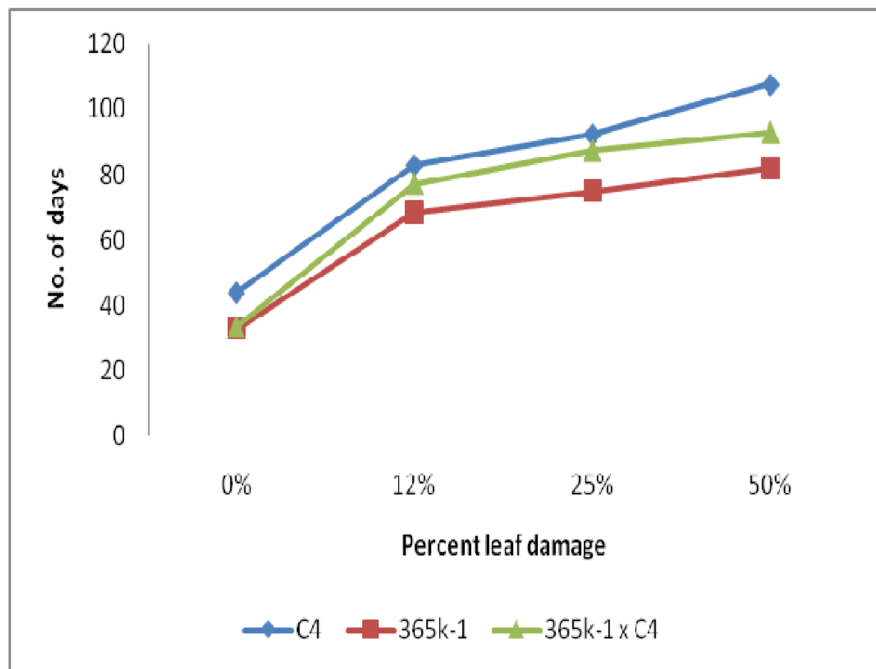
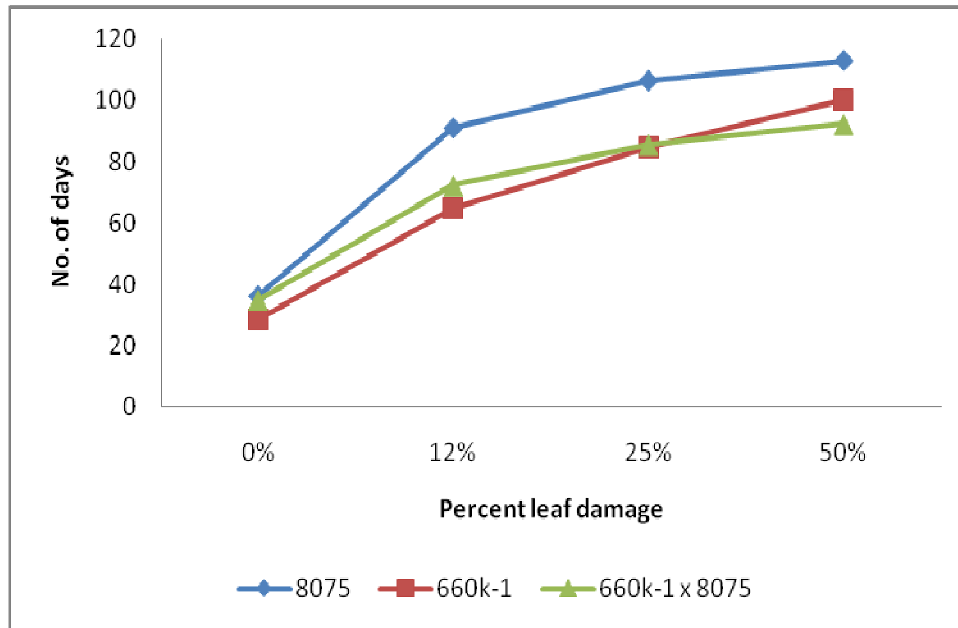


Figure 5.5. Disease development in Calcutta 4 (C4), 376k-7, 365k-1 and their progenies



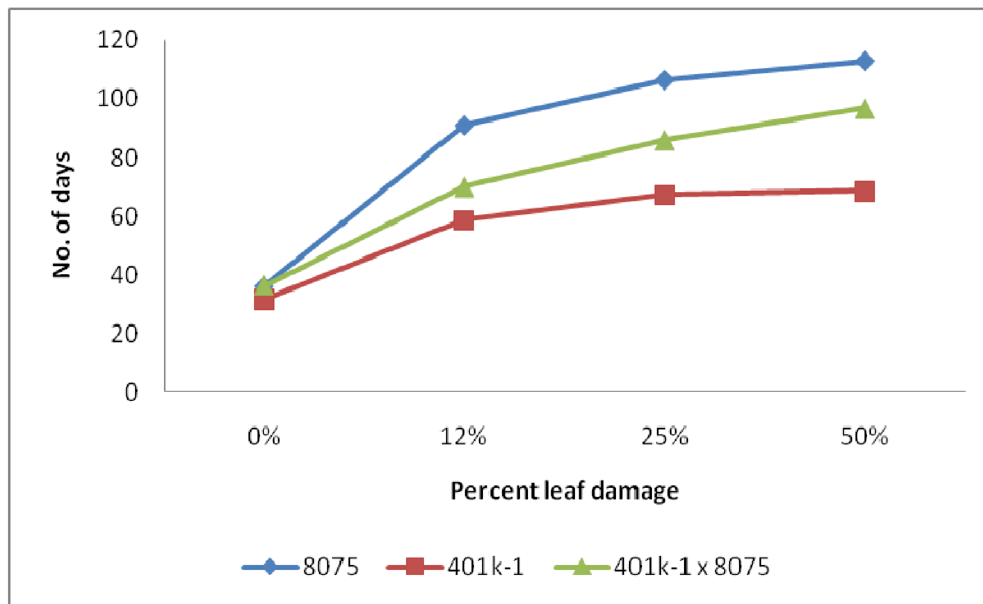


Figure 5.6. Disease development in 8075, 401k-1, 660k-1 and their progenies

5.4 Discussion

5.4.1 Genetic studies of black Sigatoka resistance in tetraploid-diploid crosses

There were significant differences in disease resistance among the parents with diploids showing more disease resistance than tetraploids. This was not surprising because the diploids that have been used in banana improvement in Uganda were selected based on disease resistance. However, the synthetic tetraploids did not show statistical differences among themselves for disease resistance. This could be because the available tetraploids were generated from triploid x diploid crosses from the same banana clone set with the same male parent (Karamura, 1998; Ssebuliba et al., 2000) and the selection was based on bunch weights.

The results in Table 5.3 showed that female tetraploids 376k-7 and 365k-1 were not significantly different from each other for total leaves at flowering. The half-sib progenies with Calcutta 4 as the common parent differed in the mean of total number leaves at flowering. Similarly in other crosses where Calcutta 4 participated, the mean number of total leaves in the progenies was more than the means of parental values. This might suggest that the male parent Calcutta 4 influenced the total leaves at flowering.

Although small differences were observed between individual crosses, when the progenies were pooled, the half-sibs of Calcutta 4 and 8075, did not show any significant differences for the youngest leaf spotted, total leaves at flowering, area under disease progress curve and the number of functional leaves at harvest. This might suggest that both male parents effectively transmitted resistance to the triploid progenies. The progeny-male parent regression for number of leaves at harvest was higher than the progeny-female parent regression. This might suggest that diploids were more influential than tetraploids in determining the number of functional leaves at harvest in the triploid progenies. Further more, disease development over time showed that it took a longer time for the diploids to develop black Sigatoka symptoms than the tetraploids. However, the progenies were in the intermediate range of the two parents. This could imply that the diploids had an effect in improving the time it took the triploid progenies to develop the damage symptoms. Diploids have been reported to transfer

black Sigatoka resistance to the triploid progenies (Rowe, 1984), and in this study diploids seem to have influenced the total leaves at harvest and disease development in triploid progenies.

5.4.2 Genetic studies of agronomic traits in the triploid progenies

The female parent 401k-1 had lower bunch weight ($P<0.05$) than female parent 365k-1. The progenies of 401k-1 x Calcutta 4 had lower bunch weights than the progenies of 365k-1 x Calcutta 4. The tetraploid female parents were taller with heavy bunch weights and plant girths than the diploid parents. The triploid progenies correlated significantly for bunch weight and plant height with the tetraploid female parents. The heritability estimates for progeny-female parent regression for bunch weight and plant height were significant ($P<0.05$). This could imply that the female tetraploid parents were influential in determining bunch weight and plant height in triploid progenies. Therefore selecting high yielding female parents could result in progenies with better performance. Orthogonal contrasts of the progenies of 8075 and Calcutta 4 differed in agronomic traits, days from flowering to harvest ($P<0.05$), and plant girth ($P<0.05$). Among the progenies of Calcutta 4, the progeny-male parent regression was 0.56 for plant girth, while among the progenies of 8075, the progeny-male parent regression for plant girth was 0.19. This might suggest that the two male parents had different breeding values for plant girth and days from flowering to harvest. It could also imply that the male parents have a significant influence on the agronomic traits in triploid progenies from tetraploid-diploid crosses. Therefore, the diploid male parents should also be improved for agronomic traits like plant girth and days from flowering to harvest. Rowe and Rosales (1993) reported improved yields in tetraploids when improved diploids were crossed with triploid bananas.

5.4.3 Family-by-Site interaction

The significant family-by-site interaction effects for bunch weight and days from flowering to harvest showed that different families were not stable across environments. For example, the families 401k-1 x 8075 and 660k-1 x 8075 had the highest bunch weights and shortest days from flowering to harvest, respectively, at Kamenyamigo. The rankings of these families changed at Kawanda site. On the contrary, at Kawanda site, the family 660k-1 x 8075 had the best bunch weight while the family, 401k-1 x

Calcutta 4 took the least days from flowering to harvest. These differences could have been due to environmental effects like rainfall (Appendix 5.2) and differences in soil fertility. Overall the Kawanda site performed better than Kamenyamigo site in terms on bunch weight of the progenies. Vuylsteke et al. (1993b) while working with plantains reported significant genotype-by-year interaction for bunch weight. Ortiz et al. (1994) reported significant genotype-by-environment interaction in tetraploid plantains for bunch weight. Some plant breeders have argued that genotype-by-environment interactions can be addressed by breeding for specific environments implying that genotypes will be promoted in those environments where they perform better. Others think that new genotypes should undergo multi-locational tests and those that perform uniformly across different environments be promoted. Ekanayake et al. (1994) advocated for selecting drought resistant hybrids for environments with drought stress. Vuylsteke et al. (1993b) also argued that plantain hybrids should be developed for each agro-ecological zone. On the other hand, Buddenhaggen (1996) advocated for wide adaptability of new banana cultivars. In Uganda, the banana producers face the same constraints of banana production, they prefer the same qualities from new banana genotypes, and the seed delivery system for bananas is not well developed to guide farmers about what genotypes to grow in which environment and where to get them. Although this study was conducted at two sites, it would advocate for testing new banana genotypes in different environments so that stable banana genotypes are promoted across a number of environments.

5.4.4 Performance of progenies within site

The analysis of performance of progenies within family will help identify the best clone in the family. If such a clone has the desirable gene combinations, then it becomes a potential variety. The within family progeny analysis indicated that the families 660k-1 x 8075 and 401k-1 x 8075 produced progenies with the heaviest bunches. The same families had progenies with the highest better parent heterosis both at Kawanda and Kamenyamigo sites. In all the crosses, the better parents were the females. This further emphasises selecting the best female parents in the improvement of bananas. The progenies of Calcutta 4 showed higher mid-parent heterosis values than progenies of 8075 for plant girth and bunch weight. The bunch weight of Calcutta 4 is far lower than

the bunch weight of 8075. These mid-parent heterosis results seemed to reflect the relative improvement of the progeny compared to the male parent.

Ortiz (1997) observed heterosis for bunch weight in triploid progenies from 4x by 2x crosses in plantains and suggested that within *Musa,* maximum heterosis could be achieved by maximising heterozygosity or crossing distantly related genotypes. The synthetic tetraploids used in this study were closely related (Table 5.3) and possibly this could explain why low heterosis values were observed.

5.4.5 Phenotypic correlations

Strong correlations were observed among disease resistance traits such as youngest leaf spotted, total leaves at flowering, number of functional leaves at harvest, and agronomic traits such as plant height, girth, and bunch weight. This could imply that it might be possible to improve the strongly correlated traits simultaneously. The purpose of breeding is to generate new banana genotypes to feed into farming communities. Banana end-users have different traits they will be looking for in new banana genotypes. For instance, farmers use banana leaves for cooking and feeding animals. Such a group of farmers would prefer banana genotypes that produce many leaves. Many leaves at flowering, higher youngest leaf spotted and many leaves retained at maturity are some of the indicators of black Sigatoka resistance in banana genotypes. Therefore, it is possible to breed black Sigatoka resistant materials that can serve other purposes in banana farming communities.

5.5 Conclusion

In conclusion the diploid male parents were more influential in determining black Sigatoka resistance in the triploid progenies than the tetraploid female parents as measured by disease development and the number of functional leaves at harvest. The tetraploid parents were taller, had larger plant girth, and heavier bunch weight than diploid male parents. The tetraploid female parent significantly influenced plant height and bunch weight in triploid progenies. These findings imply that improving agronomic traits like bunch weight in the tetraploid background would result in triploid progenies with improved bunch weights. Plant girth, finger length and finger diameter had a strong linear relationship with bunch weight in triploid progenies. The progenies 660k-1 x 8075

and 401k-1 x 8075 had progenies with the heaviest bunches and shortest days from flowering to harvest. The same families had progenies with the better parent heterosis for bunch weight. Bunch weight showed a significant genotype-by-environment interaction among the families. The families, 401k-1 x 8075 and 660k-1 x 8075 were inconsistent in performance across the two environments. This investigation recommends testing the new banana materials in different environments to identify stable genotypes to promote to farmers.

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Appendix 5.1. Rainfall (mm) and temperature (^{o}C) for Kawanda Agricultural Research Institute (KARI) and Kamenyamigo for 2007 and 2008.

	KARI site							
	Year 2007				Year 2008			
Month	Mean Minimum Temp ^{o}C	Mean Maximum Temp ^{o}C	Mean Relative humidity %	lean Rainfall in (mm)	Mean Minimum Temp ^{o}C	Mean Maximum Temp ^{o}C	Mean Relative ıumidity %	lean Rainfall in (mm)
January	16.96	27.23	81.16	3.63	17.26	27.83	75.13	0.79
February	23.55	28.11	80.07	0.66	16.90	27.46	75.10	0.73
March	17.51	28.89	81.94	1.15	17.07	26.93	83.45	3.66
April	18.37	27.98	92.83	4.97	17.67	26.55	81.93	3.37
May	17.80	34.88	96.71	7.65	17.68	26.23	88.23	5.24
June	21.88	25.51	96.53	4.99	16.96	25.33	81.90	2.18
July	16.88	25.14	88.74	3.32	16.29	25.64	82.87	2.33
August	17.27	25.58	88.10	4.44	16.79	26.10	87.84	5.23
September	17.07	26.36	90.80	7.55	17.00	27.10	86.27	8.15
October	17.31	27.18	93.00	5.89	17.68	25.74	97.03	11.12
November	17.43	26.91	93.67	7.21	16.65	27.02	96.17	4.47
December	16.57	27.70	88.00	0.83	16.97	28.19	91.74	0.57
Total				1566.20				1430.20

Kamenyamigo site

January	17.32	27.23	1.17	15.63	27.63	2.42
February	16.93	27.89	0.89	16.70	27.42	1.77
March	16.17	28.58	3.53	16.47	28.00	5.63
April	16.86	28.42	2.73	16.67	26.75	6.29
May	16.44	27.34	2.15	16.98	27.59	2.08
June	16.18	26.81	1.63	15.65	27.15	0.30
July	16.17	26.61	2.06	15.58	27.27	0.67
August	15.98	26.70	0.49	15.63	26.82	1.36
September	16.56	27.26	3.08	15.95	26.92	3.12
October	16.63	26.66	4.51	16.31	27.18	3.85
November	16.92	27.10	1.80	16.12	27.21	1.72
December	16.48	27.74	1.52	16.32	27.65	1.45
Total annual rainfall (mm)			780.70			920.10

Appendix 5.2. Orthogonal contrasts of two male parents when they combine with tetraploid female parents

Dependent Variable: Plant height

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	64	130637.7527	2041.2149	1.68	0.0037
Error	199	242134.0655	1216.7541		
Corrected Total	263	372771.8182			

R-Square	Coeff Var	Root MSE	HT Mean
0.350450	14.72379	34.88200	236.9091

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep(Site)	2	757.23426	378.61713	0.31	0.7329
Site	1	2786.29474	2786.29474	2.29	0.1318
Family	6	22287.39357	3714.56560	3.05	0.0070
Progeny(Family)	49	84539.67202	1725.29943	1.42	0.0501
Site*Family	6	6532.09108	1088.68185	0.89	0.4997

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
Progenies of (C4 vs 8075)	1	825.833273	825.833273	0.68	0.4110
Effect of C4 & 8075 with 401k-1	1	3694.770838	3694.770838	3.04	0.0830
Effect of C4(365k-1&376k-7)	1	8997.278360	8997.278360	7.39	0.0071

Dependent Variable: Plant girth

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	64	4001.800549	62.528134	2.45	<.0001
Error	201	5135.237045	25.548443		
Corrected Total	265	9137.037594			

R-Square	Coeff Var	Root MSE	GR Mean
0.437976	12.89326	5.054547	39.20301

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP(Site)	2	162.584505	81.292252	3.18	0.0436
Site	1	105.859413	105.859413	4.14	0.0431
Family	6	199.546882	33.257814	1.30	0.2579
Pltn(Family)	49	2809.385804	57.334404	2.24	<.0001
Site*Family	6	307.415929	51.235988	2.01	0.0665

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
Progenies of (C4 vs 8075)	1	0.01685210	0.01685210	0.00	0.9795
Effect of C4 & 8075 with 401k-1	1	5.31473444	5.31473444	0.21	0.6488
Effect of C4(365k-1&376k-7)	1	27.67951362	27.67951362	1.08	0.2992

Dependent Variable: Youngest leaf spotted

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Mode	6	188.4128754	2.9439512	0.90	0.6850
Error	205	670.9389764	3.2728731		
Corrected Total	269	859.3518519			

R-Square	Coeff Var	Root MSE	YLS Mean
0.219250	24.98513	1.809108	7.240741

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP(Site)	2	0.7843252	0.3921626	0.12	0.8871
Site	1	4.2854932	4.2854932	1.31	0.2538
Family	6	13.3366247	2.2227708	0.68	0.6666
Progeny(Family)	49	118.9988367	2.4285477	0.74	0.8919
Site*Family	6	25.6585441	4.2764240	1.31	0.2556

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
Progenies (C4 vs 8075)	1	0.02872874	0.02872874	0.01	0.9254
Progenies 401k-1(C4&9719)	1	1.21966596	1.21966596	0.37	0.5422
Progenies C4(365k-1&376k-7)	1	9.11405896	9.11405896	2.78	0.0967

Dependent Variable: Total leaves at flowering

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	64	204.641280	3.197520	0.80	0.8568
Error	205	823.510572	4.017125		
Corrected Total	269	1028.151852			

R-Square	Coeff Var	Root MSE	TLF Mean
0.199038	20.36713	2.004277	9.840741

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP(Site)	2	12.0978323	6.0489161	1.51	0.2243
Site	1	4.0964317	4.0964317	1.02	0.3138
Family	6	49.5483391	8.2580565	2.06	0.0599
Progeny(Family)	49	115.7545723	2.3623382	0.59	0.9854
Site*Family	6	12.6166844	2.1027807	0.52	0.7902

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
Progenies (C4 vs 8075)	1	6.93772332	6.93772332	1.73	0.1903
Progenies 401k-1(C4&9719)	1	11.83237053	11.83237053	2.95	0.0876
Progenies C4(365k-1&376k-7)	1	24.49098802	24.49098802	6.10	0.0144

Dependent Variable: Number of functional leaves at harvest

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	62	119.8704315	1.9333941	1.08	0.3640
Error	101	181.1234709	1.7933017		
Corrected Total	163	300.9939024			

R-Square	Coeff Var	Root MSE	NSL Mean
0.398249	88.91470	1.339142	1.506098

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP(Site)	2	1.14023593	0.57011796	0.32	0.7284
Site	1	1.29308577	1.29308577	0.72	0.3978
Family	6	19.28548216	3.21424703	1.79	0.1081
Progeny(Family)	47	65.44263930	1.39239658	0.78	0.8322
Site*Family	6	14.56816307	2.42802718	1.35	0.2406

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
Progenies (C4 vs 8075)	1	4.41983105	4.41983105	2.46	0.1196
Progenies 401k-1(C4&9719)	1	0.27782713	0.27782713	0.15	0.6947
Progenies C4(365k-1&376k-7)	1	13.49584278	13.49584278	7.53	0.0072

Dependent Variable: Days to flowering

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	62	65538.7540	1057.0767	1.32	0.1131
Error	92	73757.9299	801.7166		
Corrected Total	154	139296.6839			

R-Square	Coeff Var	Root MSE	DYTH Mean
0.470498	16.63166	28.31460	170.2452

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP(Site)	2	4879.55095	2439.77548	3.04	0.0525
Site	1	323.08904	323.08904	0.40	0.5271
Family	6	8877.43387	1479.57231	1.85	0.0988
Progeny(Family)	47	48678.40227	1035.71069	1.29	0.1477
Site*Family	6	12128.76813	2021.46136	2.52	0.0264

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
Progenies (C4 vs 8075)	1	3485.378270	3485.378270	4.35	0.0398
Progenies 401k-1(C4&9719)	1	214.007179	214.007179	0.27	0.6066
Progenies C4(365k-1&376k-7)	1	2791.182036	2791.182036	3.48	0.0652

Dependent Variable: transformed bunch weight (square root transformation)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	62	68.8440434	1.1103878	2.79	<.0001
Error	95	37.8435636	0.3983533		
Corrected Total	157	106.6876069			

R-Square	Coeff Var	Root MSE	transbwt Mean
0.645286	26.74199	0.631152	2.360155

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP(Site)	2	0.79387732	0.39693866	1.00	0.3730
Site	1	0.08716474	0.08716474	0.22	0.6410
Family	6	11.79163053	1.96527176	4.93	0.0002
Progeny(Family)	47	38.85856211	0.82677792	2.08	0.0013
Site*Family	6	8.33527514	1.38921252	3.49	0.0037

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
Progenies (C4 vs 8075)	1	1.34763134	1.34763134	3.38	0.0690
Progenies 401k-1(C4&9719)	1	4.38624443	4.38624443	11.01	0.0013
Progenies C4(365k-1&376k-7)	1	0.00108423	0.00108423	0.00	0.9585

Dependent Variable: Number of clusters

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	62	141.5496161	2.2830583	1.54	0.0251
Error	109	161.8631746	1.4849833		
Corrected Total	171	303.4127907			

R-Square	Coeff Var	Root MSE	CLST Mean
0.466525	17.86863	1.218599	6.819767

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP(Site)	2	2.15160134	1.07580067	0.72	0.4869
Site	1	7.02210268	7.02210268	4.73	0.0318
Family	6	16.15752272	2.69292045	1.81	0.1030
Progeny(Family)	47	96.74286906	2.05835892	1.39	0.0842
Site*Family	6	11.20012600	1.86668767	1.26	0.2833

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
Progenies (C4 vs 8075)	1	0.59449768	0.59449768	0.40	0.5282
Progenies 401k-1(C4&9719)	1	6.11279884	6.11279884	4.12	0.0449
Progenies C4(365k-1&376k-7)	1	0.01616893	0.01616893	0.01	0.9171

Dependent Variable: Total fingers per bunch

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	63	76386.9840	1212.4918	1.73	0.0061
Error	109	76367.2125	700.6166		
Corrected Total	172	152754.1965			

R-Square	Coeff Var	Root MSE	Tot_fing Mean
0.500065	25.59337	26.46916	103.4220

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP(Site)	2	308.92812	154.46406	0.22	0.8025
Site	1	362.90246	362.90246	0.52	0.4732
Family	6	7270.79846	1211.79974	1.73	0.1208
Progeny(Family)	48	57215.80064	1191.99585	1.70	0.0120
Site*Family	6	6198.60975	1033.10162	1.47	0.1936

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
Progenies (C4 vs 8075)	1	177.556454	177.556454	0.25	0.6157
Progenies 401k-1(C4&9719)	1	1567.037720	1567.037720	2.24	0.1377
Progenies C4(365k-1&376k-7)	1	3954.219978	3954.219978	5.64	0.0193

Dependent Variable: Finger diameter (cm)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	62	411.3488595	6.6346590	1.84	0.0062
Error	74	267.3016879	3.6121850		
Corrected Total	136	678.6505474			

R-Square	Coeff Var	Root MSE	FD Mean
0.606128	19.03701	1.900575	9.983577

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP(Site)	2	0.4619506	0.2309753	0.06	0.9381
Site	1	4.5670038	4.5670038	1.26	0.2645
Family	6	67.3710657	11.2285109	3.11	0.0090
Progeny(Family)	47	214.8269196	4.5707855	1.27	0.1801
Site*Family	6	30.4748041	5.0791340	1.41	0.2237

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
Progenies (C4 vs 8075)	1	15.08617368	15.08617368	4.18	0.0445
Progenies 401k-1(C4&9719)	1	47.74990497	47.74990497	13.22	0.0005
Progenies C4(365k-1&376k-7)	1	0.00826158	0.00826158	0.00	0.9620

Dependent Variable: Area under disease progress curve

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	55	4266028.921	77564.162	3.07	<.0001
Error	55	1389298.502	25259.973		
Corrected Total	110	5655327.423			

R-Square	Coeff Var	Root MSE	audpc Mean
0.754338	42.24029	158.9339	376.2613

Source	DF	Type III SS	Mean Square	F Value	Pr > F
REP(Site)	2	7653.168	3826.584	0.15	0.8598
Site	1	1298560.487	1298560.487	51.41	<.0001
Family	5	622956.516	124591.303	4.93	0.0008
Progeny(Family)	42	1136907.777	27069.233	1.07	0.4010
Site*Family	5	112625.775	22525.155	0.89	0.4931

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
Progenies (C4 vs 8075)	1	777.7895	777.7895	0.03	0.8614
Progenies 401k-1(C4&9719)	1	3068.8072	3068.8072	0.12	0.7288
Progenies C4(365k-1&376k-7)	1	581664.0898	581664.0898	23.03	<.0001

C4 = Calcutta 4

Chapter six

Evaluation of 2x by 2x banana progenies for yield and black Sigatoka resistance

Abstract

Improving the banana diploid population for black Sigatoka resistance requires an understanding of the performance of the progenies for black Sigatoka resistance, and how yield relates with agronomic and black Sigatoka resistance traits. A diploid population was generated using a random polycross design to determine the relationship between bunch weight and black Sigatoka resistance traits in 2x by 2x progenies. The half-sib progenies were significantly ($P<0.05$) different for the area under disease progress curve, bunch weight, and the days from planting to flowering. The progenies from 9719 family showed the highest level of disease resistance. The progenies of 8075 family had the highest ($P<0.05$) bunch weight and finger diameter, while the half-sib progenies of Pitu took the shortest ($P<0.05$) time from planting to flowering. The results indicated variation in family performance, therefore selection at family level would constitute the next step to produce a population with a wide range of desirable traits. Phenotypic correlations revealed strong positive relationships between bunch weight with total leaves at flowering, youngest leaf spotted, plant girth and the days from planting to flowering among the 2x by 2x progenies. Linear regression analysis indicated that girth, total fingers and finger length significantly predicted bunch weight (R^2=0.67). However, days from planting to flowering had positive indirect effects on bunch weight via plant girth. The results suggest that selection for parents with good combining ability for girth, finger length and total fingers can improve bunch weight in a diploid improvement.

6.1 Introduction

The East African highland bananas play a major role in food security in the farming communities of Uganda. About 75% of farmers in Uganda depend on bananas for food security and income. Yet the banana yields of the most commonly grown East African highland bananas have been reduced due to black Sigatoka. There are no black Sigatoka resistant cultivars among the East African highland bananas (Vuylsteke and

Swennen, 1988; Tushemereirwe, 1996; Craenen and Ortiz, 2002). Therefore efforts were undertaken to improve the East African highland bananas for black Sigatoka resistance.

The improvement of East African highland bananas requires breeding materials that are black Sigatoka resistant with improved agronomic traits. The sources of resistance for black Sigatoka were identified in wild diploid bananas (Vakili, 1968). Apart from transferring disease resistance, wild diploid bananas also transfer poor agronomic traits to their progenies/hybrids (Rowe and Rosales 1993a:b). It was also reported by Tenkouano et al. (1998) that diploids in plantain tetraploid-diploid crosses influenced bunch weight in the triploid progenies more than the tetraploid parents. Because of their influence on disease resistance and agronomic traits, banana diploid populations are being improved for these traits.

In Uganda through inter-diploid crosses (between local germplasm and accessions from international collaborators) progress has been registered in improvement of the banana diploids. Pollen fertile diploids with black Sigatoka resistance have been selected (Ssali Reuben Personal Communication). Unfortunately, some diploids with heavy bunch weights are either susceptible to black Sigatoka (Craenen and Ortiz, 1998) or have poor pollen, therefore are not useful as breeding materials. There is need to understand the relationship between black Sigatoka resistance and agronomic traits like bunch weight in the banana germplasm at Kawanda Agricultural Research Institute so that appropriate diploid improvement strategies can be designed.

Important associations that could guide on diploid improvement have been reported. Ortiz and Vuylsteke (1995) found a positive association between dwarfism and low bunch weight. Ortiz (1997) found out a positive and significant correlation between fruit size and bunch weight in diploids. The findings reported were carried out using phenotypic correlations which indicate the nature of relationship between traits. In a banana crop several traits interact to affect overall yield which is bunch weight. The understanding of both direct and indirect relationships between bunch weight and other traits will be important in the improvement of diploids for bunch weight and disease resistance.

Path analysis has been used to understand relationships between traits in crops. In guava path analysis was used to analyse seed characteristics and number of seeds in the fruit (Rajan et al., 2008) and in bambara nuts, pod yield was related to its components using path analysis (Makanda et al., 2009). In bananas, path analysis was carried out to identify an ideotype for plantain breeding (Ortiz and Langie, 1997), determine ploidy and genome effects on yield components in plantains (Baiyeri et al., 2000), and determine the contribution of banana nematodes to yield loss of East African highland bananas (Ssango et al., 2004). Path analysis enables breeders to understand the direct and indirect contributions of traits to yield. Generating information on direct and indirect contributors to bunch weight will help in selection for important traits in a diploid recurrent selection programme. Therefore the objective of the present investigation was to evaluate the 2x by 2x progenies for yield and black Sigatoka resistance, and determine the relationship between bunch weight with agronomic and black Sigatoka resistance traits in 2x by 2x progenies.

Hypotheses

The 2x by 2x progenies are not different for yield and black Sigatoka resistance, and there is a relationship between bunch weight and black Sigatoka resistance traits among the progenies.

6.2 Materials and methods

6.2.1 *Musa* clones used to generate diploid half-sib progenies

The characteristics of germplasm used to generate the diploid progenies are shown in Table 6.1. Data in this table give the summary of performance of parental materials in the pollination block. This data were compiled from 2006 to 2007 and became a basis of selection of clones to constitute parents to generate the population evaluated in this study. The following materials (Table 6.1) were selected based on their response to black Sigatoka as measured by the youngest leaf spotted to generate the 2x by 2x progenies for evaluation.

Table 6.1. Total leaves at flowering (TLF), youngest leaf spotted (YLS) number of functional leaves at harvest (NSL), plant height (HT) and bunch weight (BWT) of female parents selected to generate population for genetic analysis of 2x by 2x crosses

Clone	Total leaves at flowering	Youngest leaf spotted	Number of functional leaves at harvest	Plant height (cm)	Bunch weight (kg)
SH3142	11.2	8.8	0.2	278	8.0
3202	9.8	5.5	1.4	145	1.4
1535k-1	10.0	5.7	1.6	175	0.8
8075	9.3	5.9	0.0	200	6.1
5365	11.1	5.6	1.6	180	3.0
8615-1	11.0	5.1	1.8	185	1.0
9719	11.0	7.4	3.4	175	5.3
Wambo	13.6	7.3	5.1	259	5.0
Pitu	9.0	4.8	2.0	260	7.2
202SH	8.8	5.5	0.4	312	15.0
$LSD_{(0.05)}$	1.6	1.2	0.6	25	4.2

The male buds of the selected diploids were bagged with a cotton cloth to avoid contamination with unwanted pollen. Female flowers were also covered with white polythene bags. On the day of pollination, between 6am and 7.30am the cotton cloth was opened, pollen removed from each selected male bud and composited. The

composited pollen was used to pollinate female flowers according to Shepherd (1960). Clusters of anthers with pollen from the male parent were rubbed on the stigmas of designated female parents. The pollinated bunches were labelled with tags indicating cross number, parents and the date when pollination started. The same information was recorded in a field book. In addition, the field book had information of the day each cluster was pollinated. At physiological maturity (stage when the banana fingers have completely filled) the pollinated bunches were harvested, ripened in an enclosed room and seeds extracted. Immediately after extraction, seeds were taken to the laboratory and germinated in-vitro using the embryo culture method (Vuylsteke and Swennen, 1992). Some diploids never set seed, others set seed but the seeds never germinated. Out of ten female parents selected, plantlets were generated from 9719, 8075, 3202, 8615 and Pitu (Table 6.2).

Table 6.2. Number of plants per half-sib family that were involved in the progeny evaluation of a random polycross

	Number of plants
9719	22
8075	23
3202	40
8615	36
Pitu	29

6.2.2 Experimental design and management

The diploid progenies/families were evaluated at Kawanda Agricultural Research Institute (KARI). Kawanda Agricultural Research Institute is located at 00’ 22’’N, 00’ 32’’E at an altitude of 1193m above sea level. KARI has a bimodal type of rainfall with short rains starting in March/April to June and the long rains start in August to November/December. The strip that hosted this experiment has a sandy clay texture with a pH range of 5.1 to 5.6 (determined in water). After field ploughing with a tractor, the field was marked at a spacing of 3m by 3m. The planting holes, measuring approximately 60cm in diameter and 75cm deep were prepared. During planting hole preparation the top black soil was removed and put aside. Before planting, the black soil was put back, mixed with about 10kg of kraal manure containing 9.2% organic

matter, 0.42%N, 1093mg/kg of phosphorus, 4134mg/kg of potassium, 4860mg/kg of calcium, and 1476mg/kg of magnesium and pH of 9.7 (determined in water). The test plants were exposed to natural black Sigatoka infestation with the experimental plots planted in between the rows of the susceptible local spreader ‘Mbwazirume’. Planting was accomplished on 2nd December 2007 and the design was a randomised complete block design with two replications. Each replication had 10 to 20 plants per half-sib family. The black Sigatoka resistant check, Yangambi km 5 and the susceptible check, Grande Naine, were included in the trial as references.

Three months after planting, weeding was carried out with a hand hoe. Thereafter weeds were controlled by spraying with a systemic herbicide. During spraying, precautions were taken to make sure the herbicide did not get in contact with banana leaves. Six months after planting, desuckering was carried out to maintain 2-4 plants per mat. Dead leaves were not removed because there was need to maintain disease inoculum around plants. Also use of tools was minimised to avoid spreading banana bacterial wilt disease.

6.2.3 Data collection and analysis

Disease severity was assessed on 15th April, 29th April, and 13th May 2008. Disease assessment was carried out using the modified Stover (1971) scale. The proportion of the diseased leaf was estimated out of 100%. Each leaf was assessed individually and the overall disease damage per plant was computed. Disease damage was converted into the area under disease progress curve (AUDPC) using the formula given below as suggested by Shaner and Finney (1977):

$$AUDPC = \sum_{i}^{n=1} [(X_{i+1} + X_i)/2][t_{i+1} - t_i]$$

Where,

X_i = proportion of the host tissue damaged at i^{th} day,

t_i = the time in days after appearance of the disease at i^{th} day, and

n = the total number of observations.

Six months after planting, the number of suckers per plant was counted. At flowering, days to flowering, youngest leaf spotted (with at least 10 leaf spots of black Sigatoka),

plant girth at 100cm from ground level, total leaves, and plant height were recorded. To compare the performance of the half-sib progenies at the same growth stage, harvesting was standardised to four months after flowering. The number of functional leaves (still green) at harvest were counted and recorded. Bunch weight in kg, number of clusters per hand and number of fingers per hand were recorded. Two fingers were randomly selected from the second hand, and measured for finger length and diameter in centimetres.

The analysis was performed based on half-sibs using a random polycross model.

$Y_{ij} = \mu + B_i + F_i + P(F_j) + E_{ij}$

Where,

Y_{ij} is the response,

μ is the overall mean,

B_i is the replication effect,

F_i is the effect of the mother parent,

P(Fi) is the effect of the progeny within the half-sibs, and

E_{ij} is the random error term.

Therefore analysis of variance was generated using generalised linear model of SAS version 9.1 (SAS 2002) as shown in Table 6.3.

Table 6.3. Expected mean squares from analysis of variance for the random polycross model

Source	*df*	*Mean squares*	*Expected mean squares*
Reps	*r-1*	M_4	--
Mothers	*m-1*	M_3	$б^2_e + rб^2_{p/m} + rp\, б^2_m$
Progenies/mothers	*m(p-1)*	M_2	$б^2_e + rб^2_{p/m}$
Error	*(r-1)(mp-1)*	M_1	$б^2_e$

Source: Baker, 1986; Hallauer and Miranda, 1988.

The narrow sense heritability estimates for the different traits were computed from the estimates of genetic ($\delta^2_{p/m}$) and residual (δ^2_e) variances derived from the expected mean squares of the analysis of variance:

$\delta^2_{p/m} = (M_2 - M_1)/r$ where r is number of replications.

Then narrow sense heritability was computed as $h^2 = (\delta^2_{p/m} /(\delta^2_{p/m} + \delta^2_e)$ (Hill et al., 1998).

Standard error was calculated as suggested by Hallauer and Miranda (1988):
$SE(h^2) = 2SE(\delta^2_{p/m})/ (\delta^2_T)$,
where,

$2SE(\delta^2_{p/m})$ is the square root of the genetic variance, and
(δ^2_T) is the total phenotypic variation.

Minimum, mean, maximum, and standard deviation were generated using univariate analysis procedure in SAS version 9.1 (SAS 2002). Phenotypic correlation coefficients between disease and agronomic traits among the half-sib progenies were computed using SAS. Also a linear regression analysis of bunch weight against disease and agronomic traits was performed.

Path coefficient analyses were carried out according to Dewey and Lu (1959). Bunch weight was considered as the dependent variable. The following variables were included in the analysis:

1) Total leaves at flowering (TLF)
2) Youngest leaf spotted (YLS)
3) Plant girth (GR)
4) Days from planting to flowering (DPF)
5) Total fingers (Fingers)
6) Finger length (FL)
7) Finger diameter (FD)

The double arrows in figure 6.1 indicate the correlations between the two variables (r_{ij}) while the single arrows represent the direct influence as measured by the path coefficients (P_{ij}).

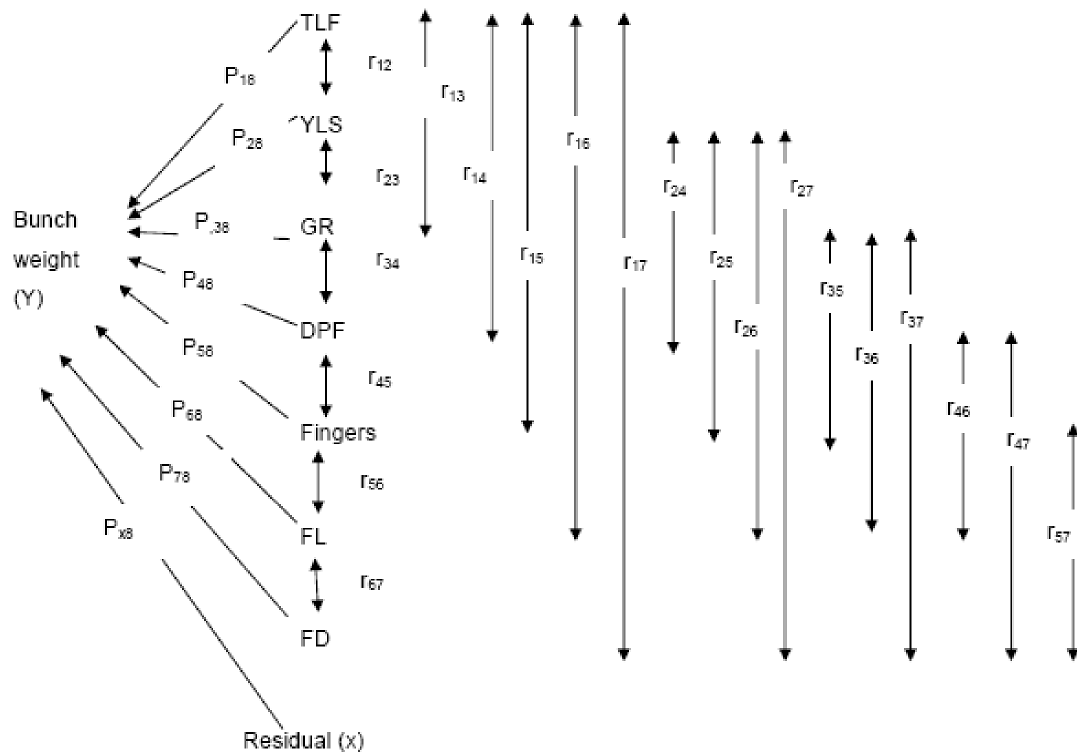

Figure 6.1. Path diagram for the relationship between bunch weight, agronomic and black Sigatoka resistance traits and agronomic traits in diploid 2x by 2x progenies

The path coefficients were estimated by solving the following simultaneous equations.

1. $r_{18} = P_{18} + r_{12}P_{28} + r_{13}P_{38} + r_{14}P_{48} + r_{15}P_{58} + r_{16}P_{68} + r_{17}P_{78}$
2. $r_{28} = r_{12}P_{18} + P_{28} + r_{23}P_{38} + r_{24}P_{48} + r_{25}P_{58} + r_{26}P_{68} + r_{27}P_{78}$
3. $r_{38} = r_{13}P_{18} + r_{23}P_{28} + P_{38} + r_{34}P_{48} + r_{35}P_{58} + r_{36}P_{68} + r_{37}P_{78}$
4. $r_{48} = r_{14}P_{16} + r_{24}P_{38} + r_{34}P_{48} + P_{48} + r_{45}P_{58} + r_{46}P_{68} + r_{47}P_{78}$
5. $r_{58} = r_{15}P_{18} + r_{25}P_{28} + r_{35}P_{38} + r_{45}P_{48} + P_{58} + r_{56}P_{68} + r_{57}P_{78}$
6. $r_{68} = r_{16}P_{18} + r_{26}P_{28} + r_{36}P_{38} + r_{46}P_{48} + r_{56}P_{58} + P_{68} + r_{67}P_{78}$
7. $r_{78} = r_{17}P_{18} + r_{27}P_{28} + r_{37}P_{38} + r_{47}P_{48} + r_{57}P_{58} + r_{67}P_{68} + P_{78}$

The indirect effects were determined as a product of the correlations with the respective path coefficients. For example the indirect effect of girth (3) through total leaves at flowering (1) was calculated as $r_{13}xP_{18}$.

6.3 Results

6.3.1 Performance of 2x by 2x half-sibs for black Sigatoka resistance and agronomic traits

Significant differences among the half-sib progenies were detected for area under disease progress curve (P<0.05) among black Sigatoka resistance traits (Table 6.4). Also the number of suckers (P<0.01), days to flowering, plant girth, bunch weight, number of clusters, and finger length of the half-sib progenies (P<0.05) were significantly different (Table 6.4). The analysis of progenies within a family indicated significant differences (P<0.05) among progenies within family for AUDPC, plant girth, plant height, and finger diameter (Tables 6.4 and 6.5). Although youngest leaf spotted and total leaves at flowering of were not statistically significant (P>0.05), the progenies within family mean square was higher than the family mean square.

Table 6.4. Mean squares for black Sigatoka resistance traits in 2x by 2x crosses planted at Kawanda Agricultural Research Institute

Source of variation	DF	Youngest leaf spotted	Total leaves at flowering	Area under disease progress curve
Replication	1	3.1	8.1	11382
Family	4	3.9	1.6	63693**
Progenies within family	83	5.0	4.7	6318*
Error	135	4.4	4.7	3728
CV(%)		27	21	30
R^2		0.68	0.65	0.82

* data significant at P=0.05; ** data significant at P = 0.01

Table 6.5. Mean squares for agronomic traits in 2x by 2x crosses planted at Kawanda Agricultural Research Institute

Source of variation	DF	Number of suckers	Days to flowering	Plant girth (cm)	Plant height (cm)	Bunch weight (kg)	No. of clusters	Finger diameter (cm)
Replication	1	13.9	30267	164	719	2.4	0.1	3.8
Family	4	9.8*	6347*	41*	11928**	2.2*	6.4**	8.6*
Progenies within family	84	1.5	1461	21*	1063*	0.9	1.3	4.3*
Error	47	1.8	1596	11	700	0.8	1.0	1.6
CV(%)		47	13	11	15	43	15	19
R^2		0.73	0.72	0.82	0.82	0.86	0.80	0.90

* data significant at P=0.05; ** data significant at P=0.01

Table 6.6 shows the response of the half-sib progenies to black Sigatoka disease as assessed by area under disease progress curve (AUDPC). Although all the families had mean AUDPC lower than that of the susceptible check, there were significant (P<0.05) differences between the family means. The half-sib progenies of 9719 had significantly (P<0.05) lower AUDPC than the rest of the family progenies implying that they were the most resistant to black Sigatoka damage. The AUDPC of half-sib progenies of 9719 was not significantly different from that of the resistant check Yangambi Km 5, but differed significantly (P<0.05) from AUDPC means of those of 8615-1 and Pitu. The half-sib progenies of Pitu showed the most susceptibility among the progenies evaluated.

Table 6.6. Mean area under disease progress curve (AUDPC) of half-sibs progenies planted at Kawanda Agricultural Research Institute between 2007 and 2008

Family	Area under disease progress curve
9719	152±12
8075	197±24
3202	177±13
8615-1	234±17
Pitu	251±14
Grand Naine (susceptible check)	375+16
Yangambi Km 5 (resistant check)	149+26
LSD $_{(0.05)}$	49

± = standard errors of the mean

The highest mean bunch weight of 3.2kg was recorded from the half-sib progenies of 8075 (Table 6.7). This bunch weight was significantly (P<0.05) higher than that of half-sib progenies of 3202, 8615-1 and Pitu. The fingers of half-sib progenies of 8075 had the largest circumference. Also the progenies of 8075 had the highest (P<0.05) mean number of clusters. However, the mean finger diameter of the half-sib progenies of 8075 was only significantly larger than the mean of progenies of 3202 and 8615-1. The half-sib progenies of Pitu took the least (P<0.05) period from planting to flowering. The progenies of Pitu on average flowered earlier (P<0.05) than the progenies of 8615-1.

Table 6.7. Mean number of suckers, days to flowering, clusters, finger diameter and bunch weight of diploid half-sib progenies planted at Kawanda Agricultural Research Institute between 2007 and 2008

Family	Suckers	Days to flowering	No. clusters	Finger diameter (cm)	Bunch weight (kg)
9719	2.2±0.22	293+7	6.9±0.20	7.0±0.37	2.3±0.56
8075	2.7±0.42	317±9	7.6±0.80	7.7±1.39	3.2±0.62
3202	2.2±0.22	308±6	7.0±0.29	5.7±0.44	1.5±0.63
8615-1	3.5±0.24	330±9	6.9±0.20	6.3±0.59	1.8±0.40
Pitu	3.5±0.24	289±6	5.8±0.20	7.3±0.41	2.1±0.42
$LSD_{(0.05)}$	1.3	41	1.2	1.3	1

± = standard errors of the mean

Table 6.8 shows within family progeny performance for girth, plant height and finger diameter. The first 20 plants from this population range from (33.9-44.6) cm for plant girth, (106.7-158) cm for plant height, and (7.4-9.5) cm for finger diameter. The progeny with the largest plant girth was from the Pitu family with a plant girth of 44.6cm. The 8075 family produced the shortest plants with mean plant height of 106.7cm. The largest fingers came from the Pitu family with a finger diameter of 9.5cm. All the families were represented in the first 20 plants arranged in descending order for plant girth. Similarly, all the families except 8615-1 got represented in the first 20 plants arranged according to height in ascending order. The family 8615-1 appeared to produce tall plants with short fingers.

Table 6.8. Within family progeny performance for plant girth (cm), height (cm) and finger diameter (cm) at Kawanda Agricultural Research Institute between 2007 and 2008.

Best plants	Progeny	Family	Girth	Progeny	Family	Height	Progeny	Family	Finger diameter
1	2	Pitu	44.6	3	8075	106.7	11	Pitu	9.5
2	10	8615-1	42.4	12	3202	111.7	2	Pitu	9.4
3	6	8615-1	39.4	10	3202	113.9	14	9719	8.6
4	19	8615-1	39.4	28	3202	127.3	1	3202	8.5
5	14	8615-1	38.4	15	Pitu	128.7	14	Pitu	8.0
6	5	8615-1	37.4	25	9719	130.3	8	3202	8.0
7	4	8075	37.4	11	3202	135.3	8	8075	7.6
8	15	3202	36.6	19	Pitu	136.7	15	8615-1	7.6
9	4	8615-1	36.4	21	9719	137.3	1	9719	7.6
10	16	8615-1	36.4	14	3202	137.3	3	9719	7.6
11	27	9719	35.6	5	3202	139.3	18	9719	7.6
12	29	9719	35.6	20	9719	142.7	19	9719	7.6
13	3	8615-1	35.6	16	3202	144.3	9	Pitu	7.6
14	7	9719	35.5	1	3202	147.5	13	Pitu	7.6
15	19	9719	35.4	9	3202	147.9	16	Pitu	7.6
16	8	8615-1	35.4	6	3202	150.5	17	Pitu	7.6
17	2	8075	35.4	16	9719	150.7	18	Pitu	7.6
18	8	8075	35.4	10	Pitu	151.5	20	Pitu	7.6
19	9	Pitu	34.4	6	Pitu	155.5	11	9719	7.4
20	8	3202	33.9	4	Pitu	158.0	12	9719	7.4
21	21	9719	33.6	12	Pitu	158.0	9	9719	7.1
22	11	Pitu	33.5	5	Pitu	158.7	13	9719	7.0
23	9	3202	33.5	3	3202	158.9	12	Pitu	7.0
24	5	Pitu	33.4	4	3202	159.8	2	8075	6.6
25	13	Pitu	33.4	8	9719	161.7	6	8615-1	6.6
26	18	Pitu	33.4	17	9719	161.7	9	8615-1	6.6
27	9	9719	33.4	6	9719	162.5	10	8615-1	6.6
28	2	3202	33.0	4	8615-1	162.7	13	8615-1	6.6
29	15	9719	33.0	11	9719	163.5	18	8615-1	6.6
30	3	Pitu	32.5	10	9719	163.7	15	9719	6.6
31	14	Pitu	32.5	17	Pitu	163.7	16	9719	6.6
32	1	8615-1	32.5	1	9719	164.7	5	Pitu	6.6
33	20	Pitu	32.4	8	3202	164.8	7	Pitu	6.6
34	8	9719	32.4	7	3202	168.6	19	Pitu	6.6
35	6	Pitu	32.0	13	9719	169.5	6	9719	6.5
36	13	9719	32.0	2	9719	169.7	1	Pitu	6.5
37	13	3202	31.5	11	8615-1	170.7	29	9719	6.4

6.3.2 Heritability estimates and correlations

Table 6.9 shows the heritability estimates of black Sigatoka and agronomic traits in 2x by 2x crosses. Apart from area under disease progress curve and the number of functional leaves at harvest which had heritability estimates of 0.52 and 0.17 respectively, disease traits had very low heritability estimates. Agronomic parameters had relatively better heritability estimates than disease traits. Total fingers per bunch registered the least heritability estimate of 0.05. The heritability estimates for the rest of the agronomic parameters ranged from low to high with plant height having the highest estimate of 0.56. The number of suckers, days to flowering, number of clusters, and finger length had heritability estimates of 0.47, 0.30, 0.37, and 0.27 respectively.

Table 6.9. Narrow sense heritability estimates of black Sigatoka resistance traits and agronomic traits in 2x by 2x crosses

Disease resistance traits		Agronomic traits	
Trait	Heritability estimate	Trait	Heritability estimate
Youngest leaf spotted	0.08(0.11)	No. suckers	0.47(0.45)
Total leaves at flowering	0.00(0.00)	Plant height (cm)	0.56(0.18)
Area under disease curve	0.52(0.21)	Plant girth (cm)	0.13(0.12)
No. functional leaves at harvest	0.17(0.18)	Days to flowering	0.30(0.33)
		Bunch weight (kg)	0.20(0.32)
		No. of clusters	0.37(0.44)
		Total fingers	0.05(0.11)
		Finger length (cm)	0.27(0.29)
		Finger diameter (cm)	0.14(0.16)

Values in parentheses are standard errors of the heritability estimates

Table 6.10 shows phenotypic correlations between agronomic and disease traits in 2x by 2x progenies. Total leaves at flowering and youngest leaf spotted had a significant ($P<0.001$) positive correlation. Bunch weight had positive and significant correlations with total leaves at flowering ($P<0.05$), youngest leaf spotted ($P<0.01$), plant height and plant girth ($P<0.001$), days to flowering and the number of functional leaves at harvest ($P<0.05$). Plant girth had positive correlations with plant height, ($P<0.001$), days from planting to flowering ($P<0.001$), total fingers ($P<0.001$) and finger diameter ($P<0.01$). Days from planting to flowering had positive relationships ($P<0.05$) with bunch weight and finger diameter. When bunch weight was regressed against disease and agronomic traits; plant girth, total fingers and finger length had a linear relationship with bunch weight (R^2=0.67) (Table 6.11).

Table 6.10. Phenotypic correlations between agronomic and disease parameters in 2x by 2x crosses

	AUDPC	TLF	YLS	HT	GR	DYF	NFL	BWT	Tot_fing	FL	FD
AUDPC	1										
TLF	-0.27*	1									
YLS	-0.38**	0.82***	1								
HT	0.20*	0.04	0.02	1							
GR	0.52***	-0.12	-0.24*	0.53***	1						
DYF	0.25*	-0.25	-0.30*	0.46***	0.63***	1					
NFL	-0.15	0.37**	0.26*	0.05	0.23*	-0.13	1				
BWT	0.04	0.34*	0.38**	0.46***	0.70***	0.27*	0.21*	1			
Tot_fing	-0.07	0.33**	0.28*	0.15	0.41***	0.10	0.15	-0.07	1		
FL	0.14	0.03	0.15	0.26*	0.30*	0.12	0.12	0.60***	0.05	1	
FD	0.15	0.10	0.19	0.26*	0.34**	0.22*	-0.05	0.56***	0.12	0.69***	1

* significant at P=0.05; ** significant at P=0.01 and *** significant at P=0.001; AUDPC = area under disease progress curve, TLF = total leaves at flowering, YLS = youngest leaf spotted, HT = plant height (cm), GR = plant girth (cm), DYF = days to flowering, NSL = number of functional leaves at harvest, CLS = number of clusters, BWT = bunch weight in kg, Tot_fing = total fingers per bunch, FL = finger length in cm, and FD = finger diameter in cm
.

Table 6.11. Stepwise regression of bunch weight against disease and agronomic parameters

Variable	Parameter estimate	Standard error	Type II SS	F Value	Pr > F
Intercept	-3.76077	0.52006	19.3619	52.29	<.0001
Girth	0.09884	0.02042	8.67694	23.43	<.0001
Fingers	0.00876	0.0033	2.61266	7.06	0.0097
Finger length (cm)	0.25137	0.04178	13.4043	36.2	<.0001

FL = Finger length
bwt = -3.76+0.098gr + 0.009total fingers + 0.25finger length; R^2=0.6664.

Table 6.12 shows direct and indirect effects affecting bunch weight in 2x by 2x banana progenies. Plant girth, total fingers and finger length had strong direct effects on bunch weight. Although the number of leaves at flowering had a negative direct effect on bunch weight, it showed a positive indirect effect through girth and total fingers. Youngest leaf spotted expressed a positive indirect effect through girth and finger length. Days to flowering had a positive indirect effect through girth, total fingers and finger length, while finger diameter had a strong positive indirect effect through finger length. Total fingers per bunch had a positive indirect contribution to bunch weight through finger length. However, the contribution of total fingers through finger diameter was negative (Table 6.12).

Table 6.12. Path analysis of direct and indirect effects affecting bunch weight in 2x by 2x progenies planted at Kawanda during 2007 to 2008

Variable	Direct effect	Indirect effects						
		TLF	YLS	GR	DYTF	TOT	FL	FD
Total leaves at flowering (TLF)	-0.0587		0.07014	0.21063	-0.039192	0.100362	0.047366	-0.0005
Youngest leaf spotted (YLS)	0.0835	-0.04931		0.21895	-0.04686	0.074688	0.103344	-0.00111
Plant girth (GR) cm	0.4379	-0.02935	-0.02004		-0.03834	0.126036	0.229322	-0.00161
Days to flowering (DYTF)	-0.0852	-0.027	0.045925	0.19706		0.077022	0.068896	-0.0006
Total fingers per bunch (TOT)	0.2334	-	-	-	-		0.036154	-0.0006
Finger length (FL) cm	0.4306	-	-	-	-	0.02355		0.19182
Finger diameter (FD) cm	-0.00503	-	-	-	-	-	0.26703	

- indirect effects were omitted because the variables develop in the later stages, so they cannot influence the other traits indirectly

Minimum, mean and maximum values of agronomic and disease traits in the 2x by 2x progenies are shown in Table 6.13. Youngest leaf spotted recorded a minimum of 3 indicating presence of susceptible clones in the half-sib families, and had a maximum of 15. The half-sib progenies retained an average of 3.6 leaves at harvest with a minimum of zero and a maximum of 9 leaves. Results from area under disease progress curve indicated there were highly resistant clones with a minimum of AUDPC of 55. Bunch weight (kg) had extremely low values (minimum 0.5kg and maximum 5kg). The bunch characteristics (finger length, finger diameter and number of clusters) showed low minimum values but the maximum values of 17, 19, and 9 indicate room for improvement in this population.

Table 6.13. Minimum, mean and maximum and values of disease and agronomic traits in 2x by 2x progenies

Trait	Minimum	Mean	Maximum	Standard deviation
Number of suckers	0	2.8	7	1.5
Area under disease curve	55	200.0	512	85.0
Total leaves at flowering	5	10.5	16	2.2
Youngest leaf spotted	3	8.1	15	2.2
Plant height (cm)	100	176.0	303	36.0
Plant girth (cm)	18	31.0	44	5.0
Days to flowering	201	299.0	437	44.0
No. of leaves at harvest	0	3.6	9	2.2
Bunch weight (kg)	0.5	2.1	5	1.0
Number of clusters	4	6.6	9	1.2
Total fingers	61	102.0	169	30.0
Finger length (cm)	4	7.2	17	2.1
Finger diameter (cm)	4	6.6	19	1.9

6.4 Discussion

6.4.1 Performance of family progenies from 2x by 2x banana crosses

The progenies of 9719 were more resistant to black Sigatoka than the progenies of Pitu based on the area under disease progress curve (AUDPC). However, all the half-sib families were significantly more resistant ($P<0.05$) than the susceptible check implying that the differences observed were at family level but did not suggest a high level of susceptibility. The minimum and maximum values also indicated a high level of variation for disease and agronomic traits which might imply that if these progenies are to be constituted into a population, response to selection would be achieved. The AUDPC had a heritability estimate of 0.52 implying that additive gene action might play a major role in the inheritance of AUDPC, therefore the performance of progenies for this trait can be predicted based on parental values.

The agronomic traits; days from planting to flowering, plant height, bunch weight, number of clusters, and finger length were significantly different among the half-sib progeny families. Results from Table 6.13 on maximum and minimum values indicate that there could be enough variability for these traits in this population. Plant height, number of clusters, and finger length had moderate to high heritability estimates, which could mean that if these progenies are constituted into a population to be advanced to the next generation, the response to selection can be predicted based on the current population. The low heritability estimates might require assessment of the breeding value of individuals before they constitute a population to be improved.

The results from the half-sib progeny evaluation indicated differences in performance for disease and agronomic traits. The differences among the families could be exploited to constitute a population with desirable traits. For example, the half-sib progenies of Pitu were the most susceptible to black Sigatoka yet they took shortest time from planting to flowering and had the biggest fingers. The half-sib progenies of 8075 had the heaviest bunch weights. The results suggest that each half-sib family have an advantage over the other. Therefore, with selection and recombination, it should be possible to accumulate the desired traits in diploid clones by selecting for multiple traits through recurrent selection.

6.4.2 Relationship between agronomic and disease resistance traits in 2x by 2x progenies

The strong positive relationships between disease traits (youngest leaf spotted, total leaves at flowering and the number of functional leaves at harvest) and bunch weight imply that a plant that has more leaves would have higher YLS implying it is more disease resistant. Similarly, more total leaves at flowering suggest that there is more photosynthetic area for bunch filling hence the strong relationship between total leaves at flowering and bunch weight (Craenen and Ortiz, 1997; Sheela and Ramachandran, 2001). It was observed that banana diploids with black Sigatoka resistance had more leaves at flowering than the susceptible diploids (Craenen and Ortiz, 1997; Tenkouano et al., 2003). Therefore total leaves at flowering could be an important trait to select for.

Although bunch weight values were very low in this population, they could be improved indirectly by improving traits associated with bunch weight. For example, Table 6.11 showed that up to 67% of the variation of bunch weight in this population was influenced by girth, number of clusters, and total fingers. Tenkouano et al. (2003) attributed higher bunch weights in banana diploids to increased fruit number. However, from the present investigation, total fingers per bunch had a negative indirect effect on finger diameter. This might suggest that when a bunch has many fingers, there will be competition for assimilates hence reduced finger size (Jullien et al., 2001ab). Yet end-users especially women prefer bananas with big fingers, because they are easy to peel. Therefore in selecting for the total number of fingers an optimum number should be considered not to compromise on the finger size.

Although days from planting to flowering did not have a positive direct effect on bunch weight, the days from planting to flowering expressed a positive indirect effect through plant girth. This means that plants which take a longer vegetative growth will attain big plant girths and this will have a direct effect on yield (bunch weight). Other studies related to days from planting to flowering, by Craenen and Ortiz (1998) indicated that early flowering shortened growth cycle of diploid bananas. On the other hand, Tenkouano (2003) reported that diploids take a longer fruit filling time than triploids and tetraploids. In the present study, we were unable to find out that relationship between early flowering and growth cycle because the bananas were harvested at a uniform

time of four months after flowering in order to compare progeny performance at a standardised stage. However, days to flowering had a positive significant relationship (r=0.27) with bunch weight and finger diameter (r=0.22). Although these correlation coefficients appear low, they were significant ($P<0.05$). Ortiz and Vuylsteke (1998) observed a positive relationship between days to flowering and bunch weight in banana plantains. This might suggest that early flowering diploid banana genotypes might have low yields than late flowering genotypes. Although early flowering is desired by banana breeders, there is a need to compromise on the appropriate time when the plants will have attained vigour to support the big banana bunch.

6.5 Conclusion

In conclusion, the half-sib families differed significantly for yield, days to flowering, and black Sigatoka resistance. The progenies from 8075, Pitu and 9719 had the heaviest bunches, took the shortest time to flowering, and were the most resistant to black Sigatoka, respectively. Therefore, the highest performing progenies should be selected and constituted into a population for further improvement through recurrent selection. Plant girth, total fingers per bunch, and finger length were important in determining bunch weight in 2x by 2x diploid progenies. However, other traits like the days from planting to flowering, total leaves at flowering, and finger diameter influenced the former traits indirectly. This implies that selection of parents with good combining ability for bunch weight traits should be carried our in a diploid improvement programme.

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Chapter seven

Overview of the Investigation and implications

7.1 Introduction

Banana yields at farm level in Uganda have declined from 20 t ha^{-1} yr^{-1} to 5 t ha^{-1} yr^{-1} mainly due to black Sigatoka. Farmers failed to adopt new black Sigatoka resistant materials which could boost banana production beyond current levels. Before this investigation it became clear that farmers' input was required in the development of new banana materials. Farmer knowledge needed to be complemented with scientific principles of quantitative genetics to produce better genotypes efficiently. The knowledge gaps identified were in quantitative disease assessment, phenotypic and genotypic variation in banana breeding populations and investigations on black Sigatoka resistance and associated traits. Therefore an investigation was undertaken to:

a) assess farmers knowledge of black Sigatoka disease and document qualities that farmers in central Uganda would desire to have in the banana genotypes to be developed for black Sigatoka resistance;

b) appraise the effectiveness of youngest leaf spotted, disease development time and area under disease progress curve in the assessment of black Sigatoka resistance in a diploid population;

c) determine the phenotypic variation of black Sigatoka resistance and agronomic traits in diploid (AA) and tetraploid (AAAA) bananas;

d) to determine the influence of tetraploid and diploid parents on black Sigatoka resistance and agronomic traits in the triploid progenies generated from tetraploid-diploid crosses, and

e) determine the relationship between bunch weight with agronomic and black Sigatoka resistance traits in 2x by 2x progenies.

7.2 Findings from the Investigation

7.2.1 Farmer assessment of traits to incorporate in black Sigatoka resistant materials

1. A survey was carried out in the two districts of Uganda (Nakaseke and Masaka districts), representing low and medium banana production zones respectively. From the survey results, farmers re-emphasised the role of bananas in their livelihoods. Farmers pointed out that bananas are an important food security crop as well as a source of income. The East African highland bananas were the most commonly grown. The farmers liked local East African highland bananas because of taste, food colour after cooking, marketability and early maturity. However, farmers reported that the local bananas were highly affected by pests and diseases, did not tolerate poor soils and drought, and produced small bunches. Only 7% of the farmers in the medium production zone and 3% in the low production zone were able to identify black Sigatoka as a banana constraint.

2. Fifty four percent and 39% percent of farmers interviewed in low banana and medium production zones, respectively, had been exposed to new black Sigatoka resistant banana materials. Farmers did not like the black Sigatoka resistant bananas despite their better yields than the local bananas. Farmers claimed that the new materials did not have good taste, required more labour to manage than the local banana materials and took long to mature.

3. Farmers were requested to suggest the traits they (farmers) would desire to have in the new black Sigatoka resistant materials to be developed. Farmers suggested the new materials should have good food (taste, colour, texture and aroma), heavy bunches, disease and pest resistance, tolerance to drought and early maturity. The relative importance of food colour, taste, texture and aroma in influencing choice of new banana materials was quantified. Out of 100%, taste was allocated 41%, texture 29%, and aroma and food colour 15% each.

7.2.2 Appraisal of methods for assessing black Sigatoka resistance in diploid banana populations

1. The three disease assessment techniques, youngest leaf spotted, disease development time and area under disease progress curve commonly used in assessment of black Sigatoka were compared using the diploid population. All the three assessment techniques were able to categorise the diploid accessions into resistant and susceptible clones in reference to the checks included in the study. However, the rankings among the three assessment techniques were not consistent.

2. The ranking of youngest leaf spotted and the are under disease progress curve were positively correlated. Also the rankings of area under disease progress curve and days it took from leaf emergence up to 25% leaf damage were strongly correlated.

3. Youngest leaf spotted and area under disease progress curve significantly explained up to 53% of total leaves at flowering. Also area under disease progress curve and youngest leaf spotted had significant linear relationships with bunch weight although R^2 of 0.24 was low.

4. Area under disease progress curve had the highest coefficient of determination (R^2=0.84) than the youngest leaf spotted (R^2 = 0.59) method. Therefore because of efficiency, ease to use and its potential to assess banana genotypes before flowering, area under disease progress curve was adopted and used in assessment of black Sigatoka in this investigation.

7.2.3 Variation in diploid (AA) and tetraploid (AAAA) populations for black Sigatoka resistance and agronomic traits

1. The diploid and tetraploid populations were investigated for their variation in black Sigatoka resistance and agronomic traits. Analysis of variance indicated that the diploid population was highly variable for plant height, youngest leaf spotted, total leaves at flowering, plant girth, number of clusters per bunch, days from flowering to harvest and area under disease progress curve. The tetraploid

population varied for plant height, plant girth, area under disease progress curve, and number of suckers.

2. Principal component analysis showed that plant height and plant girth in the diploid population had higher loadings on principal component 1 while in the tetraploid population plant height and girth had higher loadings on principal component 2. The two populations appeared to compliment each other in terms of disease and agronomic traits.
3. Total leaves at flowering, youngest leaf spotted and number of functional leaves at harvest correlated strongly in the diploid and tetraploid populations. Plant girth recorded at 100cm from the ground predicted yield in terms of bunch weight in the diploid and tetraploid populations.
4. Molecular analysis of tetraploid population using RAPD markers indicated that the clones were very closely related with genetic distances ranging from 0.04 to 0.39.

7.2.4 Genetic studies of black Sigatoka resistance and associated traits in tetraploid-diploid crosses

1. Genetic analysis of tetraploid (AAAA) and diploid (AA) progenies indicated that the half-sib progenies significantly ($P<0.05$) differed for bunch weight, plant girth and area under disease progress curve. The half-sib progenies also showed significant genotype-by-site interaction for bunch weight and days to harvest. There was a significant ($P<0.05$) within family variation for days from flowering to harvest and bunch weight.
2. The diploid male parents transferred black Sigatoka resistance to the triploid progenies as indicated by number of leaves at harvest, total leaves at flowering and disease development over time.
3. Results indicated that the tetraploid female parents were taller with heavy bunch weights than the diploid parents. On analysis of the triploid progenies from tetraploid-diploid crosses, the results suggested the tetraploid male parents

were more influential in determining bunch weight and plant height in triploid progenies than the diploid male parents.

4. There was a strong and positive correlation among the disease traits (youngest leaf spotted, total leaves at flowering and number of functional leaves at harvest). In addition, the youngest leaf spotted correlated positively with the days from planting to flowering. Days to flowering, had a negative correlation with number of fingers. Plant girth, number of fingers and finger diameter significantly predicted bunch weight in triploid bananas generated from tetraploid-diploid crosses.

7.2.5 Evaluation of 2x by 2x banana progenies for yield and black Sigatoka resistance

1. Results from evaluation studies in diploid by diploid crosses indicated that half-sib progenies were significantly different for agronomic traits (number of suckers, days from planting to flowering, plant height, bunch weight, number of clusters and finger length). Among the black Sigatoka resistance traits, significant differences were observed only in the area under disease progress curve.

2. The progenies generated by 8075 as a mother parent had the heaviest mean bunch weight. On the other hand progenies generated by Pitu and 9719 had the took the shortest time to flowering, and were the most resistant to black Sigatoka, respectively.

3. Phenotypic correlations revealed strong relationships between bunch weight with total number of leaves at flowering, youngest leaf spotted, plant height, plant girth, and days to flowering. Linear regression analysis indicated that plant girth, total fingers and finger length significantly predicted bunch weight (R^2=0.67). However, days to flowering had a positive indirect effect on bunch weight through plant girth.

7.3 Implications of the research findings and way forward

From farmers' point of view bananas with heavy bunches and good taste are essential. The bananas with heavy bunches and farmer acceptable taste were the two most important traits farmers desired to have in new banana materials with resistance to black Sigatoka. Significant variation for bunch weight was observed in the diploid population, but not in the tetraploid population evaluated. Although, there was significant variation in the diploid population for bunch weight, extremely low bunch weights were recorded in the diploid population. From the genetic studies, it appeared tetraploids are influential in determining bunch weight in triploids generated from tetraploid-diploid crosses. This study recommends that the available tetraploids in Uganda should be continuously improved for bunch weight. The inheritance of food quality parameters of colour, aroma, taste and texture was not investigated in this study though they play a major role in farmer acceptance of new banana materials. The results from farmer validation exercise for food quality traits, a clone from the cross 401k-1 x 8075 was approved by farmers for its taste. It is recommended from this investigation that farmers should be consulted using a farmer participatory approach in the cultivar development process to give feedback on traits farmers would desire in the new materials. This study also recommends that quantification and inheritance of these traits within the *Musa* breeding populations should be carried out.

The present study never investigated inheritance of food quality traits like food colour, taste and texture, however, it is believed that the synthetic tetraploids could have conserved these quality traits from the East African highland bananas. The role of synthetic tetraploids in transmitting food quality traits to the secondary triploids needs to be investigated.

Although youngest leaf spotted has been used in assessing black Sigatoka resistance, this study has found that area under disease progress curve (AUDPC) was reliable in assessing black Sigatoka resistance within *Musa*. The AUDPC was able to detect differences in black Sigatoka resistance among the triploid progenies in Chapter 5 and diploid progenies in Chapter 6. In both populations, the youngest leaf spotted method could not identify significant differences between the two populations. This might suggest that AUDPC is more precise in detecting small differences in black Sigatoka

resistance than the youngest leaf spotted method. The detection of small differences in disease resistance will be useful in the recurrent selection programme for the improvement of banana diploids. Also area under disease progress curve could select disease resistant clones before flowering to participate in the recurrent selection programme. Plant pathologists and banana breeders should assess disease severity using the area under disease progress curve method since it can be carried out before flowering and results indicated it has a higher R^2 (0.84) than YLS (0.59) which has been a commonly used method.

The most important yield component in banana is bunch weight. In this investigation, plant girth was found to predict bunch weight in the banana diploids, tetraploids and in the triploid progenies. Plant girth gives the banana plant vigour to withstand strong wind and support heavy bunches. In the diploid population plant girth was highly variable. Plant girth had low to moderate heritability estimates in the tetraploid-diploid crosses. Because of its importance, plant girth should be an important selection trait in development of banana genotypes.

Youngest leaf spotted was highly variable in the diploid population evaluated in chapter 4, yet after selecting and recombining individuals from this population and evaluating progenies in chapter 6, the half-sib progenies did not show any significant differences for this trait. This might have been caused by sterility of some of the selected materials and possibly the incompatibilities among the diploid *Musa* clones meant some selected banana diploids did not contribute their genes to the next generation. This implies that for a diploid improvement programme to be successful other traits such as pollen fertility and parthenocarpy should be considered when selecting parents to constitute a base population for further improvement.

Phenotypic characterisation of tetraploids indicated that they were variable for plant height. The heritability estimate that was based on female parent off-spring regression indicated the female parent influenced plant height in 4x by 2x crosses. This might suggest that genes for plant height can be accessed from the tetraploid female parent in 4x by 2x crosses.

Similarly, tetraploid female parents appeared to have influenced bunch weight in triploid progenies from tetraploid diploid crosses. The results indicated a better parent heterosis

of bunch weight of up to 128% from the female parent 401k-1. The phenotypic characterisation of tetraploids suggested that there was limited variation of this trait in the synthetic tetraploids currently being used in the improvement of East African highland bananas. This investigation recommends improving diversity in the synthetic tetraploids and selecting the female parents with the highest bunch weight to generate secondary triploids.

Genetic variability is very important in making genetic advances. This investigation has identified variability among traits in the breeding populations. It is very important for banana breeders to consider other methods of increasing genetic variability especially in the tetraploid populations. This can be achieved through mutation breeding, recreating more tetraploids from superior diploids by doubling the chromosome number of diploids and by making more 3x by 2x crosses which can generate more tetraploids. The current synthetic tetraploids used have limited genetic variability yet they are important in reconstituting secondary triploids.

Throughout this investigation, heritability estimates have been used to make references about the breeding materials. Some heritability estimates were extremely low, others ranged from moderate to high. The low heritability estimates could have been caused by high environmental variance and gene interactions. However, the moderate heritability estimates indicated that resistance might be improved through selection and advancing materials to the next generation. The low heritability estimates would emphasise evaluating the breeding value of parents before advancing materials to the next generation.

The significant family-by-environment interaction for bunch weight requires banana breeders to test new materials in more environments to identify stable genotypes to be promoted to a wider banana farming community. It is also recommended that studies should be conducted to classify banana production zones in Uganda based on weather conditions and soil types. From this study, analysis of progenies within the family indicated how different progenies performed in different sites. Since banana is a vegetatively propagated crop, clones with desirable gene combinations would be promoted as new varieties. From the present study, there were potential clones with better black Sigatoka resistance and bunch weights than the local check (Appendix

7.1). These materials will be advanced to a preliminary yield trial and depending on their performance they will be advanced for on-farm trials for adoption studies. Therefore the analysis of progenies within a family can help identify potential banana varieties.

Sterility, has made it difficult to generate enough numbers for genetic analysis in *Musa* species. Female sterility in bananas is believed to be a result of human selection. Sterility is associated with parthenocarpy, the ability to form fruit without fertilisation. The edible diploids are sterile and parthenocarpic. Therefore it sometimes becomes difficult to use edible diploids to improve the susceptible banana clones. The wild diploids are seed forming, and have poor agronomic traits despite having disease resistance. The elimination of poor traits, sterility and seed formation has complicated conventional banana breeding activities.

The banana seeds are highly dormant. The embryos have to be cultured on individual media to germinate them. The combined poor seed set and reduced germination of banana seeds make it difficult to generate enough numbers for detailed genetic studies.

Because of its long duration, the banana crop stays in the field for up to 18 months from planting to harvesting. This suggests there could be a lot of environmental changes influencing the same crop. This increases experimental error which results in high coefficients of variation and determination and sometimes it becomes difficult to improve the model for data analysis. Also because of the duration of the crop it demands a lot of patience on breeders to produce new varieties.

Appendix 7.1. Performance of new banana genotypes for acceptability, bunch weight and youngest leaf spotted

Cultivar code	Overall acceptability	Bunch weight (kg)	Youngest leaf spotted
K1121	2.7±1.34	6	9
K1223	2.8±0.88	14	8
K1313	3.0±0.81	15	7
K1314	3.0±0.81	9	7
K818	4.2±1.10	10	6
K914	4.3±0.57	9	6
K928	3.0±1.40	7	6
M1311	4.8±0.45	14	7
K1511 (Local check)	5.4±1.31	10	5.6

± = standard deviation;

Overall acceptability was scored on a scale of:

1 = not acceptable

6 = most acceptable