# CHARACTERIZING AN $F_2$ BANANA DIPLOID POPULATION FOR IDENTIFYING AND VALIDATING MOLECULAR MARKERS FOR WEEVIL RESISTANCE

**BY**

**ARINAITWE IVAN KABIITA**
**B.SC. BOTANY/ZOOLOGY (HONS), MAK**
**REG. NO: 2012/HD02/214U**

**A THESIS SUBMITTED TO THE DIRECTORATE OF RESEARCH AND GRADUATE TRAINING IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN PLANT BREEDING AND SEED SYSTEMS OF MAKERERE UNIVERSITY**

**NOVEMBER 2014**

## DECLARATION

This thesis titled ***characterization of an $F_2$ banana diploid population for identifying and validating molecular markers for weevil resistance*** is my original work and has never been submitted for a degree in any other university.

…… ……

**Arinaitwe Ivan Kabiita** **Date**

This thesis has been submitted for examination with our approval as supervisors

…….. ……..

**Dr. Richard Edema** **Date**

(BSc. (Agric) Hon, Msc. (Agric) MAK; PhD. (The Ohio University)
Makerere University, Kampala Uganda

…….. ……

**Dr. Alex Barekye** **Date**

(BSc. (Agric) Hon, Msc. (Agric) MAK; PhD. (University of KwaZulu Natal)
Director of Research, Kachwekano (ZARDI)
National Agricultural Research Organisation (NARO)

## DEDICATION

I dedicate this piece of work to my lovely mother Mrs. Meddy Kabiita who saw me through my education up to BSc. Botany and Zoology and for nurturing me into a real man. I also dedicate this book to my family that is my wife Irene Kebirungi and my son Sheamus Ahabwe.

## ACKNOWLEDGEMENT

I would like to thank my supervisors, Dr. Richard Edema and Dr. Alex Barekye for their commendable guidance, and advice throughout this study. I would like to appreciate the contribution of Prof. Paul Gibson, Madam Pauline Gibson and Dr. Kassim Sadik for their advice and constructive suggestions. Prof. Paul Gibson provided me with the best understanding of the subject matter and statistical analyses for this study. Pauline Gibson assisted in this work by significantly improving the English. Dr. Sadik did really help me in making corrections and putting my work in order according to the university format. I am very grateful to the administration of National Agricultural Research Laboratories (NARL)-Kawanda and the National Banana Research Programme (NBRP) for allowing me to use their station research facilities, laboratory and providing the breeding materials. I am indebted to Naboth and David the technicians who helped a lot during this study.

This work was funded by the Alliance for a Green Revolution in Africa/Programme for African Seed Systems (AGRA/PASS) project and Bioversity International -Uganda. My special gratitude goes to Dr. Richard Edema for effective coordination and administration of research funds. I thank my family members: Allen, Oscar, Charity, Rachael, Phiona, Brian and others for your prayers and encouragement. “May the Lord God be with me” Amen.

## TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

## ABSTRACT

The banana weevil (*Cosmopolites sordidus* Germar) is the most important insect pest of banana and plantain in Africa. Most East African highland bananas (EAHB) are highly susceptible to it. Sources of resistance to most pests and diseases are known to come from wild banana diploids. There is thus a need to exploit the potential of wild banana diploids in improvement of East African highland bananas by studying genetics of resistance of inter diploid crosses that could be used to improve the EAHB. The objectives of this study were a) to identify segregating weevil resistance and agronomic traits in an $F_2$ diploid banana population, b) to determine the inheritance of banana weevil resistance and agronomic traits based on an $F_2$ banana diploid population and c) to optimise SSR markers for nematode resistance for characterizing an $F_2$ diploid banana population against banana weevil resistance.

Two diploid parents *Musa acuminate* Subspecies banksii (kasaska) susceptible to banana weevil and *Musa acuminata* Subspecies microcarpa (borneo) resistant to banana weevil were crossed to generate an $F_1$ population from which one line was selfed to generate an $F_2$ diploid population. The $F_2$ population was screened against weevil resistance by artificial inoculation in a field where corm sections were inoculated with larvae at first instar and measurements taken for growth parameters after 8 days and in pot experiment where damage parameters were measured. Agronomic parameters were also studied for this $F_2$ diploid banana population.

There were significant differences ($P<0.05$) among the different genotypes for banana weevil resistance traits such as head capsule width (HCW), body length, body weight, larval mortality, total damage, peripheral damage, dead weevils and larvae retrieved. However, weevil damage parameters such as total cross sectional inner and outer damages and total cross sectional damage were non-significant. There were also significant differences ($P<0.05$) for agronomic parameters such as bunch weight, number of clusters, number of fingers, days to maturity, inner corm hardness and total corm hardness. However there were no significant differences ($P<0.05$) for number of functional leaves at flowering, number of functional leaves at harvest and youngest leaf spotted.

The nature of inheritance for all the larval resistance traits and weevil damage parameters showed a possibility of quantitative inheritance. The chi square test of goodness of fit showed body weight to be in a ratio 15:1 when tested using both resistant and susceptible parents as checks; body weight in ratio of 7:9 with resistant parent as check and 15:1 with susceptible parent as check; larval mortality in ratio of 1:3 ratio with resistant parent as check and 15:1 with a susceptible parent as check; total damage in a ratio of 3:1 with resistant parent as check, peripheral damage in a ratio of 9:7 with resistant parent as check; larvae retrieved in a ratio of 3:1 and dead weevil in ratio of 1:3 tested using a resistant parent as check and 1:15 when using a susceptible parent as a check. Knowing the nature of inheritance of segregating diploid population will assist in improving the susceptible East African highland bananas (EAHB). Among the tested 33 SSR primers, 17 primer pairs amplified the DNA of the parental materials and the $F_1$ progeny implying that they could be used in genotyping the developed diploid population especially under high resolution Gels such as Metaphor agarose or poly acrylamide gelelectrophoresis (PAGE). These markers will aid in early selections of desired cultivars which will shorten the breeding cycle and reduce on the breeding costs.

# CHAPTER ONE
# INTRODUCTION

## 1.1 Origin and distribution of bananas

Bananas and plantain originated from South East Asia, a region considered as the primary Centre of diversification of the crop and where the earliest domestication has occurred (Simmond, 1962; Sebastião *et al*., 2001; IITA, 2009). The low land areas of West Africa contain the world's largest range of genetic diversity of plantains (*Musa* AAB) (Ortiz and Vuylsteke, 1994) while the highlands of East Africa, form a secondary zone of genetic diversity for the East African highland bananas (*Musa* AAA) (Smale, 2006). The AAA dessert bananas which include Dwarf Cavendish, Lacatan, Red Banana, and Gros Michel Cavendish dessert bananas are generally concentrated in the low-lying coastal regions – in South Africa, Somalia, Ethiopia and around Lake Victoria region at a slightly higher altitude range (Karamura *et al*., 1998). Other bananas such as Kivuvu (AAB), Sukalindizi (ABB) are scatted in the region of East Africa but the AAB however are said to have had their secondary centre of origin from Polynesia where they are thought to have been carried from the Phillipines more than 4,000 years ago (De Lange, 1995).

## 1.2 Economic importance of bananas

Bananas and plantains (*Musa* spp.) are the 8$^{th}$ most important food crop in the world, and the 4$^{th}$ most important in the least developed countries with an annual global production of around 125 million tones (FAOSTAT, 2012). Only about 15 percent of this global production enters the world market, with an estimated value of more than USD 5 billion per year (Arias *et al*., 2003). Banana is the second most tradable fresh fruit in the world after apples. Latin America supplied 83.2 percent of the gross fresh bananas exported in 2009; Far East supplied 12.8 percent and Africa 3.4 percent, and the Caribbean 0.4 percent (Evans and Fredy, 2013).

Bananas represent a major staple food for 400 million people in the tropics and subtropics (INIBAP, 2000). In Africa, banana and plantain provide more than 25% of food energy requirements for around 70 million people (Vander Stichele *et al*., 2005; Evans and Fredy, 2013). Banana is a staple to an estimated 10 million Ugandans, with 66 percent of the country's urban population depending on the crop (FAO, 2011; PIBID-Uganda, 2012). Uganda is the second

largest producer of banana (9.2 million MT) in the world with one of the highest per capita consumption rates of 230-450kg/person/year (FAO, 2013). Most cooking bananas are grown by small-scale farmers either for their own consumption or for selling to the local markets.

The economic importance of the banana industry encompasses the generation of export earnings and the employment of hundreds of thousands of people in the world. In addition, the industry employs thousands of people in distribution networks and supermarkets worldwide (Evans and Fredy, 2013). In Uganda, banana is considered the most important source of rural revenue and returns to family labor to the 10 million Ugandans who depend on it. According to Bagamba (1994) and Embrechts *et al.* (1996), banana contributes 8-22% of the national agricultural revenue and provides employment to a considerable number of Ugandans. A well-managed one-hectare farm can earn up to US$1,500 dollars a year (Marlene, 2012).

**1.3 Constraints to banana production**

Banana production is limited by a number of factors and these include, biotic stresses such as banana nematodes and weevils (Gold *et al*., 2004; Ocan *et al*., 2008), black Sigatoka (Barekye, *et al*., 2009) banana bacterial wilt, Fusarium wilt (Ploetz, 2006; Biruma *et al*., 1997). Abiotic stresses include low soil fertility and drought. Low soil fertility is most important of these and is mainly due to nutrient exhaustion as a result of growing bananas season after season coupled with poor post-harvest handling as banana is a perishable crop (Rubaihayo *et al*., 2003; Lescot and Ganry, 2008; Ouma, 2009; Wachira *et al*., 2013; FAO, 2013).

The banana weevil (*Cosmopolites sordidus* Germar) is an important pest of banana, plantain and ensete. Among the pests of bananas, the banana weevil is the most serious and can cause 50-70% yield losses to priority banana production in Uganda (Rukazambuga *et al*., 1998; Kalyebara *et al*., 2005). The most destructive stage of the banana weevil is the larvae. The larvae primarily feed on the corm but will also feed on the stem under severe infestations. Adults may remain at the same mat for extended periods of time, while only a small proportion moves > 25 m within 6 months. However, weevils rarely fly. Other yield losses attributed to banana weevils include small fruit size, toppling, snapping and complete mat death (Rukazambuga *et al*., 1998).

Additionally banana weevil damage causes failure to produce suckers and shortened plantation life (Gold *et al*., 2004).

All plantains and highland banana clones appear highly susceptible to banana weevil (Gold *et al*., 1994; Gold *et al*., 1994; Ortiz *et al*., 1995; Kiggundu, 2000). This therefore threatens the food security of the people who depend on banana as main source of food and income mainly in the sub Saharan Africa. According to FAO (2011) and PIBID-Uganda (2012), an estimated 10 million people depend on banana as a staple food and as a source of income so if the problem of susceptibility to pests and diseases is not checked, these people risk famine and unemployment. Control methods used to control banana weevil such as chemical, biological and cultural methods are unviable or have not been very successful to small scale farmers because they are expensive, labour intensive and require technical knowledge to use (Gold *et al*., 1993). Therefore there is a need to use host plant resistance since it is cheap, durable and can be incorporated with other control methods. Surveys and screening trials suggest that at least some clones in other genome groups like ABB and AA are resistant or partially resistant to banana weevil (Pavis and Lemaire, 1996; Kiggundu, 2000). This implies there are opportunities to develop banana hybrids that have weevil resistance. Thus this study focused on host plant resistance as method of controlling weevils.

### 1.4 Statement of the problem

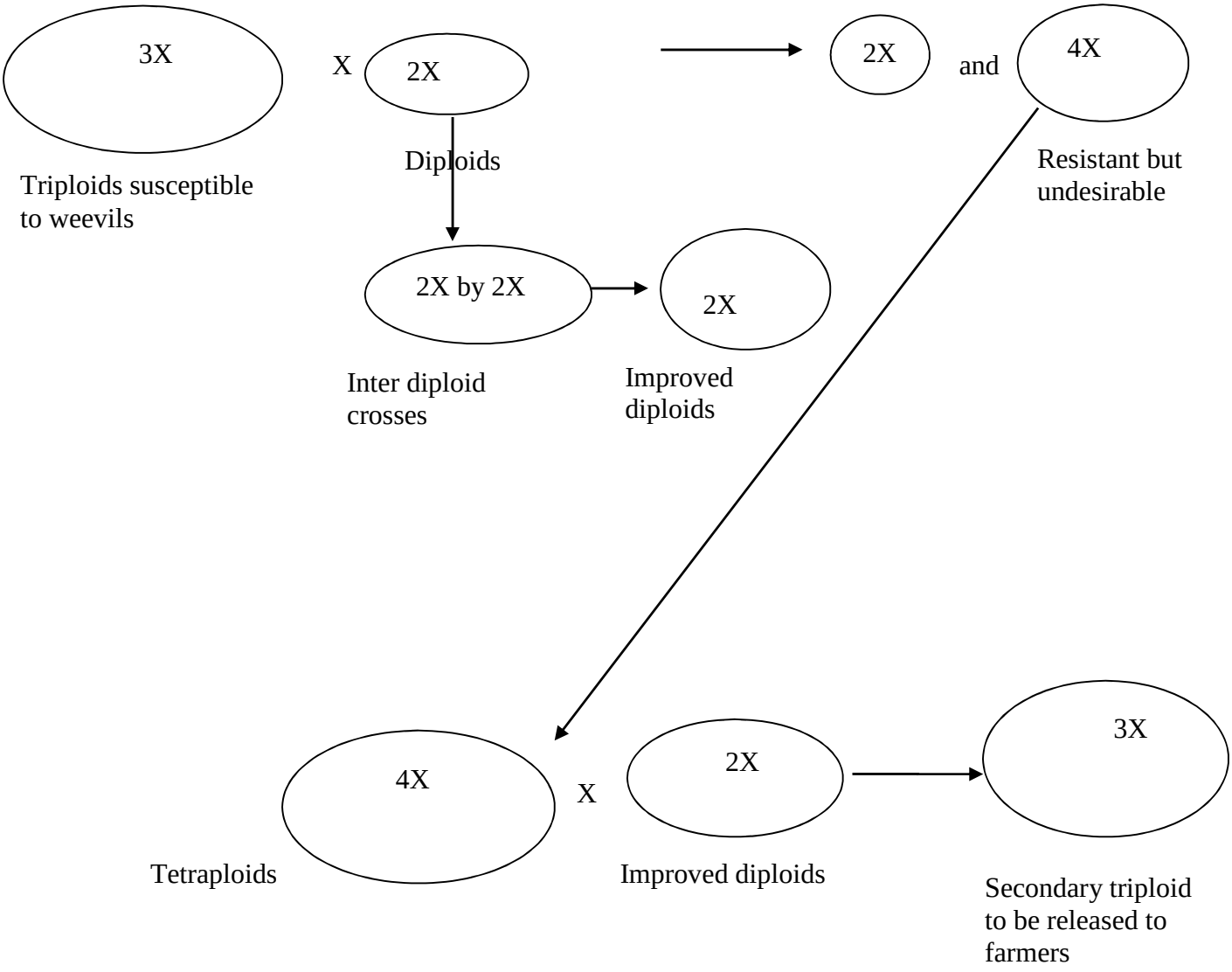
Most East African highland bananas are highly susceptible to banana weevils (Gold and Messiaen, 2000; Kiggundu, 2000; Kiggundu *et al*., 2007). Yield loss studies have indicated that the banana weevil can cause up to 50% yield loss after the 3$^{rd}$ ratoon cycle if new plantations are infested (Rukazambuga, 1996; Gold *et al*., 2004; Ocan *et al*., 2008). The loss due to banana weevil damage can go up to 100% in severe infestations (Sengooba, 1986). If this problem of yield loss by banana weevils is left un-solved, there is a great danger of food insecurity in the world and Uganda in particular. In Uganda more than 10 million people would be at risk of facing food insecurity and unemployment with women and children being the most affected. Other than food insecurity and unemployment increasing, the country as whole will be losing 1,000 tonnes of bananas generating 6 million USD as foreign exchange to Uganda (Wambi, 2008). Such problems will lead to an increase in food prices and hence increased cost of living.

Attempts to control the weevil using cultural, chemical and biological methods have not been very effective because they are labour-intensive, expensive and may not be environment friendly (Gold *et al.*, 1993) and this has left the problem still haunting millions of farmers in Uganda. Breeding for resistant varieties would be a more sustainable control to banana weevil problem. There are sources of resistance to banana weevil among wild banana diploids (Kiggundu, 2000). However, the genetics of resistance such as heritability, gene action, and linkage to other characters within the banana diploids in relation to banana weevil resistance have not been fully studied. Secondly banana improvement has challenges such as long crop duration (up to 2 years), large space requirements for evaluating progenies and poor seed set. To reduce on long breeding duration and enhance efficiency of banana breeding and selection requires employment of marker assisted selection. But markers for resistance to banana weevil have not been identified.

**1.5 Justification**

Sources of resistance to most pests and diseases are available among wild banana diploids (Kiggundu, 2000). To exploit the potential of diploids in improvement of East African highland bananas, the National Agricultural Research Organisation (NARO) and IITA adopted a methodology to improve the triploid East African bananas (Pillay *et al*., 2004). In this breeding scheme, female fertile East African highland bananas (triploids) are crossed with a wild diploid banana cultivar Calcutta-4 to generate hybrid tetraploids and diploids. The tetraploid banana hybrids that are generated are then crossed with the improved diploids to generate secondary banana triploids. This strategy utilizes the diploids to introgress resistance into the edible EAHB, so that the problems of diseases and pests such as the banana weevil are combated while retaining the farmer preferred traits in cooking bananas (EAHB).

The NARO and IITA developed banana breeding strategy (Pillay *et al*., 2004) is shown below.




Information on genetics of resistance such as heritability, gene action, and linkage to other characters within banana diploids in relation to banana weevil resistance will guide further improvement of bananas for weevil resistance.

Identification of molecular markers especially SSR markers is very useful because they are easy to analyze and are co-dominant. Speijer *et al*. (1993 ) reported a strong association between nematode and banana weevil infestation. His study found that suckers attacked by nematodes are four times more likely to be attacked by weevil. Resistance mechanism such as phenological

compounds, HPLC products, toxins and lignin have been reported for nematode resistance (Gowen & Muller-Harvey, 2000) and the same resistance mechanisms were reported for banana weevil (Kiggundu *et al*., 2003; Ocan *et al*., 2008). There is a need to find out if SSR markers for nematodes resistance have any association weevil resistance traits in *Musa spp*. Having markers that select for both nematode and banana weevil resistance is important in selecting cultivars that have resistance to both weevils and nematodes at once which saves time and resources.

## 1.6 Objectives

### 1.6.1 General objective

To contribute to development of weevil resistant banana lines through understanding genetic mechanism of resistance for weevils and identifying available SSR markers for weevil resistance.

### 1.6.2 Specific objectives

1. To identify segregating weevil resistance and agronomic traits in an $F_2$ diploid population for banana.
2. To determine the inheritance of banana weevil resistance and agronomic traits in an $F_2$ banana diploid population.
3. To identify nematode resistance SSR markers for characterizing an $F_2$ diploid banana population against banana weevil resistance

## 1.7 Hypotheses

1. The $F_2$ banana diploid population will express different resistance mechanisms both phenotypically and genotypically.
2. Banana weevil resistance traits are inherited differently in an $F_2$ diploid population.
3. SSR markers for nematode resistance can be identified for characterising an $F_2$ diploid banana population against banana weevil resistance.

# CHAPTER TWO
# LITERATURE REVIEW

## 2.1 Banana weevil and Mechanism of weevil resistance

### 2.1.1 Nature of the banana weevil

The banana weevil *Cosmopolites sordidus* (Germar, 1824) belongs to the class Coleoptera and family Curculionidae is an important pest of banana, plantain and ensete. The life cycle (egg to adult) of the banana weevil requires 30 to 40 days (Mau and Martin Kessing, 2007). The white, sausage-shaped eggs are about 1/12 inch (2 mm) long. They are deposited singly in cavities chewed by the adult females in the corm or pseudostem at ground level (Mau and Martin Kessing, 2007). After eggs are laid, they hatch in 5 to 7 days into larvae.

The larvae are stout, creamy white, legless and have reddish-brown heads. When mature they are about 1/2 inch (12 mm) long (Mau and Martin Kessing, 2007). The emerging larvae feed on the rhizome, but can also attack the true stem and occasionally the pseudostem. The larvae pass through 5-8 instars (Gold and Messiaen, 2000). Development is completed in 15 to 20 days. The larvae are the most damaging stage of the weevil in bananas. Therefore, most control measures target this stage of weevil development. The larvae then pupate within chambers that are usually close to the surface of the corms. Pupae are white and about 1/2 inch (12 mm) long (Mau and Martin Kessing, 2007). Pupation is in naked cells near the surface of the host plant (Gold and Messiaen, 2000). Pupal development is completed in about 8 days.

The adult weevil is black and measures 10-15 mm. It lives freely mostly between the leaf sheath in the soil at the base of the mat. The weevil feed at night so are nocturnal active and very susceptible to desiccation (Gold and Messiaen, 2000). Adults are reported to live for as long as 2 years and are capable of surviving for extended periods without food (Mau and Martin Kessing, 2007).

### 2.1.2 Symptoms of the banana weevil

Larvae (Grubs) feed by making irregular tunnels in the corm and root stock. The corm can be eaten up completely with tunnels, which promotes fungal infection and decay reducing its surface area

(Njau *et al*., 2011). The tunneling weakens the plant and may even cause death of suckers as well as snapping, toppling, deterioration of the root system, reduced nutrient uptake, smaller bunches, diminished vigor of followers and mat die-out (Gold and Messiaen, 2000; Gold, 2000). As a result the leaves turn yellow, wither and die prematurely. Young suckers show symptoms of wilting and die. Older plants are retarded in their growth and under heavy infestation, there is reduced yield and plants are easily blown by wind (Rukazambuga *et al*., 1998; Ocan *et al*., 2008). The larvae as they eat, tunnel through the corm and leave galleries. The galleries are used to estimate weevil damage in *Musa spp* (Gold and Messiaen, 2000; Okolle *et al*., 2009).

### 2.1.3 Control of the banana weevil

Tissue cultured plantlets are widely used in commercial banana plantations for pest and disease control. Paring suckers to remove weevil larvae and eggs and immersing pared suckers in hot water bath of 52-55$^{o}$C for 15-20 minutes kill larvae and eggs. Crop sanitation such as removal of crop residues after harvest, intercropping and mass trapping can reduce weevil densities (Masanza, 2003). However these control measures have not been very effective in controlling the banana weevil (Gold and Messiaen, 2000).

A number of predaceous beetles have been found feeding on banana weevil larvae in Southeast Asia. However, introducing these natural enemies in other banana growing areas has not been successful (Gold and Messiaen, 2000). The use of entomopathogenic fungi (e.g. *Beauveria bassiana* and *Metarhizium anisopliae*) and the entomopathogenic nematodes, *Steinerma* and *Heterorhabditis spp* for the control has shown some signs of good progress in controlling the weevil (Nankinga and Ogenga-Latigo, 1996). However the economic cost and efficacy of only being used under high weevil population densities limit their use on a larger scale.

Use of chemicals is applicable to large scale farmers who have the resources and skills required but it is not effective for our resource poor small scale farmers (Masanza, 2003). Weevils have shown some kind of resistance to chemicals making their use not an ideal control strategy (Gold and Messiaen, 2000). In view of the available banana weevil control measures, host plant resistance seems to be the most efficient, compatible and sustainable way of managing the banana weevils.

### 2.1.4 Host plant resistance mechanisms

The banana weevil is readily attracted to and freely oviposits on resistant clones. Host plant resistance appears to be primarily due to antibiosis mechanisms causing high mortality rates in the larval stage. Host plant resistance in the form of antibiosis may be expressed through larval survivorship, increased larval and weevil mortality, delayed development, reduced body size, reduced body length, reduced head capsule width (HCW) and reduced fecundity of herbivores (Gertrude *et al*., 2010).

The larval survival rate in resistant cultivars is reported to be low (Kiggundu *et al*., 2007) and estimated to be 10-20 times higher in susceptible bananas compared to resistant ones (Abera, 1997). This parameter was used in this study to determine larval resistance as in form of larval mortality after they failed to eat due to chemical compounds. Larval developmental period is prolonged in resistant genotypes (Gertrude *et al*., 2010). This could be as a result of reduced feeding, poor utilization of ingested food and toughness of the food material (Gertrude *et al*., 2010). The larvae feeding on susceptible cultivars attain a critical weight for pupation in a shorter time than larvae that fed on resistant cultivars (Gertrude *et al*., 2010). This means that larvae on susceptible cultivars weighed heavier (bigger) and are longer than the ones feeding on resistant ones. Differences in weevil survival rate were observed in experiments set by Sadik, *et al*., (2010). In his study, he reported that resistant cultivars had fewer adult weevils than in susceptible cultivar relating weevil mortality to resistance.

Kiggundu (2000) found that corm dry matter content was negatively correlated with weevil damage and therefore recommended it as a significant parameter in the resistance response. He also noted that the genetic relationships between corm hardness and weevil damage were sufficiently high and significant. Ortiz *et al*. (1995) reported that corm hardness is one of the best parameters for weevil resistance. Cultivars that had hard corms showed low levels of damage by weevils. This therefore presented a need to use corm hardness and dry matter content as mode of resistance to weevil damage as well as their correlation with weevil damage parameters

Chemically sap has been found to be rich in ions especially $K^{+}$, $Mg^{2+}$, $Cl^{-}$, and $NO^{3-}$ (Baker *et al*., 1990). These, plus other inclusions like globular vesicles and crystalloid vesicles have been found

to be osmotically active (Kallarackal *et al*., 1986). Resin/sap was found to be negatively correlated with weevil damage and recommended as significant parameters in the resistance response (Kiggundu, 2000).

As explained above, banana weevil have been reported to exist within *Musa* but their genetics of resistance have not been studied and thus there is a need to study them so as to enhance their utilization in the improvement of bananas.

## 2.2 Genetics of resistance

### 2.2.1 Heritability

Heritability is the proportion of phenotypic variance that is attributed to the genetic constitution of an organism. The traits that are easy to breed, easy to score and have stable phenotypic expressions across genotype by environment have high heritability.

There are two types of heritability: Broad sense heritability (H), the proportion of the total phenotypic variance that can be attributed to genetic constitution of an organism (all the genetic constitution) and narrow sense heritability ($h^2$), the proportion that is transmissible from parent to offspring. This is primarily additive type of gene action. There are various methods for estimating heritability such as, variance component estimation of heritability, Parent off spring estimation of heritability, $F_2$ variance-environment estimation of heritability (Falconer and Mackay, 1996).

Variance component estimation of heritability is a method that draws heritability from the variance components with the help of mean squares. This is achieved after running an ANOVA table and deriving the variance components. Genetic and phenotypic variance components developed from species that are clonally propagated crops, crops not easy to propagate clonally, families or lines containing multiple individuals are used to estimate heritabilities (Holland *et al*., 2003). Holland *et al*. (2003) further indicated that quantitative genetists have estimated heritability from ordinary least square analysis of variance (ANOVA) and variances components estimated algebraic as a function of mean squares for balanced data. For unbalanced data, the effects of unbalanced data including changes in the coefficients of variance components in the expected mean squares can be handled using Milliken and Johnson (1992), loss of independence between mean squares and

unknown distributional properties variance component estimates can be computed using softwares packages such as SAS and Genstat (Holland *et al*., 2003).

Estimation of variance components is easier to generalise, and this method is generally used to estimate genetic parameters (http://wwwpersonal.une.edu.au/~jvanderw/Estimation of variance components.pd, n.d). It is a suitable method for determining broad sense heritability and narrow sense heritability and it is an easy way of determining heritability for clonal crops since they can easily be clonally multiplied and replicated (Holland *et al*., 2003). Variance components are used to calculate broad sense heritability and repeatability (Pillay and Tenkouno, 2011).

Pavis and lemaire (1996) and Mestre (1997) noted the need for standard screening parameters and reference cultivars. Kiggundu (2000) recommended total cross sectional damage as best parameter to determine heritability of weevil resistance as it had highest heritability of all the parameters of weevil damage in East African highland bananas while Rukazambuga *et al*. (1998) suggested the use of damage to the central cylinder as this had a great impact on plant growth and yield. However this method did not include artificial method for screening for weevil resistance such as head capsule width, body weight and body length yet these could be faster, cheap and non-destructive methods.

### 2.2.2 Nature of inheritance

Continuous variation is considered a particular characteristic of quantitative traits. Quantitative traits such as plant height, yield and others carry genotypes that can be grouped in two main classes, even though continuous variation may occur within each class (Sebastião *et al*., 2001). In bananas and plantains, traits showing continuous variation are controlled by major genes (Vuylsteke *et al*., 1997). However, in bananas inheritance of resistance to black Sigatoka, parthenocarpy of the fingers and sterility, orientation of the bunch, wax in the pseudostem, male and female sterility, weight of the components of the bunch and agronomic traits such as apical dominancy, persistency of the male bracts and hermaphrodite flowers in the rachis, are governed by one or a few genes (Ortiz, 1995; Vuylsteke *et al*., 1997).

The inheritance to black Sigatoka is governed by three loci with recessive/additive effects in Plantains and Calcutta-4. The moderately resistant phenotypes correspond to homozygous alleles recessive/favorable to the three loci. The dominant allele is always present in the diploid susceptible hybrid (Sebastião *et al*., 2001). Rowe and Richardson (1975) reported that *M. acuminata* resistance to moko disease in banana was controlled by recessive genes. However, it was verified that the resistance to the race that attacks tomato was dominant in *M. acuminata* spp. banksii, and recessive in *M. acuminata* spp.microcarpa (Vakili, 1965). *Radopholus similis* nematode resistance is controlled by one or more dominant genes (Rowe, 1991).

As seen above in section 1.4, the best control method for pests and disease is host plant resistance and it has been identified to be in wild relatives of banana (banana diploids) (Kiggundu, 2000; Lorenzen *et al*., 2010). (Pillay *et al*. 2004) developed banana breeding strategy that utilizes diploids in breeding cycle where by landraces of AAA genome are crossed with wild diploids AA genome to produce tetraploids of AAAA genome. Then the tetraploids are crossed with improved diploids derived from inter diploids crosses to produce secondary triploids with desired characters. The genetics of inheritance of these inter diploid crosses have not been studied and hence need to carry out a study on inheritance of weevil resistance traits in an $F_2$ diploid population. At $F_2$, we have maximum segregation (Mendel, 1866; Mary Sarah, 2002) and therefore it is appropriate to investigate an $F_2$ population for inheritance of traits.

### 2.3 Markers for banana weevil resistance

#### 2.3.1 Molecular markers in banana breeding

Molecular markers assist selection of traits that are expressed late in the plant cycle. Research at IITA identified PCR-based molecular markers (Amplified fragment length polymorphism (AFLP) and Randomly amplified polymorphic DNA (RAPD) that discriminate between the A and B genomes and, together with flow cytometry for ploidy analysis, facilitate early selection of hybrids with putative banana or plantain labels (Tenkouano and Swennen, 2004).

Random amplified polymorphic DNA (RAPD) analysis has been particularly popular as it requires no prior knowledge of the genome (Crouch *et al*., 1998). However, AFLPs demonstrated to have multiplex ratios in a number of systems such as potato (Van Eck *et al*., 1995), rice (Cho *et al*.,

1996) and soybean (Keim *et al*., 1997). AFLP analysis also has the advantage of not requiring prior knowledge of the genome. Application of molecular markers in *Musa* have primarily concentrated on the analysis of diverse germplasm (reviewed in (Crouch *et al*., 1998). RFLP analysis may not detect sufficient polymorphism between closely related genotypes (Jarret *et al*., 1993) and it is not readily amenable to the high throughput demands of molecular breeding applications.

RAPD fragments specific for the A and B genomes of *Musa* have been identified, isolated and cloned. The RAPD markers are useful in preliminary genome classification, but they are not subspecies specific markers (Tenkouano and Swennen, 2004). According to Pushpakumari *et al*. (2009), *Musa* accessions showed considerable morphological variation but the same accession showed less genetic variation when analysed with SSR markers. Amplified fragment length polymorphisms (AFLPs) provide important information regarding genetic relationships among taxa of sections of *Musa* (Carol *et al*., 2002). Carol *et al*. (2002) further reported that the level of polymorphism in *Musa* and the number of loci generated per primer pair using AFLP compare favorably with other techniques. However, AFLPs generate huge quantities of information, which may need automated analysis and therefore computer technology, they are dominance and are technically demanding in the laboratory and, especially, in data analysis (Julian and Stephen, 1999)

The co-dominant nature of microsatellite markers and the ease with which microsatellite markers can be multiplexed (Mitchell *et al*., 1997) maintain a substantial comparative advantage for the application of this technique in marker assisted selection programs. In such applications a large number of genotypes need to be screened with a relatively small number of markers, preferably within the breeding station. Marker-assisted backcross breeding, to eliminate individuals with a large proportion of the exotic parent's genome, presents a similar logistical scenario (Crouch *et al*., 1999).

Simple sequence repeats (SSR) are ideal genetic markers for detecting differences between and within species of genes of all eukaryotes (Farooq and Azam, 2002). Some SSR makers have been validated for genotyping *Musa* accessions for resistance to *Fusarium wilt*, massr 18a (F) ,massr 18b (R) ,massr 20a (F) ,massr 20b (R) ,massr 24a (F) ,massr 24b (R) (Pushpakumari *et al*., 2009). Venkatachalam *et al*. (2008) showed in their genetic study of Indian bananas that there is a strong

linear relationship between discriminating power of microsatellite primers and their ability to distinguish genotypes. Changadeya *et al*. (2012) using SSR primers were able to cluster some genotypes of the same genome with similar morphology, fruit quality traits and that they were possibly synonymous with cultivars. SSR markers have been used successfully in banana genotyping and several maps have been developed which will help to identify markers for use in breeding programmes. Hippolyte *et al*. (2010) developed a map for SSR basing on structural heterozygosity of related diploids. Mbanjo (2012) developed an SSR map for populations segregating for nematode resistance (*Radopholus similis*). SSRs from these maps have been used in different studies like diversity in bananas basing on agro-morphological classification as well as the ploidy (Hippolyte *et al*., 2011).

Among all these markers, microsatellites have an advantage over other molecular markers because of their co-dominance and being powerful in their resolving power and therefore are becoming more popularly used (Buhariwalla *et al*., 2005; Creste *et al*., 2004). Some of the prominent features of these markers are that they are dominant fingerprinting markers and co-dominant sequence tagged microsatellites (STMS) markers (Joshi *et al*., 2011). Therefore SSR markers for weevil resistance will be a tool that is very useful for accelerating rapid assay breeding that allows the differentiation between resistant and susceptible genotypes in a reasonably short period of time.

### 2.3.2 Association of banana weevil and banana nematodes

Entomopathogenic nematodes (EPNs) effectively control a variety of economically important weevils including black vine weevil, citrus root weevil and sweet potato weevil (Georgis *et al*., 2006; Haukeland and Lola-Luz, 2010. EPNs have been explored in controlling banana weevil mainly in Australia (Treverrow and Bedding, 1993; Smith, 1995) and in Spain (Padilla-Cubas *et al*., 2010). On the Canary Islands (Spain), a study on indigenous EPNs against neonate banana weevil larvae indicated excellent control in laboratory sand bioassays (Padilla-Cubas *et al*., 2010). All isolates of the endemic EPNs are capable of infecting *Cosmopolites sordidus* larvae in laboratory experiments, and causing mortality of larvae with nematode isolates and dosage (Mwaitulo *et al*., 2011). Several of the *Heterorhabditis spp*. and *Steinernema spp*. isolates from Mkenge and Mwalusembe villages have potential for biological control of *Cosmoploites sordidus* in Tanzania (Mwaitulo *et al*., 2011).

However, most studies have shown that the adults are not particularly susceptible. Treverrow and Bedding (1993) suggested that the resistance is almost certainly due to the difficulty of nematodes entering the host rather than establishment once infection is successful. It has been widely hypothesized that high levels of banana weevil are associated with low soil fertility and nematode infestations (Speijer *et al*., 1993 ). This could explain why weevil infested suckers are more likely to be attacked by nematodes

A strong interaction between nematode infestation and the banana weevil has been observed in Kenya (Speijer *et al.* 1993, Fogain 1996), and interactions with other biotic and abiotic constraints are expected (Frison *et al*., 1997. Speijer and Fogain, (1999) suggested that an understanding of the various interactions will increase the impact of integrated pest management programmes on such pest and disease complexes. Also more knowledge is required on the interactions between the different nematode pest species and other biotic and abiotic production constraints

Nematodes feed and multiply in banana roots and corm (INIBAP, 1997). Weevils as well feed and live in the banana corm. Speijer *et al*. (1993) reported that there is a strong association between nematode and banana weevil infestation with suckers attacked by nematodes are four times more likely to be attacked by weevil. Phonological compounds, High performance liquid chromatography (HPLC) products, toxins and lignin responsible for tissue hardness are reported to be cause for resistance to nematodes (Gowen and Muller-Harvey, 2000; Fogain, 1996). The same mechanisms are reported for weevil resistance (Kiggundu, 2000; Kiggundu *et al*., 2003; Ocan *et al*., 2008; Gertrude *et al*., 2010).

Dungu (1987) suggested the need to have a super variety that is resistant to both weevils and nematodes. Mbanjo (2012) developed SSR markers for resistance to nematodes using the same resistant parent used for weevil screening. This therefore suggests a need to find out if SSR markers for nematodes resistance have any association with weevil resistance traits in *Musa spp*. Having markers that select for both nematode and banana weevil resistance is important in selecting cultivars that have resistance to both weevils and nematodes at once which saves time and resources.

# CHAPTER THREE
# PHENOTYPIC CHARACTERIZATION AND GENETIC ANALYSIS OF AN $F_2$ DIPLOID BANANA POPULATION FOR WEEVIL RESISTANCE AND OTHER TRAITS

## 3.1 Introduction

The banana weevil (*cosmopolites sordidus* Germar) is one of the most important pests of bananas. It is of great importance in Eastern Africa and Uganda in particular where the crop is a staple for many millions of people who depend on it for food and income (Gold *et al*., 1993; 1999). Attempts to control the banana weevil have been made using sanitation of the fields, cultural methods, chemical and biological methods. However, these methods seem not to be effective (Gold and Messiaen, 2000).

Host plant resistance has been suggested as a better option (Kiggundu, 2000) and some of the host plant resistance mechanisms reported include antibiosis, antixenoxis and corm hardness (Kiggundu, 2000; Gertrude, 2010). These mechanisms affect the banana weevil directly by deterring the weevil, and or making it hard for the weevil to feed or indirectly by retarding the growth of the weevil larvae (Getrude, 2010; Ortiz 1995). Other banana weevil resistance mechanisms reported include: increased larval mortality (Abera , *et al*., 2000), increased larval development time (Mesquita *et al*., 1984; Kiggundu, 2000; Getrude, 2010), and damage parameters such as cross sectional inner and outer damages (Kiggundu, 2000). The studies carried out by Kiggundu (2000) reported that banana diploids have more resistance to banana weevil compared to triploid East African highland bananas (EAHBS) suggesting that they could be exploited as source of resistance to improve the EAHBs.

The genetic variability in diploids seems to be adequate for immediate breeding purposes. There are morphological variations in height, in the vigor of suckers, in the number of hands per bunch, in the size of the fingers and sources of resistance to the main pests, diseases and nematodes (Sebastião *et al*., 2001). However, it is necessary to understand the inheritance of the resistance mechanism before breeding for host plant resistance. Understanding the nature of inheritance, heritability and gene ratio in plant breeding helps to predict which crosses to make, when to cross,

at what stage to select and which traits are easy or hard to select and the same would be of benefit to breeding for weevil resistance in bananas.

An $F_2$ population offers maximum segregation in banana where resistance mechanisms can be easily studied (Brown and Caligari, 2008; Muhammad *et al.*, 2014). However, fewer studies have been carried out to screen $F_2$ banana diploid progenies to determine their levels of resistance to banana weevil. There is little information on how the mechanisms of resistance are inherited and the number of genes involved.

The purpose of this study therefore was to:

a) Identify segregating weevil resistance and agronomic traits in an $F_2$ (*Musa acuminata* (kasaska) X *Musa acuminata* Subsp microcarpa) diploid population

b) Determine the inheritance of weevil resistance and agronomic traits in an $F_2$ (*Musa acuminata* (kasaska) X *Musa acuminata* Subsp microcarpa) diploid population.

### 3.2 Materials and methods

#### 3.2.1 Generation of segregating population

Two diploid parents *Musa acuminata* (kasaska) and *Musa acuminata* Subsp microcarpa were crossed to generate an $F_1$ population. Kasaska susceptible to weevil damage was used as the female parent whereas Microcapa resistant to weevil damage was used as the male. The $F_1$ generation that was obtained was screened for weevil resistance and was found to be moderately resistant. From the $F_1$ generation, one line was randomly selected and selfed to generate an $F_2$ segregating populations for this study.

#### 3.2.2 Field experiment

Two hundred fifty seven plants (242 $F_2$ plants, 5 Parent 1 plants, 5 Parent 2 plants and 5$F_1$ plants) were planted in a weevil free field at National Agricultural Research Laboratories (NARL) - Kawanda. NARL - Kawanda is located (0°25°N,32°32°E) and is 13km North of Kampala and at 1195m above sea level. Day length is 12 h throughout the year. Average daily temperatures are 15.8$^o$C minimum and 29.8$^o$C maximum with mean relative humidity of 76%.

Holes of size (45 X 45 X 45) cm were prepared at a spacing of (2 X 2) m. Half of the pits were filled with equal quantities of top soil and well decomposed farm yard manure before planting was done. The planting date was 14$^{th}$ September 2012. The individual plants were planted randomly in 25 columns and 11 rows. Management of weeds was by weeding and mulching. Mulch was applied two months after planting and again after 1 year. No herbicides were applied.

The plants were allowed to grow and produce as many suckers as possible without desuckering. Fourteen $F_2$ plants did not make it to stage of data collection and only 228 plants were assessed. The inoculation of the plants with weevil was done artificially in the laboratory.

The weevils were raised on corms of a susceptible cultivar (Mbwazirume) that were changed after every 3 days. Prior to egg collection, the weevils were fed on fresh peeled pseudostem of Mbwazirume where eggs where laid. The eggs were sterilised in 25mls of distilled water supplemented with 5mls of ethanol (20 percent Ethanol) and 2-3 drops of JIK were added on a petri dish. The eggs were then spread on a moistened Kitchen towel tissue in a petri dish using painters brush.

The eggs were stored at room temperature on the petri dishes and wetted daily to avoid desiccation. After 6-7 days the larvae heads emerged from the eggs and were reddish in colour at first instar.

On the fifth day from the time the eggs were inoculated, one sucker was detached from each of the 228 $F_2$ plants, their parents and $F_1$'s using a desuckering spear and the corms were dug out of the soil, cleaned and weighed to determine the weight of the whole corm. The suckers that were detached from each plant were estimated to have an average number of 7 leaves, average diameter at base of 29 cm and an average height of 137 cm.

A cube of about 3x3x3 cm was obtained from the inner ring of the corm and kept overnight. From the remainder of the corm, 150g were weighed from a representative sample, chopped into small pieces and used to determine moisture content in a laboratory.
The samples were placed in an oven at 80$^{o}$C until they were completely dry (feeling crunchy when felt with hands). The samples were then removed and weighed again as subsamples or whole samples. The dry mass of the whole sample, dry matter content of subsample and moisture content of the subsample were determined by equation 1, 2 and 3 respectively.

$$\text{Dry mass} = \frac{\text{Subsample dry mass}}{\text{Subsample fresh mass}} \times \text{Fresh mass of the whole sample (Timothy } \textit{et al.}\text{, 2005)}$$

(Equation 1)

$$\%\ \text{Dry matter content of subsample} = \frac{\text{Final Dry Weight (g)}}{\text{Initial Wet Weight (g)}} \times 100 \text{ (Mickan, 2005)}$$

(Equation 2)

% Total Moisture = 100 - % Total DM (Mickan, 2005)

(Equation 3)

The next day when the eggs had hatched, the larvae were inoculated into the corm section. The corm section was bored with four holes on one side. A larva was placed in each hole, covered with corm tissue and placed in a petri dish. The petri dish was covered well sealed with cling film

and labeled. The petri dishes were then placed in plastic boxes and kept at room temperature for eight days after which the larvae were removed for measurements. This procedure was repeated 3 times every after 30 days and these were taken as replications over time. After 8 days, the larvae were retrieved from the corm section and head capsule width, body length, body weight and larval mortality were recorded.

At harvest, a corm was taken to laboratory and corm hardness determined using digital gauge penetrometer (General FHT803 Fruit Hardness Tester for Large, Hard fruits) from Tequipment NET (USA), as described by (Ortiz *et al*., 1995; Kiggundu, 2000). The machine measured corm hardness in Newtons (N = 105 dynes). Four transversal and four longitudinal random measurements were taken on each corm fragment by punching the machine in corm using tip size of 7.9 mm. However, this tip had a blunt end and could not penetrate the corm so another tip was fabricated that had a sharper end and a mark was made at 12 mm from where the hardness would be read when reached.

### 3.2.3 Pot experiment

The 228 $F_2$ plants, their parents and $F_1$'s were allowed to grow for 7 months after which two hundred plants were selected (those that had 3 or more suckers) for initiation. A sucker from each was detached and initiated on proliferation medium, multiplied to obtain 4-8 copies, and then they were rooted on rooting medium in tissue culture laboratory. Out of the 200 genotypes, 150 genotypes made it successfully through tissue culture with 4 or more clones and were weaned in the nursery. The plantlets were left in the nursery under humid chamber for 3 weeks after which they were left to harden under shade in the nursery for 4 weeks and these were transferred to big pots (black polythene bags of 40 X 50 inches) for pot experiment. The potting mix for the pot experiment was prepared by mixing black soil and farm yard manure in ratio of 4:1, the potting mix was sterilised and left to cool before use.

The pot experiment was laid out in a Randomised Complete Block Design (RCBD) with 2 replications each replicate consisted of 2-4 lines. This means that each genotype within the two replications consisted of 4-8 plants depending on how many proliferated in the tissue culture

laboratory. The plants in the potting bags were left to grow for 3 months to attain a reasonable size before inoculation.

The pots were dressed with net bags and then 6 weevils in a ratio of 1:1 that is (3 males and 3 females) adjusted from 5 males and 5 females (Sadik *et al*., 2010) because these were tissue culture plants with small corms. The net was tightened on the pseudostem at the point where it touches the soil to avoid escape or entrance of foreign weevil and to make sure weevils do not eat the pseudostem as well. The inoculated plants were left to grow for 60 and 90 days for rep 1 and 2 respectively. After these days, the corms were uprooted and damage parameters scored for each line in a replication.

### 3.2.4 Data collection

#### 3.2.4.1 Data from the field experiment

The data collected on this experiment included head capsule width (HCW) and body length under a precision microscope calibrated with a meter scale at magnifications of x60 and x40 respectively, body weight electronic weighing scale of milligrams (mg) and corm hardness using a penetrometer.

Data on agronomic traits was collected at flowering and the following parameters were considered; flowering date, plant girth at 1m from ground, height of the plant and number of leaves at flowering. At harvest, data was collected on; harvest date, leaves at harvest, bunch weight (kg), number of clusters, fingers per cluster, and corm hardness.

#### 3.2.4.2 Data from pot experiment

The data collected from pot experiment included total damage, peripheral damage, total cross sectional damage, total cross sectional inner and outer damage, number of larvae retrieved and number of dead weevils. Data on agronomic traits was collected at termination of the experiment and this included total number of leaves, number of dead leaves and functional leaves, plant height and girth of plant at the base.

### 3.2.5 Data analysis

The field and pot experiment data were analysed by GenStat 14th edition using a linear model $Y_{ij} = Y... + T_i + R_j + e_{ij}$. Where $Y_{ij}$= $j^{th}$ observation of the $i^{th}$ treatment, Y... = Grand mean of the observations made on a certain trait, $T_i$= Treatment factor $R_j$ Replication and $e_{ij}$= Residual error. Histograms were constructed in Microsoft excel using pivot table.

#### 3.2.5.1 Determining broad sense heritability (H)

Broad sense heritability was determined using the variance component formula:

$$\text{Broad sense heritability } H = \frac{\delta G}{\delta p} = \frac{\delta G}{\delta e + (\delta G)}$$

Where δe is Residual error mean square

δe + RδG is Genotype expected mean square

$$\delta G = \frac{\text{MS Gen-MS error}}{R} \quad \text{is Genetic variance}$$

Where R is Replication

Narrow sense heritability was not determined since an $F_2$ population (only sufficient for a clonal crop) was used.

#### 3.2.5.2 Determining Heterosis

Mid-parent heterosis (MPH) and better-parent heterosis (BPH) of the $F_1$ and $F_2$ progeny were determined for total damage, peripheral damage, cross sectional outer damage, cross sectional inner damage, total cross sectional damage, HCW, body length, body weight, larval mortality, dead weevils, larvae retrieved according to Falconer and Mackay (1996) as:

Mid-parent heterosis = $F_1$- MP

---------- X 100

MP

$F_2$ mid-parent heterosis = $F_2$- MP

---------- X 100

MP

Better parent heterosis = $F_1$- BP

---------- X 100

MP

$F_2$ better parent heterosis = $F_2$- BP

---------- X 100

MP

Where $F_1$ is the mean of $F_1$'s

$F_2$ is the mean of $F_2$'s

MP (mid parent) mean of two parents

BP (better parent) mean of the better parent

### 3.3 Results

#### 3.3.1 Field response in an $F_2$ banana diploid population for banana weevil and agronomic traits

There were no significant difference (P<0.05) for agronomic traits such as girth at 1 meter, height of plant at flowering and outer corm hardness leaves at flowering, leaves at harvest (Table 1). There were significant differences (P<0.05) for agronomic parameters such as bunch weight, number of clusters, number of fingers, days to maturity, inner corm hardness and total corm hardness (Table 1).

**Table 1. Mean squares of agronomic traits in an $F_2$ banana diploid population**

| Source of variance | BW (df=187) | NC (df=187) | DM (df=187) | NF (df=187) | GM (df=191) | HP (df=191) | ICM (df=153) | OCH (df=153) | TCH (df=153) | LF (df=191) | LH (df=191) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Replication | 174.111 | 92.647 | 94022.3 | 614.365 | 5528.86 | 205433 | 76.9 | 523.2 | 250.3 | 18.988 | 15.088 |
| Genotype | 1.92* | 3.394* | 2334.7** | 9.543** | $17.73^{ns}$ | $692.8^{ns}$ | 3.4** | $9.5^{ns}$ | 4.1** | $2.726^{ns}$ | $3.529^{ns}$ |
| Error | 1.164 | 1.88 | 882 | 4.983 | 18.3 | 690.3 | 1.2 | 8.9 | 2.6 | 2.722 | 2.828 |
| Total | 2.531 | 3.511 | 2468.1 | 11.278 | 38.89 | 1476.7 | 2.5 | 10.5 | 4.0 | 2.787 | 3.454 |
| %CV | 40.85 | 17.21 | 23.76 | 12.34 | 12.62 | 10.47 | 18.3 | 38.4 | 23.5 | 16.84 | 51.59 |

* Significant at P<0.05, ** Significant at P<0.001, ns = non-significant

BW = Bunch weight, NC = Number of clusters, DM= Days to maturity, NF= Number of fingers, GM= Girth at 1 meter, HP= Height of plant, ICH= Inner corm hardness, OCH= outer corm hardness, TCH= Total corm hardness, LH= Leaves At harvest, LF= Leaves at flowering

### 3.3.2 Response of $F_2$ banana diploid genotypes to weevil growth parameters in the laboratory

There were significant differences ($P<0.05$) among the different genotypes for head capsule width (HCW), body length, body weight and larval mortality (Table 2). Dry matter content and dry mass were not significant among the genotypes.

**Table 2. Mean squares of weevil growth parameters in an $F_2$ banana diploid population**

| Source of variance | df | HCW | Body length | Body weight | Larval mortality | Dry matter | Dry mass |
|---|---|---|---|---|---|---|---|
| Replication | 2 | 1693.6 | 58409.8 | 200.3 | 20991 | 3368.9 | 1375891 |
| Genotype | 227 | 609.7** | 564.8** | 65.2** | 1056.5* | $84.0^{ns}$ | $64738.0^{ns}$ |
| Error | 378-408 | 360.7 | 400.4 | 26.1 | 840.4 | 98.1 | 65361 |
| Total | 607-638 | 457.9 | 653 | 41.3 | 980.8 | 103.8 | 69585 |
| %CV | | 34.9 | 33.9 | 57 | 50.2 | 42.9 | 36.7 |

* Significant at $P<0.05$, ** Significant at $P<0.001$, ns = non-significant

### 3.3.3 Response of $F_2$ diploid banana genotypes to weevil damage parameters under screen house screening

There were significant differences ($P<0.05$) among the different genotypes for total damage, peripheral damage, dead weevils and larvae retrieved (Table 3). These parameters show that the screened population is segregating for weevil resistance using weevil damage parameters. Weevil damage parameters such as total cross sectional inner and outer damages and total cross sectional damage were non-significant in this population but these parameters add up to total damage which is significant.

**Table 3. Mean squares of weevil damage parameters in an $F_2$ banana diploid population**

| Source of variance | df | Total damage | Peripheral damage | Total cross sectional damage | Total cross sectional inner damage | Total cross sectional outer damage | Dead weevils | Larvae retrieved |
|---|---|---|---|---|---|---|---|---|
| Replication | 1 | 2060.7 | 7120 | 36.4 | 48 | 26.5 | 87.7 | 0.3 |
| Genotype | 118 | 141.9** | 421.2** | 48.5$^{ns}$ | 46.7$^{ns}$ | 55.6$^{ns}$ | 2.4** | 0.4* |
| Error | 606 | 87.1 | 216 | 50.8 | 48.8 | 59.3 | 1.6 | 0.3 |
| Total | 725 | 98.7 | 259 | 50.4 | 48.4 | 58.6 | 1.9 | 0.3 |
| %CV | | 40 | 47 | 29.1 | 34.9 | 24.9 | 10.5 | 19.4 |

* Significant at P<0.05, ** Significant at P<0.001, ns = non-significant

### 3.3.4 Nature of inheritance for weevil resistance traits among an $F_2$ diploid banana population

Histograms for head capsule width (Figure 1), number of leaves at flowering, girth of the plants at 1M plant at flowering and height of a plant at flowering (Figure 3), and number of fingers on a bunch at harvest (Figure 4) showed normal distribution. Histograms for body weight (Figure 1), total damage, peripheral damage, cross sectional inner and outer damage and larvae retrieved (Figure 2), bunch weight and number leaves at harvest (Figure 4) were skewed to left which was towards a resistant parent. Histograms for body length (Figure 1), dead weevils (Figure 2) (Figure 3) and days to maturity (Figure 4) were skewed towards the right which was towards the susceptible parent whereas histogram for larval mortality (Figure 1) showed two distinct classes (binomial).

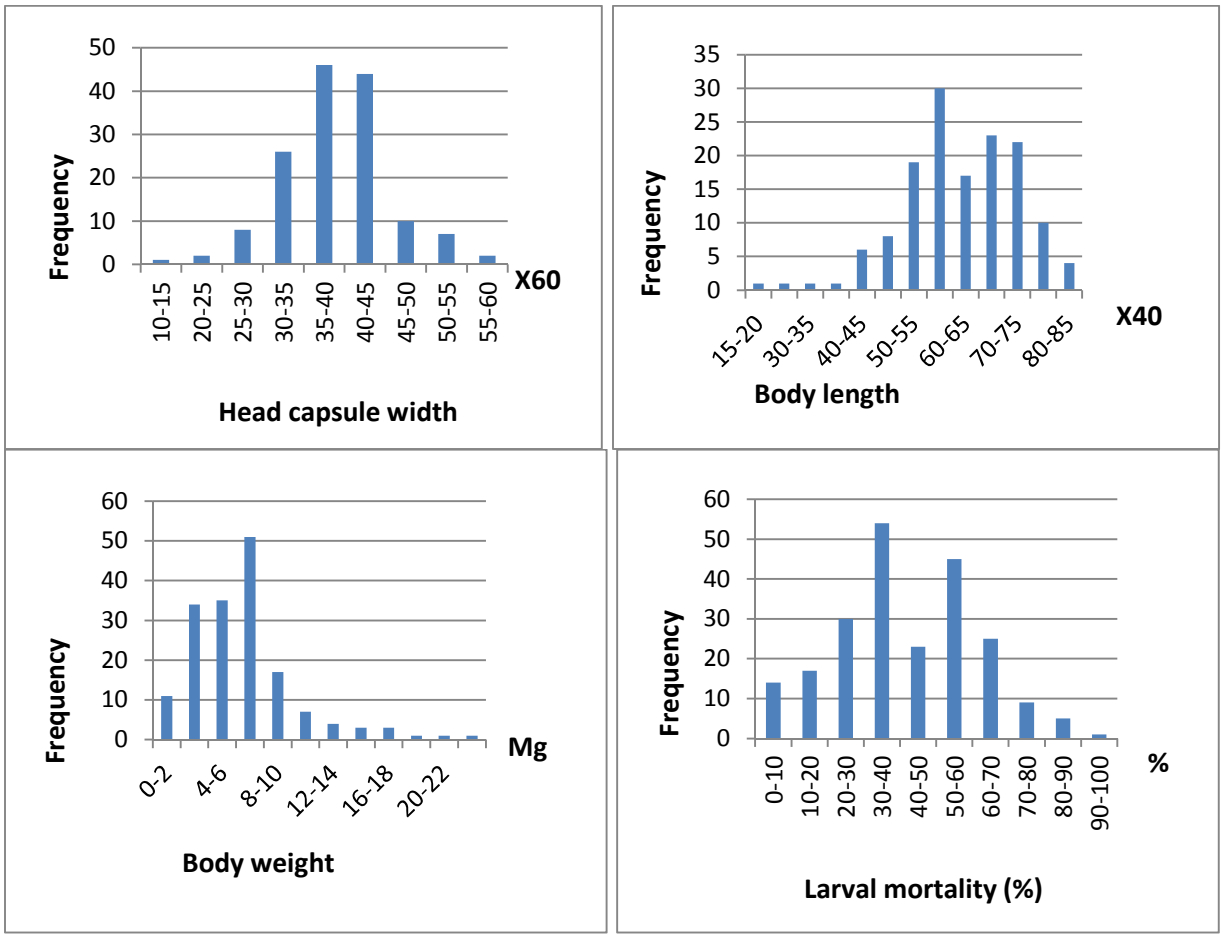



**Figure 1. Frequency of larval traits expressed among an $F_2$ banana diploid population**

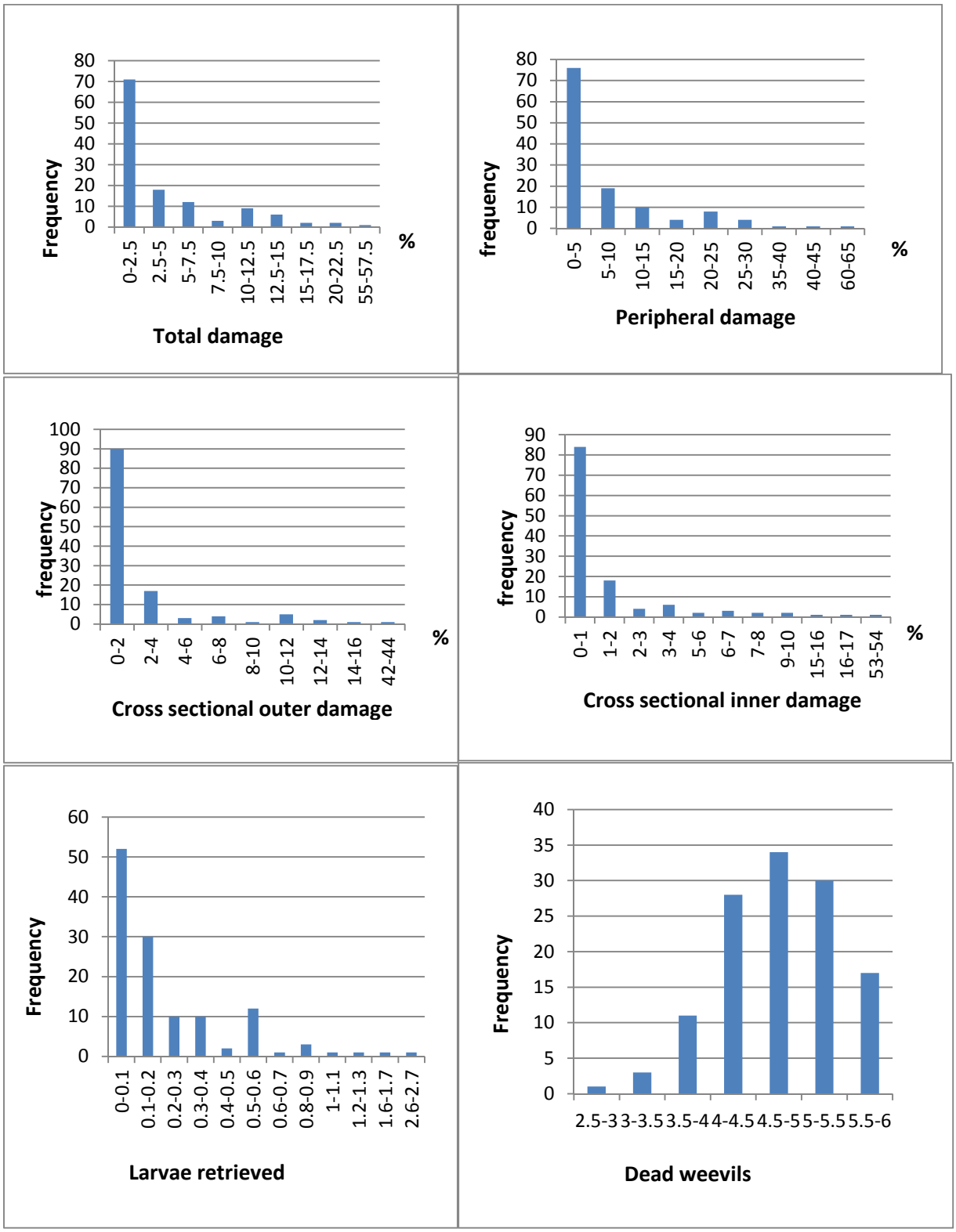



**Figure 2. Frequency of weevil damage parameters among an $F_2$ banana diploid population**

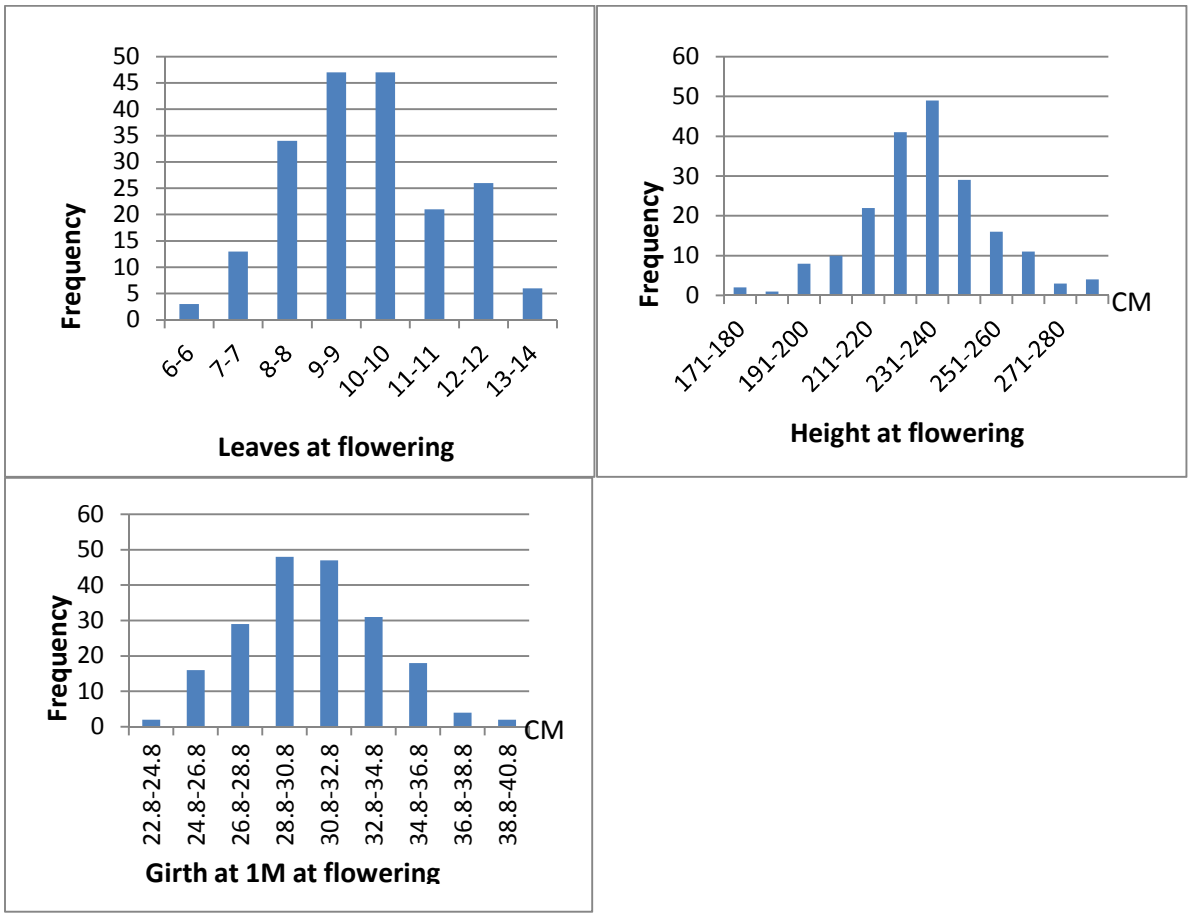



**Figure 3. Frequency of agronomy parameters expressed among an $F_2$ banana diploid population**

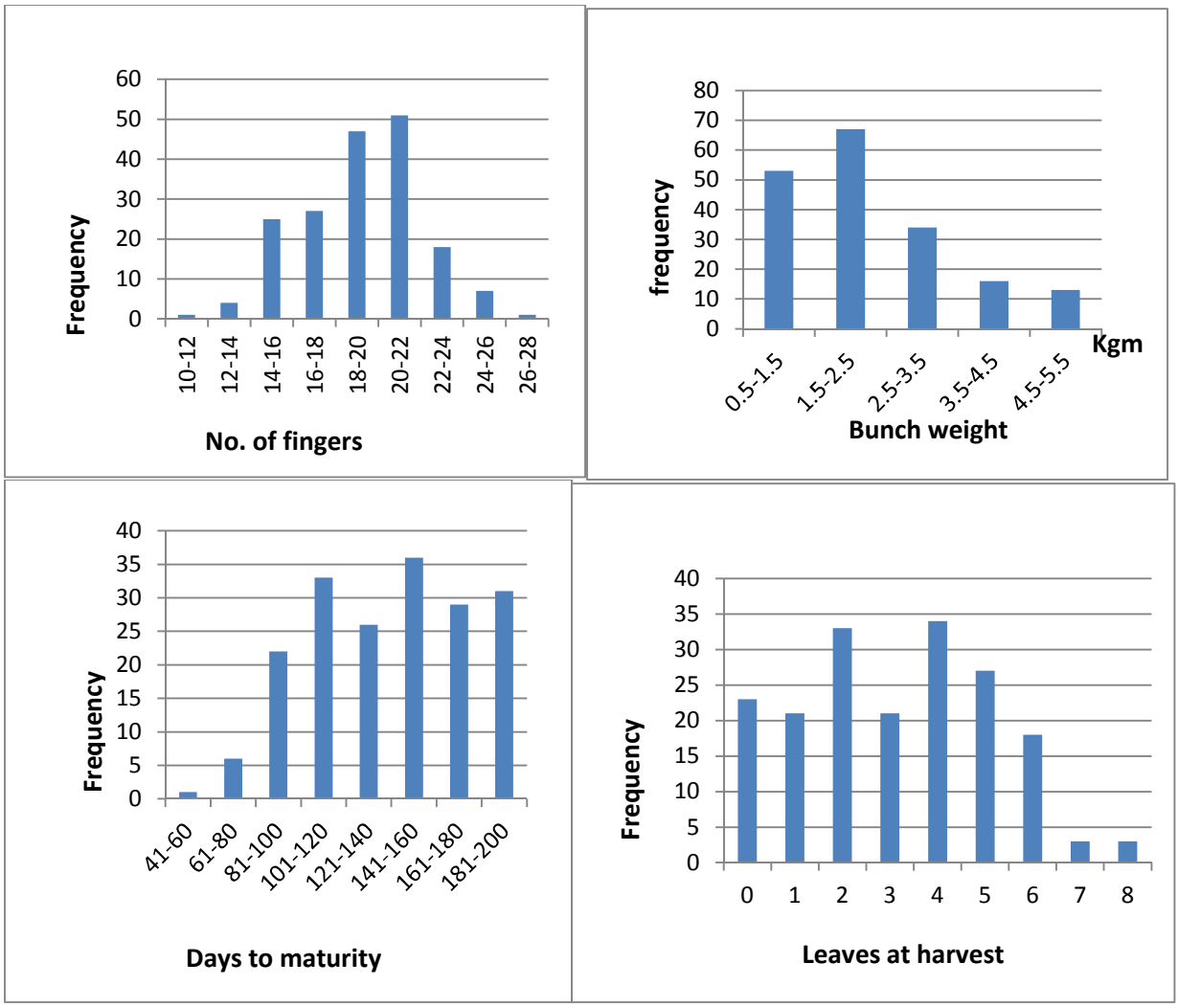



**Figure 4. Frequency of harvest parameters expressed among an $F_2$ banana diploid population**

### 3.3.5 Genetic ratios among an $F_2$ diploid banana population

Chi square for body length showed a ratio of 15:1 when performed using a resistant parent as a check (Table 4a) whereas body weight and larval mortality showed ratios of 15:1 when using a susceptible parent as a check (Table 4b and 4c) and dead weevils showed a ratio of 1:15 when performed using a susceptible parent as a check (Table 4g). Chi square for total damage and larvae retrieved showed ratios of 3:1 when performed using resistant parent as a check (Table 4d and 4f) whereas larval mortality and dead weevils showed ratios of 1:3 when using a resistant parent as check (Table 4c and 4g). Chi square for peripheral damage showed a ratio of 9:7 when using a resistant parent as a check (Table 4e) and body weight showed a ratio of 7:9 when using a resistant parent as a check (Table 4b).

**Table 4a. Genetic ratios and number of genes involved in body length in an $F_2$ banana diploid population**

| Trait | | Type of check used | Observed no. of genotypes | Total | Ratio tested | Expected Forward (e.g. 1:3) | Expected reverse (e.g. 3:1) | Chi square Calculated | Chi Dist (probability) | Significant level |
|---|---|---|---|---|---|---|---|---|---|---|
| **Body length** | Resistant parent | Resistant | 135 | 146 | 1:03 | 36.5 | 109.5 | 354.4 | 4.60E-79 | *** |
| | | Susceptible | 11 | | 3:01 | 109.5 | 36.5 | 23.8 | 1.10E-06 | *** |
| | | | | | 9:07 | 82.125 | 63.875 | 77.8 | 1.10E-18 | *** |
| | | | | | 7:09 | 63.875 | 82.125 | 140.8 | 1.80E-32 | *** |
| | | | | | 15:01 | 136.875 | 9.125 | 0.4 | 0.6 | ns |
| | | | | | 1:15 | 9.125 | 136.875 | 1852.1 | 0 | |
| | Susceptible parent | Resistant | 145 | 146 | 1:03 | 36.5 | 109.5 | 430 | 1.60E-95 | *** |
| | | Susceptible | 1 | | 3:01 | 109.5 | 36.5 | 46 | 1.20E-11 | *** |
| | | | | | 9:07 | 82.125 | 63.875 | 110 | 9.70E-26 | *** |
| | | | | | 7:09 | 63.875 | 82.125 | 183.2 | 9.90E-42 | *** |
| | | | | | 15:01 | 136.875 | 9.125 | 7.7 | 0.01 | * |
| | | | | | 1:15 | 9.125 | 136.875 | 2158.1 | 0 | |

* Significant at P<0.05, ** Significant at P<0.001, *** Significant at P<0.0001, ns = non-significant

**Table 4b. Genetic ratios and number of genes involved in body weight in an $F_2$ banana diploid population**

| Trait | | Type of check used | Observed no. of genotypes | Total | Ratio tested | Expected Forward (e.g. 1:3) | Expected reverse (e.g. 3:1) | Chi square Calculated | Chi Dist (probability) | Significant level |
|---|---|---|---|---|---|---|---|---|---|---|
| **Body weight** | Resistant parent | Resistant | 76 | 171 | 1:03 | 42.75 | 128.25 | 34.5 | 4.30E-09 | *** |
| | | Susceptible | 95 | | 3:01 | 128.25 | 42.75 | 85.1 | 2.80E-20 | *** |
| | | | | | 9:07 | 96.1875 | 74.8125 | 9.7 | 1.00E-04 | *** |
| | | | | | 7:09 | 74.8125 | 96.1875 | 0 | 0.8 | ns |
| | | | | | 15:01 | 160.313 | 10.6875 | 709.5 | 2.60E-156 | *** |
| | | | | | 1:15 | 10.6875 | 160.313 | 425.7 | 1.40E-94 | *** |
| | Susceptible parent | Resistant | 158 | 171 | 1:03 | 42.75 | 128.25 | 414.3 | 4.30E-92 | *** |
| | | Susceptible | 13 | | 3:01 | 128.25 | 42.75 | 27.6 | 1.40E-07 | *** |
| | | | | | 9:07 | 96.1875 | 74.8125 | 90.8 | 1.60E-21 | *** |
| | | | | | 7:09 | 74.8125 | 96.1875 | 164.4 | 1.20E-37 | *** |
| | | | | | 15:01 | 160.313 | 10.6875 | 0.5 | 0.5 | ns |
| | | | | | 1:15 | 10.6875 | 160.313 | 2165.9 | 0 | |

* Significant at P<0.05, ** Significant at P<0.001, *** Significant at P<0.0001, ns = non-significant

**Table 4c. Genetic ratios and number of genes involved in larval mortality in an $F_2$ banana diploid population**

| Trait | | Type of check used | Observed no. of genotypes | Total | Ratio tested | Expected Forward (e.g. 1:3) | Expected reverse (e.g. 3:1) | Chi square Calculated | Chi Dist (probability) | Significant level |
|---|---|---|---|---|---|---|---|---|---|---|
| **Larval mortality** | Resistant parent | Resistant | 62 | 226 | 1:03 | 56.5 | 169.5 | 0.7 | 0.4 | ns |
| | | Susceptible | 164 | | 3:01 | 169.5 | 56.5 | 272.7 | 2.90E-61 | *** |
| | | | | | 9:07 | 127.125 | 98.875 | 76.3 | 2.50E-18 | *** |
| | | | | | 7:09 | 98.875 | 127.125 | 24.4 | 7.60E-07 | *** |
| | | | | | 15:01 | 211.875 | 14.125 | 1696.3 | 0 | |
| | | | | | 1:15 | 14.125 | 211.875 | 173.1 | 1.60E-39 | *** |
| | Susceptible parent | Resistant | 211 | 226 | 1:03 | 56.5 | 169.5 | 563.3 | 1.60E-124 | *** |
| | | Susceptible | 15 | | 3:01 | 169.5 | 56.5 | 40.6 | 1.80E-10 | *** |
| | | | | | 9:07 | 127.125 | 98.875 | 126.5 | 2.40E-29 | *** |
| | | | | | 7:09 | 98.875 | 127.125 | 226 | 4.30E-51 | *** |
| | | | | | 15:01 | 211.875 | 14.125 | 0.1 | 0.8 | ns |
| | | | | | 1:15 | 14.125 | 211.875 | 2927 | 0 | |

* Significant at P<0.05, ** Significant at P<0.001, *** Significant at P<0.0001, ns = non-significant

**Table 4d. Genetic ratios and number of genes involved in total damage in an $F_2$ banana diploid population**

| Trait | | Type of check used | Observed no. of genotypes | Total | Ratio tested | Expected Forward (e.g. 1:3) | Expected reverse (e.g. 3:1) | Chi square Calculated | Chi Dist (probability) | Significant level |
|---|---|---|---|---|---|---|---|---|---|---|
| **Total damage** | Resistant parent | Resistant | 95 | 124 | 1:03 | 31 | 93 | 176.2 | 3.30E-40 | *** |
| | | Susceptible | 29 | | 3:01 | 93 | 31 | 0.2 | 0.7 | ns |
| | | | | | 9:07 | 69.75 | 54.25 | 20.9 | 4.90E-06 | *** |
| | | | | | 7:09 | 54.25 | 69.75 | 54.4 | 1.60E-13 | *** |
| | | | | | 15:01 | 116.25 | 7.75 | 62.2 | 3.20E-15 | *** |
| | | | | | 1:15 | 7.75 | 116.25 | 1047.8 | 7.50E-230 | *** |
| | Susceptible parent | Resistant | 123 | 124 | 1:03 | 31 | 93 | 364 | 3.70E-81 | *** |
| | | Susceptible | 1 | | 3:01 | 93 | 31 | 38.7 | 4.90E-10 | *** |
| | | | | | 9:07 | 69.75 | 54.25 | 92.9 | 5.40E-22 | *** |
| | | | | | 7:09 | 54.25 | 69.75 | 154.9 | 1.50E-35 | *** |
| | | | | | 15:01 | 116.25 | 7.75 | 6.3 | 0.01 | * |
| | | | | | 1:15 | 7.75 | 116.25 | 1828.1 | 0 | |

* Significant at P<0.05, ** Significant at P<0.001, *** Significant at P<0.0001, ns = non-significant

**Table 4e. Genetic ratios and number of genes involved in peripheral damage in an $F_2$ banana diploid population**

| Trait | | Type of check used | Observed no. of genotypes | Total | Ratio tested | Expected Forward (e.g. 1:3) | Expected reverse (e.g. 3:1) | Chi square Calculated | Chi Dist (probability) | Significant level |
|---|---|---|---|---|---|---|---|---|---|---|
| **Peripheral damage** | Resistant parent | Resistant | 81 | 124 | 1:03 | 31 | 93 | 107.5 | 3.40E-25 | *** |
| | | Susceptible | 43 | | 3:01 | 93 | 31 | 6.2 | 0.01 | * |
| | | | | | 9:07 | 69.75 | 54.25 | 4.1 | 0.06 | ns |
| | | | | | 7:09 | 54.25 | 69.75 | 23.4 | 1.30E-06 | *** |
| | | | | | 15:01 | 116.25 | 7.75 | 171 | 4.40E-39 | *** |
| | | | | | 1:15 | 7.75 | 116.25 | 738.5 | 1.20E-162 | *** |
| | Susceptible parent | Resistant | 123 | 124 | 1:03 | 31 | 93 | 364 | 3.70E-81 | *** |
| | | Susceptible | 1 | | 3:01 | 93 | 31 | 38.7 | 4.90E-10 | *** |
| | | | | | 9:07 | 69.75 | 54.25 | 92.9 | 5.40E-22 | *** |
| | | | | | 7:09 | 54.25 | 69.75 | 154.9 | 1.50E-35 | *** |
| | | | | | 15:01 | 116.25 | 7.75 | 6.3 | 1.00E-02 | * |
| | | | | | 1:15 | 7.75 | 116.25 | 1828.1 | 0 | |

* Significant at P<0.05, ** Significant at P<0.001, *** Significant at P<0.0001, ns = non-significant

**Table 4f. Genetic ratios and number of genes involved in Larvae retrieved in an $F_2$ banana diploid population**

| Trait | | Type of check used | Observed no. of genotypes | Total | Ratio tested | Expected Forward (e.g. 1:3) | Expected reverse (e.g. 3:1) | Chi square Calculated | Chi Dist (probability) | Significant level |
|---|---|---|---|---|---|---|---|---|---|---|
| **Larvae retrieved** | Resistant parent | Resistant | 102 | 124 | 1:03 | 31 | 93 | 216.8 | 4.50E-49 | *** |
| | | Susceptible | 22 | | 3:01 | 93 | 31 | 3.5 | 0.06 | ns |
| | | | | | 9:07 | 69.75 | 54.25 | 34.1 | 5.30E-09 | *** |
| | | | | | 7:09 | 54.25 | 69.75 | 74.7 | 5.40E-18 | *** |
| | | | | | 15:01 | 116.25 | 7.75 | 27.9 | 1.20E-07 | *** |
| | | | | | 1:15 | 7.75 | 116.25 | 1222.6 | 7.40E-268 | *** |
| | Susceptible parent | Resistant | 123 | 124 | 1:03 | 31 | 93 | 364 | 3.70E-81 | *** |
| | | Susceptible | 1 | | 3:01 | 93 | 31 | 38.7 | 4.90E-10 | *** |
| | | | | | 9:07 | 69.75 | 54.25 | 92.9 | 5.40E-22 | *** |
| | | | | | 7:09 | 54.25 | 69.75 | 154.9 | 1.50E-35 | *** |
| | | | | | 15:01 | 116.25 | 7.75 | 6.3 | 1.00E-02 | * |
| | | | | | 1:15 | 7.75 | 116.25 | 1828.1 | 0 | |

* Significant at P<0.05, ** Significant at P<0.001, *** Significant at P<0.0001, ns = non-significant

**Table 4g. Genetic ratios and number of genes involved in dead weevils in an $F_2$ banana diploid population**

| Trait | | Type of check used | Observed no. of genotypes | Total | Ratio tested | Expected Forward (e.g. 1:3) | Expected reverse (e.g. 3:1) | Chi square Calculated | Chi Dist (probability) | Significant level |
|---|---|---|---|---|---|---|---|---|---|---|
| **Dead weevils** | Resistant parent | Resistant | 31 | 124 | 1:03 | 31 | 93 | 0 | 1 | ns |
| | | Susceptible | 93 | | 3:01 | 93 | 31 | 165.3 | 7.70E-38 | *** |
| | | | | | 9:07 | 69.75 | 54.25 | 49.2 | 2.30E-12 | *** |
| | | | | | 7:09 | 54.25 | 69.75 | 17.7 | 2.60E-05 | *** |
| | | | | | 15:01 | 116.25 | 7.75 | 1000.3 | 1.60E-219 | *** |
| | | | | | 1:15 | 7.75 | 116.25 | 74.4 | 6.40E-18 | *** |
| | Susceptible parent | Resistant | 8 | 124 | 1:03 | 31 | 93 | 22.8 | 1.80E-06 | *** |
| | | Susceptible | 116 | | 3:01 | 93 | 31 | 310.8 | 1.50E-69 | *** |
| | | | | | 9:07 | 69.75 | 54.25 | 125 | 5.20E-29 | *** |
| | | | | | 7:09 | 54.25 | 69.75 | 70.1 | 5.70E-17 | *** |
| | | | | | 15:01 | 116.25 | 7.75 | 1612.8 | 0 | |
| | | | | | 1:15 | 7.75 | 116.25 | 0 | 0.9 | ns |

* Significant at P<0.05, ** Significant at P<0.001, *** Significant at P<0.0001, ns = non-significant

### 3.3.6 BS-CGD [Broad sense heritability]

The heritabilities for agronomic parameters such as inner corm hardness, days to maturity, number of fingers and clusters were relatively high, 48%, 45%, 31% and 28.7%, respectively (Table 5). Body weight had highest heritability of 33.4% compared to other larval resistance traits such as head capsule width, body length, and larval mortality with values of 12, 10 and 7.9 %, respectively (Table 6). For pot experiment, peripheral damage and total damage had heritabilities of 32% and 24%, respectively (Table 7). Other parameters assessed in pot experiment like dead weevils, larvae retrieved, total cross sectional damage and total inner and outer damages had low heritabilities of less than 21% (Table 7).

**Table 5. Heritabilities of agronomic parameters of an $F_2$ diploid banana population**

| PARAMETER | HERITABILITY (%) |
|---|---|
| Inner corm hardness | 48.0 |
| Days to maturity | 45.2 |
| Number of fingers | 31.4 |
| Number of clusters | 28.7 |
| Bunch weight | 24.5 |
| Total corm hardness | 22.5 |
| Leaves at harvest | 11.0 |
| outer corm hardness | 3.4 |
| Girth at 1 meter | 1.6 |
| Height of a plant | 0.2 |
| Leaves at flowering | 0.1 |

**Table 6. Heritabilities of larval resistance traits among an $F_2$ diploid banana population**

| PARAMETER | HERITABILITY (%) |
|---|---|
| Body weight | 33.4 |
| Body length | 12.0 |
| HCW | 10.0 |
| Larval mortality | 7.9 |

**Table 7. Heritabilities of weevil damage parameters among an $F_2$ diploid banana population**

| PARAMETER | HERITABILITY (%) |
|---|---|
| Peripheral damage | 32.0 |
| Total damage | 24.0 |
| Dead weevils | 20.0 |
| Larvae retrieved | 14.0 |
| Total cross sectional outer damage | 3.2 |
| Total cross sectional damage | 2.3 |
| Total cross sectional inner damage | 2.2 |

### 3.3.7 Heterosis

At $F_1$ larval resistance traits head capsule width (HCW) and larvae retrieved had a negative mid parent heterosis while body length, body weight and larval mortality had high positive heterosis (Table 8). Better parent heterosis (Heterobeltiosis) at $F_1$ was positive for all the weevil resistance traits, head capsule width, body length, body weight, larvae retrieved and larval mortality (Table 8).

At $F_2$, the $F_2$ mid-parent heterosis for all weevil damage parameters, total damage, peripheral damage, total cross sectional damage, cross sectional inner and outer damage, and weevil resistance parameters, head capsule width, body length, body weight and larvae retrieved were all negative and high except for larval mortality and dead weevils that had high positive values

(Table 8). Better parent heterosis (heterobeltiosis) at $F_2$ was also negative for all weevil resistance parameters and weevil damage parameters except for larval mortality and peripheral damage which were negative (Table 8).

**Table 8. Heterosis of weevil resistance traits in an $F_2$ diploid banana population**

| Type of heterosis | Total damage | Peripheral damage | Cross sectional outer damage | Cross sectional inner damage | Total cross sectional damage | HCW | Body length | Body weight | Larval mortality | Dead weevils | Larvae retrieved |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $F_1$-MPH | -52.5 | -45.2 | -44.7 | -76.8 | -61.9 | -6.8 | 9.7 | 2.8 | 42.9 | 37.1 | -93.3 |
| $F_1$-BPH | 158.0 | 215.0 | 99.5 | 80.5 | 92.9 | 10.0 | 28.2 | 50.4 | -16.7 | 27.1 | -73.3 |
| $F_2$-MPH | -87.2 | -81.5 | -92.9 | -95.5 | -94.3 | -17.7 | -32.2 | -32.9 | 38.6 | 19.9 | -87.6 |
| $F_2$-BPH | -30.5 | 6.2 | -74.5 | -65.3 | -71.3 | -2.8 | -20.7 | -1.8 | -19.2 | 11.1 | -50.4 |

### 3.3.8 Phenotypic correlations among an $F_2$ banana diploid population

The girth at base (GP) of the inoculated plant was positively correlated with height of the plant (HP), inner diameter (ID) of corm and outer diameter of corm (OD) assessed and was significant at P<0.001 (Table 9). However, girth at base was negatively correlated with peripheral damage (PD), total damage, total cross sectional damage (TXD), total cross sectional inner and outer damage (TXI, TXO) and was significant at P<0.05. Height of the plant was positively correlated with outer diameter and was significant at P<0.001 but it was negatively correlated with peripheral damage, total damage, total cross sectional damage, total cross sectional inner and outer damages and significant at P<0.001. Inner diameter was positively correlated with outer diameter of the corm and was significant at P<0.001

The number of eggs found in the corm was positively correlated with total cross sectional damage and significant at P<0.001 (Table 10). The number of larvae retrieved was positively correlated with peripheral damage, total damage, total cross sectional damage and was significant at P<0.001. All the weevil damage parameters were directly and positively correlated with each other, peripheral damage was positively correlated with total damage, total cross sectional damage, total cross sectional inner and outer damages and was significant at P<0.05 (Table 10). Total damage was positively correlated with total cross sectional damage, total cross sectional inner and outer damages and was significant at P<0.001. Total cross sectional damage was positively correlated with total cross sectional inner and outer damages and was significant at P<0.001. Also total cross sectional inner damage was positively correlated with total cross sectional outer damage and was significant at P<0.001 (Table 10).

Larval resistance parameters, body length was positively correlated with body weight, head capsule width, peripheral damage and total damage and was significant at P<0.05 but the correlation was very low for peripheral damage and total damages (0.23 and 0.25) respectively (Table 11). However, body length was negatively correlated with larval mortality and was significant at P<0.001. Body weight was positively correlated with head capsule width (HCW) and was significant at P< 0.001 and body weight was negatively correlated with larval mortality and significant at P<0.001. Head capsule width was negatively correlated with larval mortality and was significant at P<0.05 but the correlation was very low.

Bunch weight was positively correlated with number of clusters and number of leaves at flowering and was significant at P<0.001 (Table 12). Days to maturity of bunch were negatively correlated with leaves at harvest and significant at P< 0.05 but the correlation was very low at 0.2. Girth of plant at 1meter was positively correlated with height of the plant and significant at P<0.001. Height of a plant at flowering was negatively correlated with leaves at flowering and was significant at P<0.001.

**Table 9. Correlations of growth parameters and weevil damage parameters among an $F_2$ diploid banana population**

| | | GP | HP | ID | OD | PD | TD | TXD | TXI | TXO |
|---|---|---|---|---|---|---|---|---|---|---|
| GP | 1 | - | | | | | | | | |
| HP | 2 | 0.46** | - | | | | | | | |
| ID | 3 | 0.35** | 0.23 | - | | | | | | |
| OD | 4 | 0.40** | 0.38** | 0.86** | - | | | | | |
| PD | 5 | -0.22* | -0.35** | 0.08 | -0.07 | - | | | | |
| TD | 6 | -0.29* | -0.40** | 0.01 | -0.11 | 0.95** | - | | | |
| TXD | 7 | -0.34** | -0.39** | -0.11 | -0.16 | 0.61** | 0.82** | - | | |
| TXI | 8 | -0.32** | -0.36** | -0.10 | -0.13 | 0.58** | 0.79** | 0.99** | - | |
| TXO | 9 | -0.37** | -0.42** | -0.13 | -0.18* | 0.65** | 0.84** | 0.99** | 0.95** | - |
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |

GP = Girth of the plant, HP = Height of the plant, ID = Inner diameter, OD = Outer diameter, PD = Peripheral damage, TD = Total damage, TXD = Total cross sectional damage, TXI = Total cross sectional inner damage and TXO = Total cross sectional outer damage

**Table 10. Correlations of weevil damage parameters and corm hardness among an $F_2$ diploid banana population**

| | | Dead wev | Eggs | Inner CH | larvae | Outer CH | PD | TD | TXD |
|---|---|---|---|---|---|---|---|---|---|
| Dead_wev | 1 | - | | | | | | | |
| Eggs | 2 | -0.08 | - | | | | | | |
| Inner_CH | 3 | -0.05 | 0.03 | - | | | | | |
| Larvae | 4 | 0.01 | 0.07 | 0.09 | - | | | | |
| Outer_CH | 5 | -0.10 | 0.02 | 0.15 | -0.09 | - | | | |
| PD | 6 | 0.09 | -0.03 | 0.09 | 0.69** | 0.06 | - | | |
| TD | 7 | 0.08 | 0.08 | 0.12 | 0.68** | 0.07 | 0.97** | - | |
| TXD | 8 | 0.02 | 0.37** | 0.15 | 0.29* | 0.09 | 0.39** | 0.60** | - |
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |

Dead_Wev = Dead weevils, Inner_CH = Inner corm hardness, Outer_CH = Outer corm hardness, PD = Peripheral damage, TD = Total damage and TXD = Total cross sectional damage

**Table 11. Correlations of larval resistance mechanisms, total damage and peripheral damage among an $F_2$ diploid banana population**

| | | Body len | Body we | Dead wev | Eggs | HCW | larvae | Larval_M | PD | TD |
|---|---|---|---|---|---|---|---|---|---|---|
| Body_len | 1 | - | | | | | | | | |
| Body_we | 2 | 0.63** | - | | | | | | | |
| Dead_wev | 3 | -0.12 | -0.08 | - | | | | | | |
| Eggs | 4 | 0.20 | 0.06 | -0.10 | - | | | | | |
| HCW | 5 | 0.49** | 0.35** | -0.08 | 0.04 | - | | | | |
| Larvae | 6 | 0.30** | 0.08 | -0.10 | 0.31* | 0.05 | - | | | |
| Larval_M | 7 | -0.49** | -0.33** | 0.08 | -0.02 | -0.20* | -0.21* | - | | |
| PD | 8 | 0.23* | -0.01 | 0.01 | 0.16* | 0.07 | 0.73** | -0.07 | - | |
| TD | 9 | 0.25* | 0.02 | -0.03 | 0.27* | 0.06 | 0.79** | -0.09 | 0 .95** | - |
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |

Body_len = Body length, Body_we = Body weight, HCW = Head capsule width, Larval_M = Larval mortality, PD = Peripheral damage and TD = Total damage

**Table 12. Correlations of growth parameters and yield parameter among an $F_2$ diploid banana population**

| | | BW | Cluster | DM | Fingers | GF | HF | LF | LH |
|---|---|---|---|---|---|---|---|---|---|
| BW | 1 | - | | | | | | | |
| Cluster | 2 | 0.34** | - | | | | | | |
| DM | 3 | 0.03 | 0.02 | - | | | | | |
| Fingers | 4 | 0.04 | 0.01 | 0.13 | - | | | | |
| GF | 5 | 0.02 | 0.07 | -0.02 | 0.05 | - | | | |
| HF | 6 | -0.01 | 0.07 | -0.01 | -0.08 | 0.36** | - | | |
| LF | 7 | 0.32** | 0.03 | -0.02 | 0.13 | 0.15 | -0.36** | - | |
| LH | 8 | 0.14 | 0.19* | -0.23* | -0.02 | 0.09 | -0.02 | 0.10 | - |
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |

BW = Bunch weight, DM = Days to maturity, GF = Girth at flowering, HF = Height at flowering, LF = Leaves at flowering and LH = Leaves at harvest.

* Significant at P<0.05, ** P<0.001, ns non-significant

## 3.4 Discussion

### 3.4.1 Segregation of weevil resistance and agronomic traits in an $F_2$ diploid banana population

Agronomic parameters, number of leaves at flowering and number of leaves at harvest were not significant among the $F_2$ diploid banana population indicating that the population was not segregating for these parameters. The significant differences for agronomic parameters such as bunch weight, number of clusters, number of fingers, and days to maturity (Table 1) was an indication that the population was segregating for growth (agronomic) and yield traits and this could be utilised in banana breeding to provide potential for high yielding and early maturing parents. Morphological traits like corm hardness, corm size, physiological or other traits like chemical compounds of the host plant may affect the insect population and growth by negatively affecting its biology such as larval growth in weight and length or reducing the severity of attack (Ortiz *et al*., 1995). Significant differences were reported in corm hardness, both inner and outer corm hardness for euploid hybrids (Ortiz *et al*., 1995) who also reported increased corm hardness among resistant cultivars. This study found significant differences in inner and total corm hardness (arrived at after summing up inner and outer corm damages) showing that these parameters could be used to characterise the $F_2$ diploid banana population.

Host plant resistance in the form of antibiosis can be expressed as increased mortality, delayed development, reduced body size and reduced fecundity of the weevils and weevil larvae (Gertrude, 2010). When the larval resistance traits were used to screen the $F_2$ diploid population, head capsule width, body length, body weight and larval mortality were significant among the genotypes implying that the population screened was segregating. The above traits therefore can be used to screen populations for weevil resistance. This method is an indirect way of measuring resistance because it depends on the ability or inability of the larvae to feed on a corm tissue. The strategy is that larvae that feed on a susceptible corm tissue will feed a lot and consequently will grow faster in terms of body weight, body length and head capsule width while the larvae that feed on resistant corm tissue will feed little and have reduced body size, weight and consequently will die faster. So we expect more mortality in resistant corm tissue.

Weevil damage parameters such as total damage, peripheral damage and traits like dead weevils and larvae retrieved were significant among the genotypes screened in a pot experiment indicating that the population was segregating for weevil resistance and could be used to screen diploid populations for weevil resistance within a short period in a screen house other than waiting for long duration in the field basing on the method developed by (Sadik *et al*., 2010).

Peripheral damage that is the damage on the outer part of the corm and total damage which is the overall damage on a corm arrived at after summing all damage indices were significant and can be used on weevil assessment in the diploids. These results show that most of the damage on diploid corms was not so much inside the corm and that larvae attempted to eat but never penetrated so deep as reported in the case for triploids (Kiggundu, 2000) where the damage was significant in the cross sectional inner and outer damages. The inner and outer cross sectional damages in the segregating population were non-significant indicating that they are not good parameters for assessing weevil damage but they are necessary since they contribute to the total damage which was significant. Peripheral damage was significant and this was evident when assessing as the larvae attempted to penetrate the corm but could not so they ended up on the outer side of the corm. Dead weevil and larvae retrieved were significant implying that either of the two is an indicator of weevil resistance and mechanisms that lead to weevil mortality or reduction in number of larvae retrieved should be investigated.

The artificial method of screening for weevil resistance in this study could be a good method since it encourages picking a sucker (clone), assessing it and leaving the rest of the clones in the field undestructed and can be used for more related studies unlike the pot experiment and experiment under natural infested fields which are destructive experiments and the genotypes are lost making it complicated to acquire new one for more studies.

### 3.4.2 Nature of inheritance for weevil resistance and agronomic traits in an $F_2$ diploid banana population

Histograms on the nature of inheritance for weevil, larval resistance and agronomic parameters in banana $F_2$ population for head capsule width, number of leaves at flowering, girth of a at 1M plant at flowering, height of a plant at flowering and number of fingers on a bunch at harvest

showed normal distribution indicating that these parameters confirm to a mendelian ratio of 9:3:3:1.

Histograms for body weight, total damage, peripheral damage, cross sectional inner and outer damage, larvae retrieved, bunch weight and number of leaves at harvest were skewed to left which is towards a resistant parent. This shows that when the $F_2$ diploid population was screened with the above parameters, most of the genotypes are on the side of resistant parent with few on the side of the susceptible parent. Ortiz *et al*. (1995) reported diploid hybrids to be more resistant compared to polyploids when Calcutta-4 was crossed with plantains.

Histograms for body length, dead weevils and days to maturity were skewed towards the right which is towards the susceptible parent. This indicates that when the $F_2$ diploid population is screened with the above parameters, most of the genotypes are on the side of susceptible parent. All the above histograms show quantitative inheritance which is an indication of two genes involved except for the histogram of larval mortality showed two distinct classes (binomial distribution) which shows qualitative inheritance an indication of one gene involved.

### 3.4.3 Genetic ratios for weevil resistance traits among $F_2$ diploid banana population

The genetic ratios involved in for weevil resistance show that body length (when tested using resistant parent as a check), larval mortality (when tested using resistant parent as a check), number of dead weevils (when using a susceptible parent as check) and body weight (when using a susceptible parent as check) is due to a form of epistasis called duplicate dominant epistasis that causes a ratio of 15:1 whereas peripheral damage and body weight (when tested using resistant parent as a check) showed that the ratios of these traits are due to a form of epistasis called duplicate recessive epistasis causing a ratio of 9:7. Epistasis can cause unexpected phenotypic ratios to appear since an allele at one genotype masks the effect of another allele on another locus.

The ratios for total damage (when tested using resistant parent as a check) and larvae retrieved (when tested using resistant parent as a check), larval mortality and dead weevils (when using a

susceptible parent as check) showed that these traits are controlled by a single gene making a ratio of 3:1.

### 3.4.4 Heritabilities for weevil resistance traits and agronomic in an $F_2$ diploid banana population

Heritability for inner corm hardness can be used a good parameter to determine hardness of a corm since it had a high heritability of 48.0%. Days to maturity, number of fingers and number of clusters are good parameters to determine selection for yield since they have high heritabilities. For larval resistance traits, body weight had the highest heritability of 33.4% and for weevil damage parameters peripheral damage and total damage had highest heritabilities of 32.4 and 24% and this shows that for these parameters with high heritabilities, most of their inherited component is genetic and can be used for selection among selection in diploid breeding populations. The part that is transferred to the off springs is higher body weight compared to other parameters and therefore it is better parameter to use in the study of weevil resistance compared to the rest of the above parameters.

Body weight and peripheral damage are a better estimator compared to the rest since its heritability is high. Kiggundu (2000) carried out heritabilities in banana triploids for different weevil resistance traits and found out that total inner damage had the highest heritability of 0.87 and recommended it as better option for estimating heritability; however this is best done in a destructive experiment and normally done at harvest which takes alittle bit longer. In this study inner damage was done on plants in pot experiment and was found to be 0.022 which is way far smaller. Furthermore, Kiggundu (2000) found heriabilities of other weevil resistance traits like inner cross section damage (Upper) 0.35, Inner cross section damage (Lower) 0.34, Outer cross section damage (Outer) 0.29, Outer cross section damage (Lower) 0.29, in this study these were found to be much more less and this study shows other parameters that have high heritabilities that can be used in the study of diploid segregating populations.

### 3.4.5 Heterosis for weevil resistance traits in an $F_2$ diploid banana population

High heterosis was recorded for all parameters at $F_2$ implying that the diversity of the parents used was most expressed in $F_2$ but less at $F_1$, therefore inter diploid banana crosses should continue up to $F_2$ if better results for heterosis and maximum segregation is to be achieved.

### 3.4.6 Phenotypic correlations for weevil resistance and agronomic traits in an $F_2$ diploid banana population

Girth at base (GP) of the plant can be used to predict height of the plant, inner diameter of corm and outer diameter of corm because the correlations are positive and significant at $P<0.001$ and girth at base can also be used to predict peripheral damage, total damage, total cross sectional damage, total cross sectional inner and outer damage since the correlations with these parameters are negative and significant at $P<0.05$. Height of the plant is positively correlated with outer diameter and is negatively correlated with peripheral damage, with total damage, total cross sectional damage, total cross sectional inner and outer damages at significant at $P<0.001$ which means that height of the plant can be used to predict the above parameters. Inner diameter is positively correlated with outer diameter of the corm and is significant at $P<0.001$ and therefore inner diameter can be used to predict outer diameter. These results imply that as the girth and height of the plant increases, the damage indices decrease and so the bigger the plant the less the damage. Although the correlations were low between 0.22-0.45, they were significant mostly at $P< 0.001$ (Table 9).

Number of eggs found in the corm is positively correlated with total cross sectional damage at a significance of $P<0.001$. Number of larvae retrieved is positively correlated with peripheral damage, total damage, total cross sectional damage and is significant at $P<0.001$ (Table 10). These results show that the more the eggs and larvae found in the corm, the more severe the damage on the corm. When weevils lay eggs at the peripheral of the corm and in pseudostem sheaths and when they hatch, the larvae burrow into the corm making tunnels while feeding causing damage to the corm.

The weevil damage parameters weevil damage parameters were directly and positively correlated with each other (Table 9). Peripheral damage is positively correlated with total damage, with total

cross sectional damage, with total cross sectional inner and outer damages and are significant at $P<0.001$ and this was also reported by Kiggundu (2000) and any one of these above damage parameters may be a sufficient measure of weevil resistance.

Larval resistance traits are positively correlated with each other and significant at $P<0.05$ these are body length, body weight, head capsule width meaning that any one of these can be used to predict larval resistance (Table 11). Body length is positively and significantly correlated with peripheral damage and total damage and but the correlation are very low for peripheral damage and total damages (0.23 and 0.25) respectively so body length might not be good predictor of total damage and peripheral damage. However body length, body weight and head capsule width are negatively correlated with larval mortality and significant at $P<0.001$. This means that one of the above larval resistance parameters can be used as a measure of larval resistance. For larval mortality, increase in one the above parameters, means there is a decrease in larval mortality and logically if the above larval resistance traits increase, this means that the larvae are feeding well on good food and therefore the chances of dying are fewer.

Bunch weight is positively correlated with number of clusters and number of leaves at flowering and is significant at $P<0.001$ (Table 12). This implies that the more the number of clusters the bigger the bunch weight and that if a plant has many leaves a flowering, photosynthesize will be continuous over a long period of time and food will be deposited in the bunch which is the sink much more than those with few leaves and this this will lead bigger bunches. Days to maturity of bunch are negatively correlated with leaves at harvest and significant at $P< 0.05$ (Table 12) but the correlation is very low at 0.2 meaning that days to maturity is not a good predictor of leaves at harvest. But this can be explained in a way that when a plant retains many leaves up to harvest, then it matures faster and this can be attributed to photosynthesis rate which increase food formation in the bunch and thus reduce maturity rate of the bunch. Girth of plant at 1meter is positively correlated with height of the plant and significant at $P<0.001$ (Table 12) and this implies that as the plant increases in height, it also increases in girth so growth is uniform above the ground. Height of a plant at flowering is negatively correlated with leaves at flowering and is significant at $P<0.001$ (Table 12), so if a plant grows taller, it forms few leaves.

There were low and non-significant correlation (< 0.2) between corm hardness and weevil damage parameters in this study (Table 10) and this was also reported by Ortiz *et al*. (1995) in segregating plantain populations and this means corm hardness is not one of the weevil damage assessment parameters. Ortiz *et al*. (1995) also reported that there was a highly significant correlation between longitudinal outer and inner corm hardness and this study found no high correlations between inner and outer corm hardness. Kiggundu (2000) reported coefficient of infestation (visual), was related to inner corm hardness by r=4.5 and Pavis and Minsot (1993) found a small negative correlation (-4.7) between corm hardness and weevil damage parameters. The correlations between bunch weights, number of fingers and number of clusters is a good indication since all these those parameters contribute to yield in bananas.

### 3.5 Conclusion

Larval resistance traits such as head capsule width, body length, body weight and larval mortality, and damage parameters such as total damage and peripheral damage showed variation within in the F2 population and thus parameters segregating. Also bunch weight, number of clusters, fingers and corm hardness are segregating agronomic traits. The traits above are good parameters for screening diploid populations for weevil resistance.

The histograms for larval resistance traits, damage parameters and agronomic traits revealed that the nature of inheritance is quantitative and the skewedness to the resistant parents indicates that most the genotypes in this $F_2$ population were resistant. Body weight, Peripheral damage and total damage had high heritabilities and this implies that they are mostly genetically controlled. These traits can easily be improved by use of plant breeding methods. These traits genetic ratios deviated from the normal mendelian ratio of 9:3:3:1 which indicates that the gene controlling these traits is dependent on the presence one or more modifier genes on another chromosome (Epistasis). High heterosis was observed at $F_2$ indicating that diploid banana crosses should continue up to $F_2$ if better results for heterosis and maximum segregation is to be achieved.

Larval resistance traits such as body weight, body length, head capsule width and larval morality are significantly and highly correlated to each other. This shows that any one of these traits can be used to study resistance and also predict other traits. Damage traits such as total damage, peripheral

damage and total cross sectional damages are also significantly and highly correlated to each other meaning that anyone of them can be used to study resistance and also predict other damage traits.

### 3.6 Recommendations

Improved male parents could be used for further breeding activities and segregating population for further genetic and molecular marker associations.

Biochemical studies should be carried out to identify chemical factors responsible for weevil resistance.

### 3.7 Challenges

Timing the desuckering to obtain corm pieces for inoculation with larvae at first instar with hatching of larvae. Corm pieces where to be obtained a day before hatching of the eggs but hatching of the eggs is dependent on temperature. During cold weather the eggs would hatch after 7 or 8 days where as during hot weather the eggs would hatch after 6 days.

It was hard to ascertain if all the weevils used for pot experiment were of the same age for uniformity. The weevils were trapped from the field, sexed and used in the experiment so these could have been of different ages leading to differences in their capacity to lay eggs which hatch into the larvae that directly infect the corms

# CHAPTER FOUR
# OPTIMISATION OF PRIMERS FOR CHARACTERISING AN $F_2$ DIPLOID BANANA POPULATION AGAINST WEEVIL RESISTANCE

## 4.1 Introduction

Molecular characterization is a science that includes DNA sequence data and the mapping of particular functions on the plant chromosome and on the inserted DNA. This particular function of a chromosome or inserted DNA can be detected by use of a molecular marker. A marker, in this context, is an identifier of a particular aspect of phenotype and/or genotype; its inheritance can easily be followed from generation to generation.

Molecular markers are powerful tools for fingerprinting, assessing genetic variation and studying relatedness among cultivars of many species (Changadeya *et al*., 2012). Markers increase the effectiveness in breeding and significantly shorten the development time of plants, therefore plant breeders are considering molecular marker assisted selection (MAS, MAB) a useful additional tool in plant breeding programs to make selection more efficient (Bueren *et al*., 2010).

The improvement of bananas has several challenges, banana is long duration crop and this has hindered banana conventional breeding because the breeding cycle takes a long time to develop a product. In addition *Musa* breeding is based mainly on phenotypic mass recurrent selection and the high levels of heterozygosity within *Musa* makes it difficult to identify ideal parental material and also large populations are required for selection of individual clones with good agronomic traits (Pillay *et al*., 2012). The genes for resistance to diseases and pests are present in wild diploid species and are introgressed into elite banana. These wild species carry many undesirable traits such as low yield and non-parthenocapy which are carried along with resistance genes during crossing. Eliminating these unwanted traits requires several backcrosses that lengthen the breeding process as well (Pillay *et al*., 2012).

Therefore the use of molecular marker assisted breeding will improve the efficiency of *Musa* breeding and conservation hence overcoming some of the challenges through conventional improvement of bananas. DNA markers are being sought for several characters of importance in

*Musa* including resistance to pests and diseases (Pillay *et al*., 2012). But to date very few markers have been linked to traits of interest in *Musa* and are limited to markers for disease resistance and the main genomes. The examples of molecular markers that have been applied in *Musa* genetic studies include: Restriction fragment length polymorphism (RFLP) randomly amplified polymorphic DNA (RAPD) markers (Pillay *et al*., 2001; Tenkouano and Swennen, 2004) and simple sequence repeats (SSR) (Farooq and Azam, 2002).

Amplified Fragment Length Polymorphisms generate huge quantities of information which may need automated analysis and, therefore computer technology, they are dominant often cluster at the centromeres and telomeres and are technically demanding in the laboratory especially, in data analysis, randomly amplified polymorphic DNAs are dominant, they have a problem of lack of a priori knowledge on the identity of the amplification products, and they are poorly reproduced and co-migrate together hence poor differentiation. Restriction fragment length polymorphisms need large amounts of DNA, automation not possible, low levels of polymorphism in some species, are time consuming, especially with single-copy probes and are costly mostly during distribution of probes to collaborating laboratories (Kumar *et al*., 2009).

Simple Sequence Repeat (SSR) markers are routinely used for diversity analysis and molecular breeding in many crops because of their high level of polymorphism, co-dominant nature, efficiency and cost effectiveness (Pushpakumari *et al*., 2009). Research into many of biotic and abiotic stresses of banana will be aided by the use of markers leading to the improvement of this economically important crop (Mbajo *et al*., 2012). There have been studies in *Musa* where SSRs have been employed successfully for example high quality 846,762 sequences were developed for analysis of the interaction of *Mycosphaerella musicola* with leaf transcriptome of *Musa acuminata* (Marco *et al*., 2013). SSRs have also been used in investigating phylogenetic relationships in *Musa* (Hippolyte *et al*., 2010), SSRs have also been used in identifying morphological variation and *Fusarium* wilt tolerance accessions in desert bananas (Pushpakumari *et al*., 2009) and in many more studies in *Musa*. Due to the disadvantage of AFLP and RFLP mentioned above, SSR markers present themselves advantageous for use in this study.

Kumar *et al*. (2009) indicated the process of developing markers is a very time-consuming and costly process mostly when suitable probes or sequence data for primer construction are unavailable. Therefore using markers that have already been developed for nematode resistance to be validated on a population already developed for weevil resistance will help save time and minimize on the marker development costs.

Some SSR have been developed for nematode resistance (Mbajo *et al*., 2012).These SSR markers were developed using a mapping population that developed and segregated for nematode (*Radopholus simili*s) and later a map for these SSRs was constructed by Mbanjo (2012). Speijer *et al*. (1993 ) reported a strong association between nematode and banana weevil infestation, he reported that suckers attacked by nematodes are four times more likely to be attacked by weevil. Resistance mechanism like phonological compounds, HPLC products, toxins and lignin have been reported for nematodes (Gowen & Muller-Harvey, 2000) and same mechanism reported for banana weevil (Kiggundu *et al*., 2003; Ocan *et al*., 2008). Having markers that select for both nematode and banana weevil resistance is important in selecting cultivars that have resistance to both weevils and nematodes at once which saves time and resources. The objective of this study therefore is to optimise nematode resistance SSR markers for characterizing an $F_2$ diploid banana population against banana weevil resistance on a population that was developed from a cross of (*Musa acuminata* (kasaska) X *Musa acuminata* (borneo)).

## 4.2 Materials and Methods

### 4.2.1 Population development

The $F_2$ population used for molecular studies was developed as described in chapter three (Section 3.2 sub section 3.2.1).

### 4.2.2 Genomic DNA extraction

The experiment was carried out at the National Agricultural Research Laboratories-Kawanda, Uganda. Two parents *Musa acuminata* Subsp banksii (kasaska) and *Musa acuminata* Subsp microcarpa (borneo) and an $F_1$ accessions were collected. Immature unopened banana cigar leaves were taken for DNA extraction.

DNA was extracted according to the CTAB (Centylmethyl Ammonium bromide) procedure (Weising, *et al*., 1995), which was modified for *Musa* by Samarasinghe, *et al*. (2001). One gram of each leaf samples was ground with treated fine Lake sand and 2mL pre-warmed (60°C) 4% CTAB extraction buffer (0.1% ß–mercaptoethanol was replaced with 1% sodium sulphite w/v). Samples were incubated at 65$^{o}$C for 15min in a prewarmed incubator and centrifuged at 13,000rpm for 5 minutes. The supernatant (750 ul) was transferred to a fresh 2ml centrifuge tube (Eppendorf) and an equal volume of Chloroform:Isoamyl (24:1) was added. The sample was vortexed for 3 minutes and then centrifuged at 13,000 rpm for 5min. From these samples, 600ul of the supernatant was pipetted off and the separation step with chloroform:isoamyl was repeated. The supernatant (450 ul) was pipetted and an equal amount of glacial isopropanol was added. Samples were left in -20$^{o}$C freezer overnight and then centrifuged at 13,000 rpm. Supernatant was replaced with 400ul of 70% ethanol as a washing step. Washing was carried out at 13,000 rpm and pellet was air dried. Pellet was resuspended in PCR water and quantified using a Nano-Drop 2000 (Thermo Scientific).

For running PCR reactions, aliquots were diluted to a working stock of 50ng/uL. PCR amplification was carried out with 33 simple sequence repeats (SSR) primers (Table 13). Final concentration in the reaction solution was 0.5pM for each forward and reverse primer, GoTaq premix was used at a rate of 10uL per reaction and 1ul of DNA template in a 20ul reaction volume. PCR was performed according to Buhariwalla *et al*. (2005) using 94°C for 2 min for the initial denaturing and then 35 cycles of (30 seconds at 94$^{o}$C for denaturing phase, 30 seconds at 55°C for

annealing, 30 seconds at 72°C for extension) and a final extension at 72°C for 5 min. However, due to the range of 12°C in the Tm across the 33 primer pairs, 4 groups separated by a Tm range of 3°C were created. Each group would have a different annealing temperature during the PCR reaction as below.

### 4.2.3 SSR primers used in the study

**Table 13. Primers names and sequences that were selected for use in optimisation using the two contrasting parents and $F_1$.**

| ID | Marker Name | Sequence | Yield(ug) | Yield(nmol) | Vol.for 100pmol/ul | Tm (°C) |
|---|---|---|---|---|---|---|
| | | **Forward primer (5'-3')** | | | | |
| 1 | Ta6838 | gggtcatctcgccagggat | 407 | 69.7 | 697 | 61 |
| 2 | Ta3550 | ccctgatcgatcccaatcggag | 561 | 83.9 | 839 | 64 |
| 3 | Dn239853 | gggccttccattgggagaaag | 560 | **85.9** | 859 | 61.8 |
| 4 | Ta6942 | ctgcaaggagctggaccc | 378 | 68.6 | 686 | 60.5 |
| 5 | mMA513019043 | gttaacggccacctgcatgg | 524 | 85.7 | 857 | 62.7 |
| 6 | mMA513049034 | aggccattcattccttaagggtgg | 634 | 85.9 | 859 | 62.9 |
| 7 | mMA513035997 | gaggaccaatctgcgttcgc | 424 | 69.3 | 693 | 61.4 |
| 8 | Ta6456 | ggctgctgaaggcaagaactg | 447 | 68.6 | 686 | 61.8 |
| 9 | Ta1069 | agagaagcgactttgtcatgcctc | 462 | 62.8 | 628 | 62.7 |
| 10 | Ma513047166 | tctcggtcgctttgtttggga | 384 | 59.5 | 595 | 59.8 |
| 11 | Ta1693 | gcaatctggtactccacctggtga | 495 | 67.5 | 675 | 64.4 |
| 12 | Ma513051273 | caagggaagtgaacagaaacctctcc | 506 | 63.4 | 634 | 64.8 |
| 13 | Ma513049385 | tcgtcgagcaaggcaaatgc | 432 | 70.3 | 703 | 59.4 |
| 14 | mMa-1-27 | tgaatcccaagtttggtcaag | 500 | 77.5 | 775 | 55.9 |
| 15 | Ma513049385 | tcgtcgagcaaggcaaatgc | 438 | 71.1 | 711 | 59.4 |
| 16 | mMaCIR214 | ccattgagagatcaaccc | 327 | 60 | 600 | 53.7 |
| 17 | mMa1-32 | cacgtaaacaaggaggtgatc | 416 | 64.1 | 641 | 57.9 |
| 18 | Ta3455 | atgacgaggcgggctcac | 395 | 71.2 | 712 | 60.5 |
| 19 | Ta3816 | tgggttgggtgccatgac | 382 | 68.4 | 687 | 54.5 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 20 | mMa-1-24 | gagcccattaagctgaaca | 399 | 68.7 | 687 | 54.5 |
| 21 | Ma513049196 | gggcttctttcgttagcggga | 498 | 76.8 | 768 | 61.8 |
| 22 | Ma513036168 | cgcagtagcagcaggcag | 430 | 77.3 | 773 | 60.5 |
| 23 | Ma513045122 | cgctctgtggcaggactg | 441 | 79.7 | 797 | 60.5 |
| 24 | Ta3135 | cccatttggccaacacttgca | 470 | 74.3 | 743 | 59.8 |
| 25 | Ta5199 | gcaccaaatcctataagcatagaggcctt | 589 | 66.5 | 665 | 65.3 |
| 26 | Ma513050182 | accaaatcccgtcaccgct | 409 | 72.1 | 721 | 58.8 |
| 27 | Ma513046502 | agccatggacgggctctc | 352 | 64 | 640 | 60.5 |
| 28 | Ma513050081 | gtgcgctccatcgttgttgag | 637 | 98.8 | 988 | 61.8 |
| 29 | Ma513041952 | agcatggccacgagcgtc | 350 | 63.5 | 635 | 60.5 |
| 30 | Ma513050212 | ggctgcttcgttgccaagac | 421 | 68.9 | 689 | 61.4 |
| 31 | Ma513045018 | cgtttccgtcaattacaacgtcagc | 517 | 68.3 | 683 | 63 |
| 32 | Ma513050755 | ggctctttggtgggagggag | 544 | 86.6 | 866 | 63.5 |
| 33 | Ta7676 | acgaggccacccagtgat | 359 | 65.4 | 654 | 58.2 |

| **ID** | **Marker Name** | **Sequence** | **Yield(ug)** | **Yield(nmol)** | **Vol.for 100pmol/ul** | **Tm (°C)** |
|---|---|---|---|---|---|---|
| | | **Reverse primer (5'-3')** | | | | |
| 34 | Ta6838 | ggagcggtcactaccaccg | 469 | 80.9 | 809 | 63.1 |
| 35 | Ta3550 | gacaacgcccgccacgaa | 339 | 62.2 | 622 | 60.5 |
| 36 | Dn239853 | tcgacatcctcccggatcc | 435 | 76.5 | 765 | 61 |
| 37 | Ta6942 | cgagaggacgacacgacgtc | 369 | 60 | 600 | 63.5 |
| 38 | mMA513019043 | acggagcagtaacacgggattg | 649 | 95 | 950 | 62.1 |
| 39 | mMA513049034 | gctgcagctgacccaatcg | 476 | 82.4 | 824 | 61 |
| 40 | mMA513035997 | acgcagcacaagtcgtcca | 376 | 65.2 | 652 | 58.8 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 41 | Ta6456 | cagggcttggaaggcaagg | 501 | 84.2 | 842 | 61 |
| 42 | Ta1069 | ggttcacaacaaagaggaatagaacgtctg | 699 | 75.2 | 752 | 65.2 |
| 43 | Ma513047166 | tcgttgcctcgattcgaggaatg | 354 | 50.1 | 501 | 62.4 |
| 44 | Ta1693 | gaagcatgcatggctaaggagg | 627 | 91.2 | 912 | 62.1 |
| 45 | Ma513051273 | agcttcctgtcgatgaggctg | 590 | 91.5 | 915 | 61.8 |
| 46 | Ma513049385 | ggagaggtcaggcacgaagg | 495 | 78.8 | 788 | 63.5 |
| 47 | mMa-1-27 | caaacacatgtccccatctc | 464 | 77.7 | 777 | 57.3 |
| 48 | Ma513049385 | ggagaggtcaggcacgaagg | 524 | 83.4 | 834 | 63.5 |
| 49 | mMaCIR214 | ctatttgacgttggtggtc | 433 | 74.1 | 741 | 54.5 |
| 50 | mMa1-32 | cgacagatttaagattggatca | 452 | 66.7 | 667 | 54.7 |
| 51 | Ta3455 | ggagaggagtgagcgagaactg | 540 | 77.8 | 778 | 64 |
| 52 | Ta3816 | agggcagtttctcgagacgtc | 461 | 71.4 | 714 | 61.8 |
| 53 | mMa-1-24 | ccgacagtcaacatacaataca | 393 | 59 | 590 | 56.5 |
| 54 | Ma513049196 | tcacggcgacgagctgct | 399 | 72.5 | 725 | 60.5 |
| 55 | Ma513036168 | gccacagcaggatccacc | 423 | 77.7 | 777 | 60.5 |
| 56 | Ma513045122 | gcaccgattggtcgaattagcg | 478 | 70.5 | 705 | 62.1 |
| 57 | Ta3135 | gggaggcaacattcccctc | 424 | 73.4 | 734 | 61 |
| 58 | Ta5199 | tccgacttcgaaggctgagac | 418 | 65.1 | 651 | 61.8 |
| 59 | Ma513050182 | cgagcgctcatcactgagttcg | 542 | 80.8 | 808 | 64 |
| 60 | Ma513046502 | agaaagaccccacttcgagcc | 374 | 58.8 | 588 | 61.8 |
| 61 | Ma513050081 | gccactacccaatgcatcgag | 552 | 86.9 | 869 | 61.8 |
| 62 | Ma513041952 | ggacagtctccgagtcggttc | 547 | 84.9 | 849 | 63.7 |
| 63 | Ma513050212 | ggatcgcgagacatcgtgtacc | 534 | 78.9 | 789 | 64 |
| 64 | Ma513045018 | gtgcagctactgccacagc | 386 | 66.9 | 669 | 61 |
| 65 | Ma513050755 | accctggtctgattcgatttggtg | 602 | 81.7 | 817 | 62.7 |
| 66 | Ta7676 | tccacgcatgcacacagg | 459 | 84.2 | 842 | 58.2 |

### 4.2.4 Primer optimisation

The 33 selected primer pairs were optimized with the parental and $F_1$ DNA using agarose gels to obtain optimal working annealing temperatures and working primer concentrations. The two contrasting parents used in this study for primer optimisation were *Musa acuminata* (kasaska) used as a female and is susceptible to weevil damage and *Musa acuminata* (borneo) used as a male and is resistant to weevil damage. The $F_1$ used was developed from a cross of *Musa acuminata* (kasaska) and *Musa acuminata* (bormeo).

The range between the melting temperatures of the 33 primers was 12 degrees, necessitating optimization of the annealing temperatures for the PCR run. This was done by running a gradient PCR for all the primers using extracted $F_1$ genomic DNA as the template. The annealing temperatures tested ranged from 50$^o$C to 67.5$^o$C with a difference of 2.5$^o$C between each set. Three PCR's were run to account for the large number of primers versus the number of wells available on the thermocycler. Previously, DNA concentrations were varied (20ng, 50ng, 100ng) to test for the optimum amount to use in amplification reactions.

**Table 14. PCR master mix used to run gradient PCR.**

| Component | Stock concentration | Working conentration | Working Volume | 8 Reactions |
|---|---|---|---|---|
| GoTaq | 2.0X | 1.0X | 10.0µL | 80.0µL |
| Primer F | 10.0µM | 0.25µM | 0.5µL | 4.0µL |
| Primer R | 10.0µM | 0.25µM | 0.5µL | 4.0µL |
| H2O | | | 8.0µL | 64.0µL |
| DNA Template | | 100.0ng | 1.0µL | |

### 4.2.5 Electrophoresis Gels

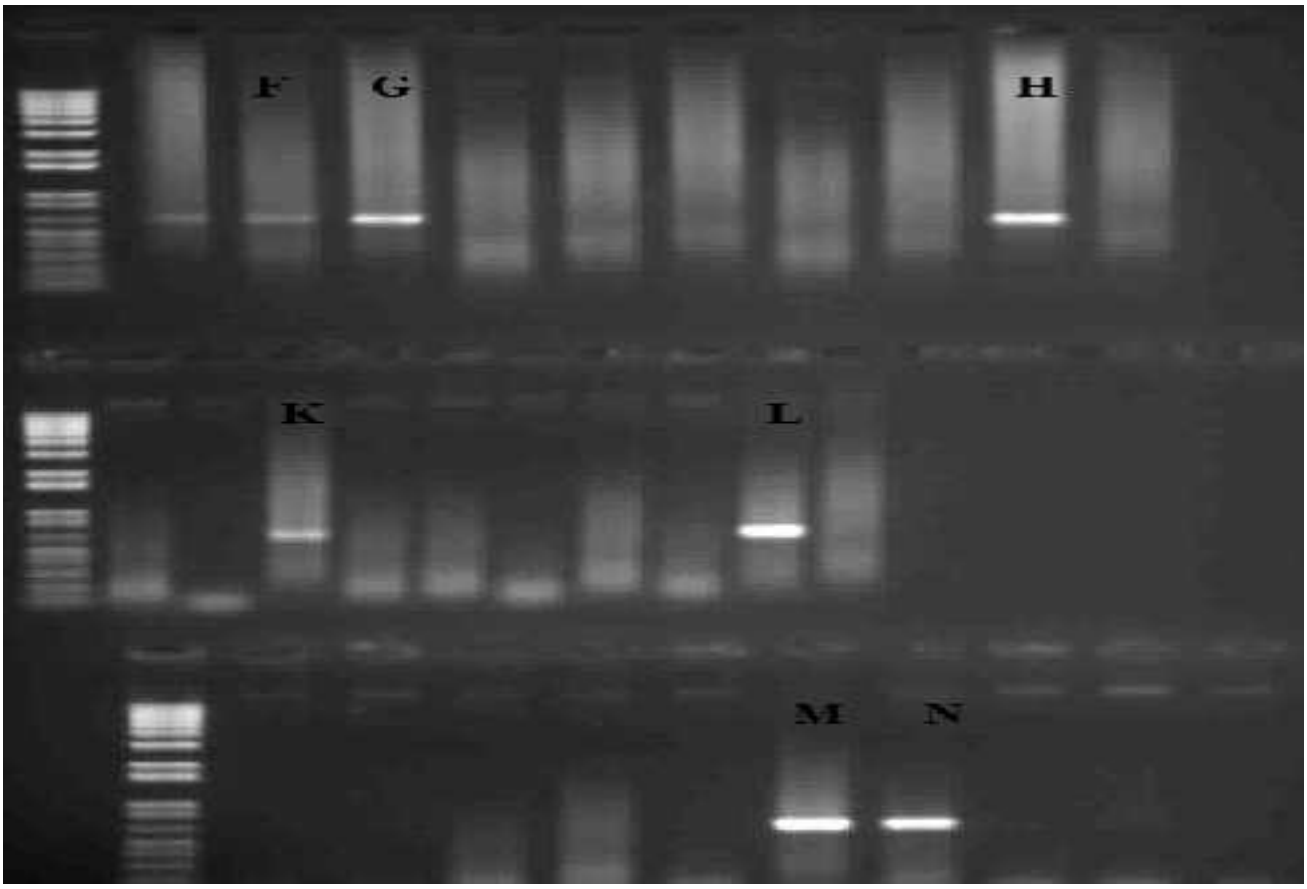
2.5% agarose gels made with 1X TAE buffer were prepared. 3micro litres of products were run at 100V for 45min along with a 50bp ladder (Fermenetas) and water as negative control. After electrophoresis, the gels were stained in 0.5% ethidium bromide for 15 min and then viewed through a gel documentation machine to visualize the bands.

### 4.3 Results

When the primers were optimised, good results were obtained with 100ng of template DNA where by 3 primer pairs amplified at 50$^{o}$C, 7 amplified at 55$^{o}$C, 4 amplified at 57$^{o}$C and 3 amplified at 60$^{o}$C. Therefore a total of 17 primer pairs amplified at different annealing temperature as shown in Table 15.

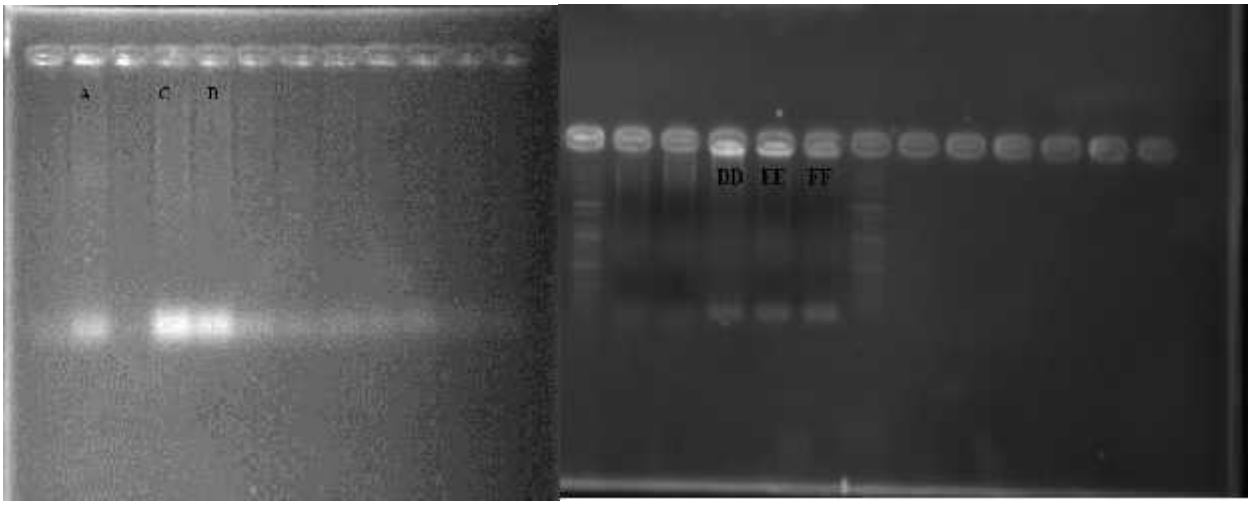
#### 4.3.1 Gradient PCR gels showing primer annealing temperatures

The gradient PCR gels show that 7 primers amplified the template DNA at 55.0$^{0}$C (Figure 5a), 3 primers amplified at 50.0$^{0}$C (Figure 5b), 3 primers amplified at 60.0$^{0}$C (Figure 5b) and 4 primers amplified at 57.5$^{0}$C (Figure 5c). Details of the primers are shown in Table 15 on page 66 below.



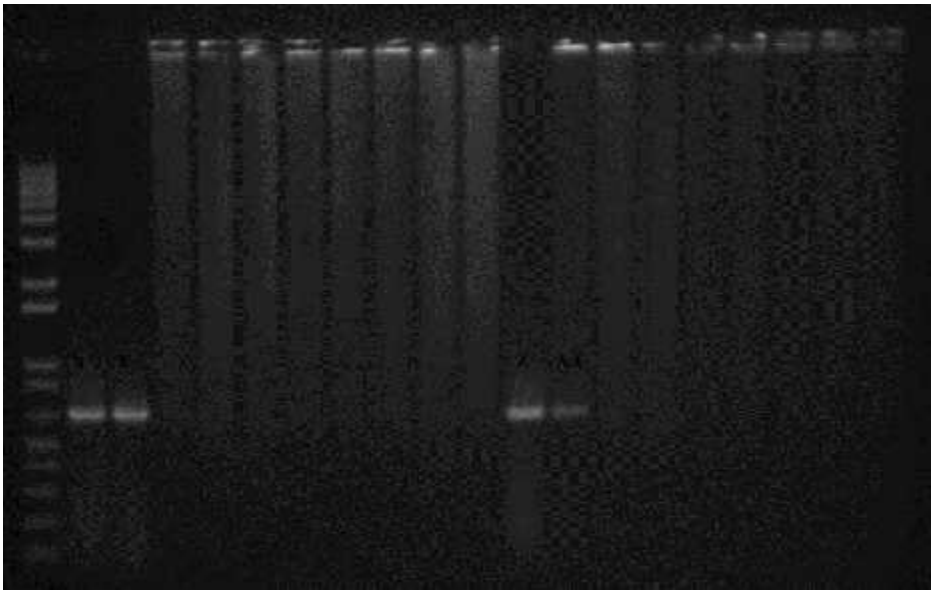

A

**Figure 5a. Gels of different gradient PCR that show primers F, G, H, K, L, M and N which amplified at 55ºC**




B C

**Figure 5b. Gels of different gradient PCR that show primers A, C, D and DD, EE, FF which amplified at 50ºC and 60ºC respectively.**



D

**Figure 5c. Gels of different gradient PCR that show primers X, Y, Z and AA which amplified at 57.5ºC.**

**Table 15. Amplified primer pairs and their respective annealing temperature**

| Code | Primer name | Forward | Reverse | Annealing Temperature |
|---|---|---|---|---|
| A | mMaCIR214 | ccattgagagatcaaccc | ctatttgacgttggtggtc | 50.0°C |
| C | mMa-1-24 | gagcccattaagctgaaca | ccgacagtcaacatacaataca | 50.0°C |
| D | mMa-1-27 | tgaatcccaagtttggtcaag | caaacacatgtccccatctc | 50.0°C |
| F | Ta7676 | acgaggccacccagtgat | tccacgcatgcacacagg | 55.0°C |
| G | Ma513050182 | accaaatcccgtcaccgct | cgagcgctcatcactgagttcg | 55.0°C |
| H | Ma513049385 | tcgtcgagcaaggcaaatgc | ggagaggtcaggcacgaagg | 55.0°C |
| K | Ta3135 | cccatttggccaacacttgca | gggaggcaacattcccctc | 55.0°C |
| L | Ta6942 | ctgcaaggagctggaccc | cgagaggacgacacgacgtc | 55.0°C |
| M | Ta3455 | atgacgaggcgggctcac | ggagaggagtgagcgagaactg | 55.0°C |
| N | Ma513036168 | cgcagtagcagcaggcag | gccacagcaggatccacc | 55.0°C |
| X | Ma513050081 | gtgcgctccatcgttgttgag | gccactacccaatgcatcgag | 57.5°C |
| Y | mMA513019043 | gttaacggccacctgcatgg | acggagcagtaacacgggattg | 57.5°C |
| Z | Ta1069 | agagaagcgactttgtcatgcctc | ggttcacaacaaagaggaatagaacgtctg | 57.5°C |
| AA | mMA513049034 | aggccattcattccttaagggtgg | gctgcagctgacccaatcg | 57.5°C |
| DD | Ta3550 | ccctgatcgatcccaatcggag | gacaacgcccgccacgaa | 60.0°C |
| EE | Ta1693 | gcaatctggtactccacctggtga | gaagcatgcatggctaaggagg | 60.0°C |
| FF | Ma513051273 | caagggaagtgaacagaaacctctcc | agcttcctgtcgatgaggctg | 60.0°C |

### 4.3.2 Electrophoresis gels for primers run against parental DNA

The Gel of primers coded A,C,D,F,G,H,I,K,L,M,N,X,Y,Z,AA,,DD,EE and FF below amplified on parental an$F_1$s. The bands were however on the same level in the gels and are single bands for all the parents and $F_1$'s that were tested indicating that the primers could not separate the two contrasting parents and the $F_1$ Primer pairs mMaCIR214, mMa-1-24, mMa-1-27, Ta7676, Ma513050182, Ma513049385, Ma513049385, Ta3135, Ta6942, Ta3455, Ma513036168, Ma513050081, mMA513019043, Ta1069, mMA513049034, Ta3550, Ta1693 and Ma513051273 which showed amplification in both parentals and $F_1$ using agarose gels can further be studied with PAGE to select primers that are co-dominant.

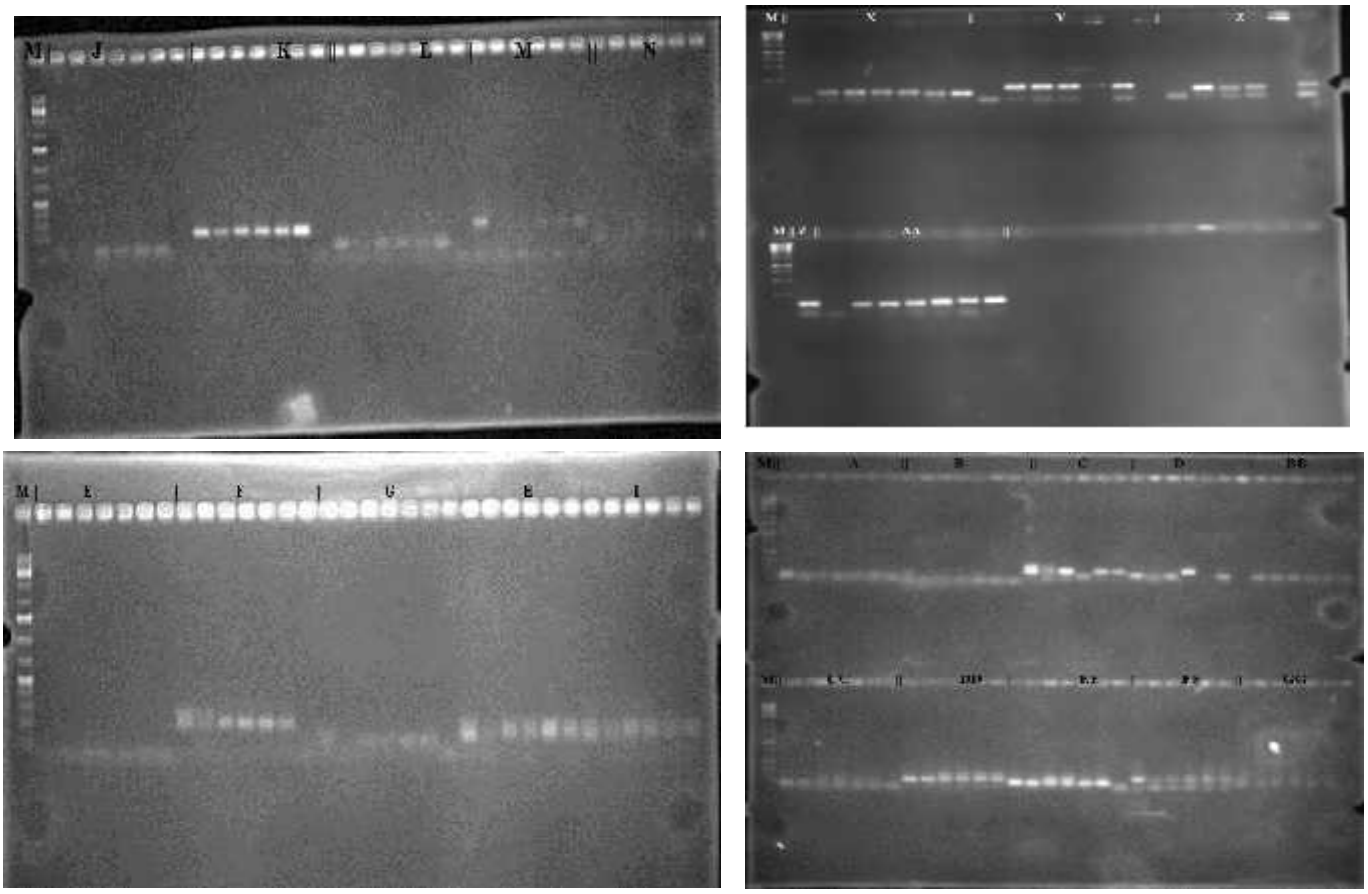


**Figure 6. Primers bands that amplified in both contrasting parents and $F_1$ of plants used to generate weevil segregating population**

## 4.4 Discussion

Primer pairs mMaCIR214, mMa-1-24, mMa-1-27, Ta7676, Ma513050182, Ma513049385, Ma513049385, Ta3135, Ta6942, Ta3455, Ma513036168, Ma513050081, mMA513019043, Ta1069, mMA513049034, Ta3550, Ta1693 and Ma513051273 all amplified the P1, P2 and $F_1$ DNA but the bands did not differentiate between contrasting parents and their $F_1$. This was most possibly due to low resolution of Agarose Gels. Nevertheless the above primer pairs appear to have an association with weevil resistance. Further studies need to be conducted with methods that have a higher resolution such as Metaphor agarose gels or poly acrylamide gelelectrophoresis (PAGE) (Muhammad *et al*., 2008; Xinwang *et al*., 2009) where the bands can segregate well to reveal polymorphism and co-dominance. Such studies would enable the above SSR primers to screen the whole population and identify which ones are associated with weevil resistance or susceptibility.

## 4.5 Conclusion

The primers for nematode resistance amplified the parental and $F_1$ DNA for weevil resistance. Though the bands did not differentiate between the contrasting parents and their $F_1$, there seem to be a possibility that SSR markers for nematode resistance can be optimized for characterizing an $F_2$ diploid banana population against banana weevil resistance

# CHAPTER FIVE
# CONCLUSION AND RECOMMENDATIONS

## 5.1 Conclusion

Larval resistance traits and weevil damage parameters such as body weight, body length, head capsule width, total damage, peripheral damage, larvae retrieved and dead weevil were segregating among the $F_2$ diploid population meaning that they can be used to screen segregating weevil populations and hence can be used as markers for weevil resistance. The developed $F_2$ diploid banana population that was screened in this study therefore segregates for weevil resistance.

Histograms for larval resistance and weevil damage parameters body weight, body length, head capsule width, total damage, peripheral damage, cross sectional inner and outer damages, larvae retrieved and dead weevil show that the parameters are quantitatively inherited. Larval resistance traits, body weight had the highest heritability of 33.4% and for weevil damage parameters peripheral damage and total damage had highest heritabilities of 32.4 and 24% and these parameters could be good parameters for estimating weevil resistance in weevil segregating populations. The genetic ratios of these parameters show that they are epistatic which results in decreased phenotypic classes.

Larval resistant parameters were correlated to each other and weevil damage parameters were correlated to each other as well. However larval and weevil damage parameter were not correlated with black Sigatoka, agronomy and harvest parameters so they cannot be used to predict each other.

## 5.2 Recommendations

Larval resistance traits and weevil damage parameters such as body weight, body length, head capsule width, total damage, peripheral damage, larvae retrieved and dead weevil are good parameters for screening populations against weevil resistance.

A detailed study needs to be conducted with a method that has a higher resolution mostly poly acrylamide gelelectrophoresis (PAGE) where the obtained bands can segregate well reveal bands

that are polymorphic and co-dominant can easily be identified and used to screen the whole population.

Furthermore, the population should be well replicated with more than 5 replication and the issue using sampling as replication is eliminated and further more to obtained more accurate and consistent agronomic and harvest data, a separate population be set aside so that at least 3 crop cycles be evaluated and also a field under natural infestation be set up to asses weevil damage at harvest. The issue of genotype by environment should also be considered since genotypes tend to perform differently in different environments.

## REFERENCES


Abera, A., Gold, C. S., Kyamanywa, S. & Karamura, E. B., 2000. Banana weevil *cosmopolitus sordidus* (Germar) oviposition preference, timing of attack and larval survivorship in mixed cultiar trial in Uganda. *Acta Horticulturae*, 540, 487-496.

Abera, A., 1997. Oviposition prefrences and timing of attack by Banana weevil (*Cosmopolites Sordidus* Germar) in East African Highland Bananas (*Musa spp*). Msc.Thesis, Makerere University- Kampala.

Arias, P., Dankers, C., Liu, P. & Pilkauskas, P., 2003. The world banana economy: 1985-2002. FAO Commodity studies 1, FAO. Roma, CIRAD, INRA.

Bagamba, F., 1994. Resource allocation efficiency in a banana based cropping system in Uganda. M.Sc. Thesis, Makerere University- Kampala.

Baker, A. D., Kallarackal, J. & Millburn, J. A., 1990. Water relations of the banana II:Physiochemical aspects of the latex and other tissue fluids. *Australian Journal of Plant Physiology*, 17, 57-68.

Barekye, A., Tongoona, P., Derera, J. & Tushemereirwe, W. K., 2009. Variation in diploid (AA) and tetraploid (AAAA) banana populations for black Sigatoka resistance and agronomic traits. *African Crop Science Journal*, 9, 509-516.

Biruma, M., Pillay , M., Tripathi, L. & Blomme, G., 1997. Banana *Xanthomonas wilt*: a review of the disease, management strategies and future research directions. *African Journal of Biotechnology*, 6, 953–962.

Brown , J. & Caligari, P., 2008. An introduction to plant breeding. s.l.:Wiley-Blackwell Publishers.

Bueren, E. T., Backes, G., Vriend, H. & Ostergard, H., 2010. The role of molecular markers and marker assisted selection in breeding for organic agriculture. *Euphytica*, 175, 51–64.

Buhariwalla, H. K., Jarret, R. L., Crouch, J. H. & Ortiz, R., 2005. Isolation and characterisation of microsatellite markers from *Musa balbisiana*. *Molecular Ecology Notes*, 5, 327-330.

Carol, W., Ruth George, K., Argent, O. & Sing, K. L., 2002. Assesment of the validity of sections in *Musa* (*Musaceae*) using AFLP. *Annals of Botany,* 90, 231-238.

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

Cho, G. Y., Blair, W. M., Panaud, O. & McCouch, R. S., 1996. Cloning and mapping of variety-specific rice genomic DNA sequences:amplified fragment length polymorphism (AFLP) from silver stained polyacrylamide gels. *Genome,* 9, 373–378.

Creste, S., Neto, A. T., Vencovsky, R. & Silva, S. O., 2004. Genetic diversity of *Musa* diploid and triploid accessions from the Brazilian banana breeding programme estimated by microsatellite markers. *Genetic Resources and Crop Evolution*, 51, 723-733.

Crouch, H. J., Vuylsteke, D. & Ortiz, R., 1998. Perspectives on the application of biotechnology to assist the genetic enhancement of plantain and banana (*Musa spp*.). *Electronic Journal of Biotechnology,* 1, http://www.ejb.org.

Crouch, K. H., Crouch, H. J., Jarret, L. R. & Ortiz, R., 1998. Segregation of microsatellite loci from haploid and diploid gametes in *musa*. *Crop Science,* 38, 211-217.

Crouch, J. H., Crouch, H. K., Constandt, H. & Gysel, A. V., 1999. Comparison of PCR-based molecular marker analyses of *Musa* breeding populations. *Molecular Breeding,* 5, 233–244.

De Lange, E., 1995. De Lange, E. Banana and plantain: the earlier fruit crops? 1: 6-8., Montepellier, France: http://bananas.bioversityinternational.org/files/files/pdf/publications/ar95en.pdf.

Dungu, J. C., 1987. The weevil borer and nematode problems in *Musa* in Uganda. Bujumbura, Burundi., INIBAP.

Embrechts, J., Bananuka, J. A., Couveur, Y. & Bagamba, F., 1996. Banana sub sector review, Republic of Uganda.Commissioned by the European Union (STABEX), Service Contract No.SC126196, Kampala.

Evans, E. & Fredy, B., 2013. Banana Market, Florida.

Falconer, D. S. & Mackay, T. F., 1996. Introduction to quantitative genetics.. Fourth edition edition. England.: Prentice Hall. Peterson Education Limited.

FAO, 2011. Examination of technical feasibility of analyzing and monitoring the economic significance of banana varieeties other than Cavendish. Yaoundé, Cameroon, Bioversity Intenational,CIRAD and IITA.

FAO, 2013. Fight against black Sigatoka must continue to save small Caribbean banana farms. *News article,* Rome.

FAOSTAT, 2012. The Agrricultural production, FAO.

Farooq, S. & Azam, F., 2002. Molecular markers in plantbreeding –1: concepts and characterization. *Pakistan journal of biological sciences*, 5(10), 1135-1140.

Fogain, R., 1996. Investigations on possible mechanisms of resistance to nematodes in *Musa*. *Euphytica*, 375-381, 92.

Frison, E. A., Orjeda, G. & Sharrock, S. L., 1997. PROMUSA: a Global Programme for *Musa* Improvement. Gosier, Guadeloupe, PROMUSA.

Georgis, R., Koppenhofer, M. A., Lacey, A. L. & Belair, G., 2006. Successes and failures in the use of parasitic nematodes for pest control. *Biological Control*, 38, 103-123.

Gertrude, N., Gold, S. C. & Alison, G., 2010. Survivorship and development rates of banana weevils reared on excised plant material of different banana cultivars. *International Journal of Tropical Insect Science,* 30(2), 77–83.

Gold, C. S., 2000. Biology and integrated pest management of banana weevil,*Cosmopolites sordidus* (Germar). In: A. B. Molina, V. N. Roa & M. A. Maghuyop, eds. Advancing banana and plantain R&D in Asia and Pacific. Bangkok: 28-33.

Gold, C. S., Kagezi, G. H. & Night, G., 2004. The effects of banana weevil, *Cosmopolites sordidus* (Germar) damage on highland banana growth, yield and stand duration in Uganda. *Applied Biology* , 145, 263-269.

Gold, C. S., Latigo, M. O. & Tushemereirwe, W., 1993. Farmer perceptions of banana pest constraints in Uganda: In: Results from a rapid rural appraisal. Ibadan,Nigeria: Biological and Integrated Control of Highland Banana and Plantain.

Gold, C. S. & Messiaen, S., 2000. The banana weevil, *Cosmopoites sordidus*, Montpellier, FRANCE: INIBAP.

Gold, C. S., Nemeye, P. & Coe, R., 1999. Recognition and duration of larval instars of banana weevil, *Cosmopolites sordidus* Germar, in Uganda.. *African Entomology Journal*, 7, 49-62.

Gold, C. S., Speijer, P. R., Karamura, E. B. & Rukazambuga, D. N., 1994. Assessment of banana weevils in East African highland banana systems and strategies for control. In; Banana Nematodes and Weevil Borers in Asia and the Pacific. R.V. Valmayor, R.G.Davide,J.M.Stanton, N.L.Treverrow,V.N.Roa ed. Los Banos,Philippines: INIBAP.

Gowen, R. S. & Muller-Harvey, I., 2000. Investigation into biochemical basis for nematode resistance in roots of the *Musa* cultivars in Rhodophorous Similis infection. *Journal of Agricultural Food Chemistry,* 48,5297-5301.

Haukeland, S. & Lola-Luz, T., 2010. Efficacy of the entomopathogenic nematodes *Steinernema kraussei* and *Heterorhabditis megidis* against the black vine weevil *Otiorhynchus sulcatus* (Coleoptera:Curculionidae) in open field grown strawberry plants.. *Agricultural and Forest Entomology*, 12, 363-369.

Hippolyte, I., Bakry, F. & Seguin, M., 2010.. A saturated SSR/DArT linkage map of *Musa acuminata* addressing genome rearrangements among bananas. *BioMed Central LTD,* 10-64.

Hippolyte, I., Jenny, C., Gardes, L. & Bakry, F., 2011. Foundation characteristics of edible *Musa* triploids revealed from allelic distribution of SSR markers. *Annals of Botany*, 1-15.

Holland, J. B., Nyquist, W. E. & Cervantes-Martinez, C. T., 2003. Estimating and interpreting heritability for plant breeding. An update. In: J. Janick, ed. *Plant Breeding Reviews.* 2nd ed. John Wiley and Sons, Inc, 9-112.

http://wwwpersonal.une.edu.au/~jvanderw/Estimation of variance components.pd, n.d

IITA, 2009. Banana & Plantain, Ibadan, Oyo State, Nigeria: IITA.

INIBAP, 1997. Screening of *Musa* germplasm for resistance and tolerance to nematodes. Montepellier France: INIBAP.

INIBAP, 2000. International Network for the Improvement of Banana and Plantain. Banana: food for the poor, Montpellier, France.

Jarret, L. R., Vuylsteke, R. D., Gawel, J. N. & Dunbar, J. L., 1993. Detecting genetic diversity in diploid bananas using PCR and primers from a highly repetitive DNA sequence. *Euphytica*, 68, 69–76.

Joshi, S. P., Prabhakar, K., Ranjekar, K. P. & Gupta, S. V., 2011. Molecular markers in plant genome analysis. *http/www.ias.ac.in/currsci/jul25/articles 15.htm*, 1-19.

Julian, R. P. & Stephen, H. A., 1999. Amplified Fragment Length Polymorphisms and Microsatellites: A phylogenetic perspective. In: M. E. Gillet, ed. Which DNA Marker for Which Purpose*?*. Great Britain: Department of Plant Sciences, University of Oxford, South Parks Road, Oxford OX1 3RB, Great Britain, 1-22.

Kallarackal, J., Garlick, P. R. & Milburn, J. A., 1986. Characterisation of the structural inclusions in the latex of banana (*Musa spp.*). *Canadian Journal of Botany*, 64, 2591-2601.

Kalyebara, R., Wood, S. & Abodi, P., 2005. The potential economic benefits of improved banana productivity in Uganda: an industry-scale analysis. Forthcoming in IFPRI Research Report by Smale et al., 2006. Kampala-Uganda: IFPRI.

Keim, P., Schupp, M. J., Travis, E. S. & Clayton, K., 1997. A high-density soybean genetic map based on AFLP markers. *Crop Science,* 37, 537–543.

Kiggundu, A., 2000. Host plant reactions and resistance mechanisms to banana weevil, *Cosmopolites sordidus* (Germar) in Ugandan *Musa* germplasm. Msc.Thesis, University of the Orange Free State- South Africa:.

Kiggundu, A., Gold, C., Labuschagne, M. T. & Vuylsteke, D., 2007. Components of resistance to banana weevil (*Cosmopolites sordidus*) in *Musa* germplasm in Uganda. *Eurpoe PubMed Central,* 122(1), 27-35.

Kiggundu, A., Tushemereirwe, W. & Kunert, K., 2003. Enhancing banana weevil (*Cosmopolites sordidus*) resistance by plant genetic modification: A perspective. *African Journal of Biotechnology,* 2 (12), 563-569.

Kumar, P., Gupta, V. K., Misra, A. K. & Modi, D. R., 2009. Potential of Molecular Markers in Plant Biotechnology. *Plant Omics Journal,* 2(4), 141-162.

Lescot, T. & Ganry, J., 2008. Plantain cultivation in Africa :Production and research in the past two decades., Montpellier - France: CIRAD.

Lorenzen, J., Tenkouan, A., Bandyopadhyay, R. & Vroh, B., 2010. Overview of Banana and Plantain (*Musa spp*.) Improvement in Africa: *Acta Horticulturae,* 879, 595-603.

Marco, A. P., Viviane, O. C., Flavia, L. E. & Cristiane, C. T., 2013. Analysis of the leaf transcriptome of *Musa acuminata* during interaction with *Mycosphaerella musicola*: gene assembly, annotation and marker development. . *BioMed Central,* 14(78), 14-78.

Marlene, . F. .C., 2012. *MATOOKE: FEEDING UGANDA.* [Online]
Available at: http://www.marlenefrancia.com/Matoke%20Feeding%20Uganda.html

Mary Sarah, M. P., 2002. An introduction to recombination and linkange analysis. Presented by Yue Wang and Zheng Yin, Chicago:

Masanza, M., 2003. Effect of crop sanitation on banana weevil *Cosmopolites sordidus* (Germar) populations and associated damage. PhD. thesis, Wageningen Universiteit- Netherlands

Mau, R. F. & Martin Kessing, J. L., 2007. *Cosmopolites sordidus* (Germar). Honolulu, Hawaii: Crop Knowledge Master.

Mbanjo, E. N., Tchoumbougnang, F., Mouelle, A. S. & Oben, J. E., 2012. Molecular marker-based genetic linkage map of a diploid banana population (*Musa acuminata* Colla). *Euphytica*, 188, 369–386.

Mendel,. G., 1866. Versuche über Plflanzenhybriden. Verhandlungen des naturforshenden vereines in Brun, Bd IV fur das jahr 4:3-44, 1865.(Reprinted as experiments in plant hybridization in bateson,w.1909. mendels principals of heredity). Bateson, ed. experiments in plant hybridization. Cambridge: Cambridge University Press. 317-361.

Mesquita, M. A., Alvers, E. J. & Caldes, R. C., 1984. Resistance of banana cultivars to *Cosmopolites sordidus* (GERMAR). *Fruits,* 39 (4), 254-257.

Mestre, J., 1997. A coefficient to evaluate infestations of banana weevils, *Cosmopolites sordidus*. *Fruits,* 52, 135-140.

Mickan, F. E., 2005. Dry Matter Content of Conserved Forages: Measurement of DM Content. (Frank Mickan, Ellinbank 2005 and reviewed December 2008).

Miliken, G. A. & Johnson, D. E., 1992. Analysis of messy data. In: Designed experiments. New York: Chapman and Hall.

Mitchell, E. S., Kresovich, S., Jester, A. C. & Hernandez, J. C., 1997. Application of multiplex PCR and fluorescence-based, semi-automatic allele sizing technology for genotyping plant genetic resources. *Crop Science,* 37, 617-624.

Muhammad, J. B., Naila, G., Wajid, A. J. & Raja, Z. B., 2014. Genetic Constitution of Multigenic Traits in $F_2$ Populations of Intrahirsutum Crosses. *Advances in Agricultural Sciences,* 2(2), 35-47.

Muhammad, A., Mehboob ur, R., Javed, M. I. & Yusuf, Z., 2008. High resolution Metaphor agarose gel electrophoresis for genotypying with microsatellite markers.. *Pakistan Journal of Agricultural Science,* 45(1), 75-79.

Mwaitulo, S., Haukeland, S., Saethre, M. G. & Maerere, A. P., 2011. First report of entomopathogenic nematodes from Tanzania and their virulence against larvae and adults of the banana weevil *Cosmopolites sordidus* (Coleoptera: Curculionidae). *International Journal of Tropical Insect Science,* 31(3), 154-161.

Nankinga, C. M. & Ogenga-Latigo, M. W., 1996. Effect of Method of application on the effectiveness of Beauveria bassiana against the banana weevil, *Cosmopolites sordidus*. *African Journal of Plant Protection*, 21, 12-21.

Njau, N., Mwangi, M., Gathu, R. & Mbaka, J., 2011. Banana weevil (*Cosmopolites sordidus*) reduces availability of corms for seedling production through macropropagation technology. *Journal of Animal and Plant Sciences,* 12(1), 1537-1542.

Ocan, D., Mukasa, H. & Rubaihayo, R. P., 2008. Effects of banana weevil damage on plant growth and yield of East African *Musa* genotypes. *Applied Biosciences,* 9(2), 407 - 415.

Okolle, J. N., Fansi, G. H., Lombi, F. N. & Lang, P. S., 2009. Banana entomological research in Cameroon: How far and what next?. *African journal of plant science and biotechnology*, 3(1), 1-19.

Ortiz, R., Vuylsteke, D., Dumpe, B. & Ferris, R. B., 1995. Banana weevil resistance and corm hardness in *Musa* germplasm. *Euphytica*, 86, 95-102.

Ortiz, R. & Vuylsteke, R. D., 1994. Future strategy of *Musa* improvement. In: Banana and plantain breeding: Priorities and strategies, Montpellier, France, 40-42.: INIBAP.

Ouma, G., 2009. Intercropping and its application to banana production in East Africa: A review. *Plant Breeding and Crop Science,* 1(2), 013-015.

Padilla-Cubas, A., Hernandes, A. C. & Garcia del Pino, F., 2010. Laboratory efficacy against neonate larvae of the banana weevil (*Cosmopolites sordidus*) of two indigenous entomopathogenic nematode species from the Canary Islands (Spain). *International Journal of Pest Management*, 56, 211–216.

Pavis, C. & Lemaire, L., 1996. Resistance of *Musa* germplasm to the banana borer weevil, *Cosmopolites sordidus* Germar (Coleoptera: Curculionidae). *Infomusa*, 5, 3-9.

Pavis, C. & Minost, C., 1993. Banana resistance to the banana weevil borer *Cosmopolites sordidus*: Role of pseudostem attractivity and physical properties of the rhizome. In: J. Ganry, ed. Breeding Banana and Plantain for Resistance to Diseases and Pests. Montpellier, France: CIRAD, 129-142.

PIBID-Uganda, 2012. Banana Industrial Development-Presidential Initiative on Banana Industrial Development (PIBID), Kampala,Uganda: PIBID.

Pillay, M. & Tenkouno, A., 2011. Banana Breeding: Progress and Challenges., IBADAN: s.n.

Pillay, M., Ashokkumar, K., Shunmugam & Elayabalan, 2012. A Case for Molecular Breeding in *Musa*. *Taylor & Francis Group, LLC.*

Pillay, M., Ssebuliba, R., Hartmann, J. & Vuylsteke, D., 2004. Conventional breeding strategies to enhance the sustainability of *Musa* biodiversity conservation for endemic cultivars.. *African Crop Science Journal,* 12, 63–69.

Ploetz, C. R., 2006. *Fusarium Wilt* of Banana Is Caused by Several Pathogens Referred to as *Fusarium oxysporum f.* sp. cubense. *Phytopathology*, 96(6), 653-656.

Pushpakumari, W. H., Samarasinghe, L. W. & Senanayake, G. S., 2009. DNA typing of dessert banana cultivar 'KOLIKUTTU' ('SILK') accesions by microsatellite markers. *Tropical Agricultural Research & Extension,* 12(2), 1-4.

Rowe, R. P., 1991. Avances genéticos em banana e plátano. *Augura Bogotá,* 17(1), 19-83.

Rowe, R. P. & Richardson, L. D., 1975. Breeding bananas for disease resistance, fruit quality and yield.. La Lima, Honduras: Tropical Agriculture Research Service. (SIATSA. Bull. 2).

Rubaihayo, P., Frison, E., Shah, D. & Atkinson, H. J., 2003. Improved Production of Bananas and Plantains in sub-Saharan Africa, Kampala , Uganda: AATF.

Rukazambuga, N. D., 1996. The effect of banana weevil (*Cosmopolites sordidus* Germar) on the growth and productivity of bananas (*Musa* AAA-EA) and the influence of host vigour on attack. PhD. Thesis, University of Reading- United Kingdom.

Rukazambuga, N., Gold, C. S. & Gowen, S. R., 1998. Yield loss in East African highland banana (*Musa spp*., AAA-EA group) caused by the banana weevil, *Cosmopolites sordidus* Germar. *Crop Protection,* 17, 581-589.

Sadik, K., Moses, N. & Michael, P., 2010. A screening method for banana weevil (*Cosmopolites sordidus,* Germar) resistance using reference genotypes.. *African Journal of Biotechnology,* 9 (30), 4725-4730.

Samarasinghe, W. G., Rukshanthi, J. D., Nahfees, M. A. & Muhunthan, R., 2001. A Laboratory Manual on DNA typing using RAPD & SSR technologies with silver stained PAGE. *PGIA, Unversity of Peradeniya,* 1-22.

Sebastião, S. O., Manoel, S. T., Élio, A. J. & Marcelo, B. L., 2001. Banana breeding program at Embrapa. *Crop Breeding and Applied Biotechnology,* 1(4), 399-436.

Sengooba, T., 1986. Survey of banana pest problem complex in Rakai and Masaka Districts in Uganda. Namulonge, Uganda: Namulonge Research Station.

Simmonds, W. N., 1962. The Evolution of the Bananas. London. Longmans.

Smale, M., 2006. Assessing the impact of crop genetic improvement in sub-Saharan Africa: Research context and highlights. *Genetic Resources Policies Briefs,* 19.

Smith, D., 1995. Banana weevil borer control in south eastern Queensland. *Australian Journal of Experimental Agriculture,* 35, 1165–1172.

Speijer, P. R. & Fogain, R., 1999. *Musa* and *Ensete* nematode pest status in selected African countries. In Gold, C S; Karamura, E B; Sikora, R A (eds), Mobilising IPM for sustainable banana production in Africa. Proceeding of a workshop on banana IPM held in Nelspruit, South Africa, 99-108.

Speijer, R. P., Budenberg, W. J. & Sikora, R. A., 1993 . Relationships between nematodes,weevils, banana and plantain cultivars and damage. Published online 2008. *Annuals of Applied Biology,*123, 517-525.

Tenkouano, A. & Swennen, L. R., 2004. Progress in breeding and delivering improved plantain and banana to african farmers. *Horticultural Science Focus,* 44(1), 1-7.

Timothy, P., Walker, S. & Sandra, B., 2005. Sourcebook for Land Use, Land-Use Change and Forestry Projects.

Treverrow, N. L. & Bedding, A. R., 1993. Development of a system for the control of the banana weevil borer, *Cosmopolites sordidus* with entomopathogenic nematodes. In: R. A. Bedding, R. Akhurst & H. K. Kaya, eds. In Nematodes and the Biological Control of Insect Pests. East Melbourne. CSIRO Publication, 41–47.

Vakili, G. N., 1965. Inheritance of resistance in *Musa acuminata* to bacterial wilt caused by the tomato race of *Pseudomonas solanacearum*. *Phytopathology,* 55, 1206-1209.

Van Eck, J. H., van der Voort, R. J., Draaistra, J. & Bakker, J., 1995. The inheritance and chromosomal localization of AFLP markers in a non-inbred potato offspring. *Molecular Breeding,* 1, 397–410 .

Vander Stichele, M., Van der Wal, S. & Oldenziel, J., 2005. Critical Issues in the Fresh Fruit and Vegetable chain, Amsterdam: Stichting Onderzoek Multinationale Ondernemingen(SOMO), Centre for Research on Multinational Corporations.

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

Vuylsteke, D., Ortiz, R., Shaun, F. B. & Crouch, H. J., 1997. Plantain Improvement. *Plant Breeding Reviews,* 14, 267-320.

Wachira, M. P., Kimenju, W. J., Kiarie, W. J. & Kihurani, 2013. Incidence of pests and diseases affecting banana in a commercial banana production setting in kenya. ,. *International Journal of Research In Agriculture and Food Sciences,* 1(1), 1-7.

Wambi, M., 2008. TRADE: Kibuzi Bananas Follow Ugandans to London, Kampala: Inter press service-News agency.

Weising, K., Nybom, H., Wolff, K. & Meyer, W., 1995. DNA isolation and purification . In: DNA fingerprinting in plants and fungi. Boca Raton, Florida: CRC Press, 44-59.

Xinwang, W., Timothy, R. A., Phillip, W. A. & James, S. M., 2009. A new electrophoresis technique to separate microsatellite alleles. *African Journal of Biotechnology,* 8(11), 2432-2436.