

**RESISTANCE OF BANANA TO *RADOPHOLUS*  
*SIMILIS* IN A DIPLOID POPULATION**

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Academic year 2015-2016

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## **ACKNOWLEDGEMENT**

I would like to thank my supervisors Dr Brigitte Uwimana and Dr. Danny Coyne for accepting me to conduct this study in Uganda, and for their guidance and advice throughout this study. Thanks to LaSalle Institute of Polytechnic for the financial support throughout the program.

I would also like to thank my supervision team at the International Institute of Tropical Agriculture who guided me throughout the months of the research. Special thanks go to James Kisaakye, Violet Akech, Radisras Nkurunziza and Olim Expenditor for their continuous support, guidance, encouragement and friendship.

Most of all I thank the staff in the breeding team at IITA- Uganda field station (Sendusu). Their dedication to work and their friendship has contributed greatly to the completion of this program. My special thanks to Harriet Nabatanzi, Catherine Nasuuna, and Catherine Nakayenga.

Lastly but not least, I thank all my friends in the different parts of the world for their encouragement and my parents for their love, support and constant prayers throughout this entire process.

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## **ABSTRACT**

Banana is an important food crop in the world. Its production is hindered by biotic and abiotic stress. Biotic stress includes phytopathogen microorganisms and pests. Burrowing nematode (*Radopholus similis*) is one of the important pathogens in banana production. Resistant cultivars have to be identified, in order to utilize them in the breeding program the nature of its inheritance of gene(s) need to be understood.

The objective of the current research is to study the inheritance of plant resistance gene to *R. similis* in a diploid banana population derived by crossing diploid Kasaska and Borneo. F<sub>2</sub> genotypes were evaluated with the individual root inoculation method using an *R. similis* population from Namulonge. It was found that there was segregation of resistance gene in F<sub>2</sub> diploid population, heritability was 91% and 74% for nematode count and percentage necrosis respectively.

The results show that the genes controlling resistance in banana is highly heritable and quantitative hence it will be effective during breeding and selection. Data generated are promising in finding QTL associated with *R. similis* resistance in banana

## CHAPTER ONE

### INTRODUCTION

#### 1.1 Banana: History and Production

Banana, a giant herbaceous and perennial plant, belongs to the genus *Musa*, family *Musaceae*. It grows well in humid tropical and subtropical regions (Ortiz, 2013). There are more than 1000 cultivars of banana in the world which are mainly triploids ( $2n = 3x = 33$ ), seedless, often sterile and parthenocarpic (Heslop-Harrison, 2011). It is believed that banana originated from South East Asia a primary centre of diversification (Jones, 2000). Bananas came from two wild diploids ( $2n = 22$ ); *Musa acuminata* Colla (AA) and *M. balbisiana* Colla (BB) and have been spread throughout the humid tropics (Valmayor, 2000). Edible bananas originated from intra- and interspecific hybridization of those two diploid wild bananas (Simmonds, 1995). There is great diversity of banana and plantains in sub-Saharan Africa, the humid lowlands of West Africa are dominated by Plantain AAB, Central Africa and East African highlands are dominated by AAA cooking and beer banana also known as East African highland Banana (EAHB). These two eco-regions harbour the world's greatest diversity of plantains and highland bananas and are considered secondary centres of banana diversification (Swennen, 1990).

Banana is vegetatively propagated and is characterised with small holder farming. In the world production, dessert bananas contribute about 53% of total production while cooking bananas contribute 47% (Lescot 1998). Banana world market is dominated with the Cavendish cultivars, regional and national markets are dominated by; plantains in West Africa and EAHB in East Africa. In Uganda, 75% of farmers grow bananas (Tushemereirwe *et al.*, 2001). Eighty five percent of banana production in Uganda is from the group EAHB, 11% of Pisang awak (ABB) and Kisubi (AB) used for juice/beer, 1% plantains (AAB) and Bluggoe (ABB) used for roasting and cooking, 3% of Gros Michel, Red/Green Red (AAA) and Sukali Ndizi (AAB) used for dessert (Karamura *et al.*, 1996).



## 1.2 Importance of banana

Banana is the fourth most important food crop globally (after rice, wheat and maize). It is grown in more than 130 countries over a harvestable area of over 10 million hectares and an annual production of more than 143 million tonnes (FAOSTAT, 2016), (Table 1). Fifty two percent of production comes from Asia, 33.3% from America, 14.5% from Africa, 3.2% from Europe and oceanic (FAOSTAT, 2016). Uganda has been reported as the first producer of cooking banana in world (FAOSTAT, 2016). The Great Lakes region of Africa is the largest producer and consumer of bananas in Africa (Smale, 2006) where per capita consumption of banana ranges from 230 to 450 kg person<sup>-1</sup> year<sup>-1</sup> (FAOSTAT, 2012). The fruiting habit of banana gives it great potential to bridge the hunger gap between crop harvests; hence it provides food and income security to farmers engaged in its production and trade especially in developing countries (PBS & UNCST, 2007). In Uganda banana is one of the most important cash crop and contributes up to 22% of the national agriculture rural revenue (Embrechts, 1996). Banana is a staple food for Ugandans and 66% of the country's urban population depends on this crop (PIBID-Uganda, 2012).

Table 1: Production, yield and harvestable area for bananas and plantain

	<b>Production (tons)</b>	<b>Harvestable area(ha)</b>
<b>Uganda</b>	9,504,029	1,785,303
<b>East Africa</b>	21,939,278	3,402,418
<b>Africa</b>	44,307,349	5,903,186
<b>world</b>	143,834,510	10,524,615

Source: FAOSTAT (2016)

Nutritionally, banana provides energy and nutrient to the human body. It is rich in water 75%, carbohydrate 23%, fibre 2.5%, fat 0.5% and protein 1% (Mohapatra *et al.*, 2010). Banana is a good source for the following vitamins: carotene, vitamin E, thiamine (B1), riboflavin (B2), niacin, pyridoxine (B6), folic acid, pantothenate, biotin and vitamins C. It is also rich in minerals such as sodium, potassium, calcium, magnesium, phosphorus, iron, copper, zinc, chloride, manganese and iodine (Robinson, 1996)

Bananas are consumed as fresh fruit or cooked; they can also be processed as chips, fries, fritters purees, jams, ketchup and alcohol (Barky *et al.*, 2009). Banana and plantain fibres are used in the production of handicrafts. Peels are used as animal feeds and leaves for wrapping food stuffs and as mulch (Frison and Sharrock, 1999). Innovations to extract starch from pseudostems have also been tried (Fondi, et al., 2010).

### 1.3 Constraints to banana production

Banana production is greatly affected by both abiotic and biotic factors. The biotic constraints are the pests and diseases (Vuylsteke *et al.*, 1993) while the major abiotic constraints are low fertility and drought (Wachira *et al.*, 2013)

In Uganda, production and yield of banana has declined despite an increase in the area harvested from 2003-2013 (Table 2). This could be attributed to, among other factors, the disease and pest constraints.

**Table 2: Banana production trend in Uganda (2003-2013)**

Year	Area harvested (ha)	Yield (kg/ha)	Production (tonnes)
2003	1,661,000	58,399	9,700,000
2004	1,670,000	58,000	9,686,000
2005	1,675,000	54,000	9,045,000
2006	1,677,000	53,989	9,054,000
2007	1,678,000	55,012	9,231,000
2008	1,680,000	55,780	9,371,000
2009	1,682,000	56,552	9,512,000
2010	1,700,000	56,176	9,550,000
2011	1,715,000	55,977	9,600,000
2012	1,700,000	54,118	9,200,000
2013	1,649,347	54,120	8,926,308

Source: FAOSTAT Date: Sun May 15 08:34:20 CEST 2016

#### 1.3.1 Disease constraints to banana production

Black Sigatoka disease also known as black leaf streak caused by *Mycosphaerella fijiensis* attacks almost all varieties of Musa (Arzanlou *et al.*, 2007), it causes significant yield losses of up to 50% on plantains (Mobambo *et al.*, 1993) and 37% loss on East African Highland bananas (Tushemereirwe, 1996). In Uganda, black sigatoka alone can reduce yield by 30-50%; hence it is considered as a major threat to the country's food security (Gale, 2012).

Banana bacterial wilt (BBW) is caused by a bacterium *Xanthomonas campestris* pv. *Musacearum*. The disease is characterised by yellowing and complete wilting of the plant starting with the most peripheral leaves (Tushemereirwe *et al.*, 2003). The economic impact of banana bacterial wilt is not fully understood but its impact on food security in the Uganda is very significant (Biruma *et al.*, 2007)

Fusarium wilt also known as Panama disease is caused by the soil fungus *Fusarium oxysporum* f.sp. cubense. It causes disruption of translocation and systemic foliage symptoms in bananas which eventually leads to collapse of the crown and pseudostem (Jeger *et al.*, 1995). Kangire (1998) reported that Fusarium wilt of banana caused banana bunch weight reduction of up to 78% in severely affected plants due to poor development of fingers.

Viral diseases such as banana bunchy top virus (BBTV) is one of the most popular disease in the world, it has spread from Fiji to many parts of the world, with up to 90% destruction of crop in Queensland (Fist, 1970). Banana bunchy top disease occurs in 36 countries in Africa, Asia, and Oceania (Kumar *et al.*, 2011). In Africa, BBTD is most common at elevations below 1300m and sparsely above 1700m in the hills in eastern DRC. Records of losses from BBTD outbreaks in Africa are lacking (Kumar *et al.*, 2011).

Banana streak virus (BSV) causes chlorotic streak disease and is known to be the most widely distributed virus affecting banana and plantain around the world contributing to yield losses up to 90% on Poyo (AAA, Cavendish subgroup) (Daniells *et al.*, 2015). Banana streak virus can be transmitted by mealy bugs (Kubiriba *et al.*, 2001).

Banana bract mosaic disease (BBMD) caused by the banana bract mosaic virus (BBrMV), was first reported in 1979 in the Philippines (Magnaye & Espino, 1990). Currently it has spread in many countries in Asia and southern America. Yield losses of between 30% and 70% in India and Philippines were reported (Cherian, *et al.*, 2002). The virus is transmitted through infected planting materials and by several aphid species (Kumar *et al.*, 2015). Bananas are also known to be susceptible to five other viruses of minor significance, such as Abaca mosaic virus, Abaca bunchy top virus, Banana mild mosaic virus, Banana virus X, and Cucumber mosaic virus (Kumar *et al.*, 2015).

## 1.3.2 Pests of Banana

### 1.3.2.1 Banana weevils (*Cosmopolites sordidus*)

The banana weevil, *Cosmopolites sordidus* Germar is an important pest in banana. Banana weevil damage is caused by the larvae which feed and create tunnels into the banana corms resulting into snapping of plants Figure 1, prolonged maturation rates and reduced yields. Severe infestations by this damaging insect pest can lead to total crop failure resulting into 100% yield loss (Sengooba, 1986). Kiggundu *et al.*, (2007) reported that EAHB are highly susceptible.



Figure 1: A snapped banana stem following severe infestation by the banana weevil, *Cosmopolite sordidus*

### 1.3.2.2 Banana nematodes

The production of bananas in Africa is mostly threatened by the presence of plant-parasitic nematodes; burrowing nematode *Radopholus similis* [Cobb] Thorne, root-lesion nematode *Pratylenchus. goodeyi* [Sher] et Allen, and spiral nematode *Helicotylenchus. multicinctus* [Cobb] Golden (Speijer et al., 1994). Nematodes pose a serious threat to sustainable banana production. They feed, multiply and migrate with-in the banana roots and the corm tissue, this results into necrotic lesions and results into poor root development, thus reducing the plant's ability to uptake enough water and nutrients, this leads to delayed flowering, ratooning and reduced bunch size. Furthermore, destruction of the plants roots by the nematodes results into reduced plant anchorage leading to plant toppling especially during bunch filling (Stover and Simmonds, 1987). Plant parasitic nematodes contribute losses of up to 70% on plantains and

cooking bananas in Africa (Tripathi *et al.*, 2015). The most destructive species in Uganda is *R. similis* (Barekye *et al.*, 2000).

#### **1.4 *Radopholus similis* (Cobb) Thorne**

The burrowing nematode *R. similis* (Cobb) Thorne is the most economically important nematode parasite of banana. It belongs to the family Pratylenchidae, order Tylenchida and class Secernentea (Siddiqui, 2000). The order Tylenchida contains the most important plant-parasitic nematodes in the world (Luc *et al.*, 1990). It attacks the plant's roots and rhizomes resulting in whole plant toppling and reduced yield (Gowen *et al.*, 2005). *R. similis* causes yield losses of 30-60% and this is associated with high nematode count (Plowright *et al.*, 2013). *R. similis* and *H. multicinctus* are reported to cause yield losses up to 50% in Costa Rica and Panama, 90% in Nigeria, over 56% in Ghana, and 31-58% in Uganda depending on the varieties (Mukasa *et al.*, 2006).

*R. similis* is widespread in most tropical and subtropical banana and plantain growing areas in the world (Sarah *et al.*, 1996). This includes Africa, Asia, Central and South America Caribbean islands and Pacific (Jones *et al.*, 2013). It is still absent in many countries growing banana such as Israel, the Canary Islands, the Cape Verde Islands, Cyprus, Crete and Mauritius (Marin *et al.*, 1998). Its spread world-wide is believed to be from the distribution of infected banana planting materials (Price, 2000), its spread can be limited by quarantine and the use of *R. similis*- free planting material (Price, 2000).

##### **1.4.1 Morphology**

*R. similis* is wormlike, about 0.65 millimeters long, 25 micrometers wide and colourless (Agrios, 2005). The species exhibits pronounced sexual dimorphism. Male nematodes possess a raised lip region and poorly developed stylet. The male has a sharp, curved spicule, enclosed in a bursa, or sac (Figure 1A and B). Females have a heavily sclerotized and thickened framework. The female stylet is robust with three distinct knobs. The vulva, the opening of the reproductive system, is located slightly below mid body (figure 2A and B) (Agrios, 2005). Juveniles are often present in both root and soil samples.

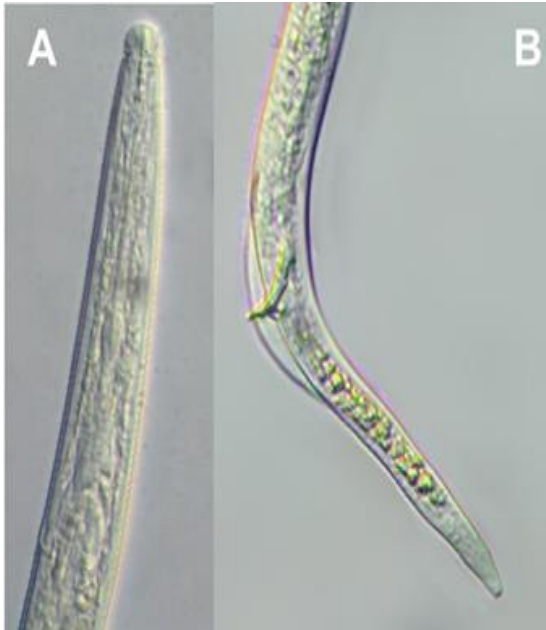


Figure 2: Illustration showing lip region (A) and spicule (B) of male *Radopholus similis*

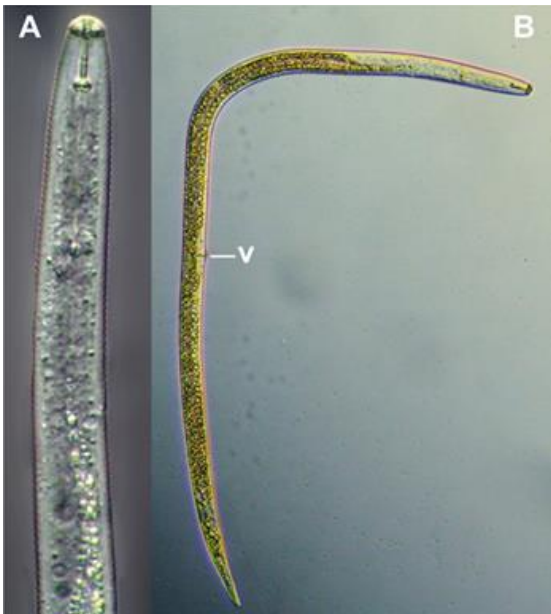


Figure 3: Illustration showing lip region and vulva of female *Radopholus similis*

Photograph by Nicholas Sekora, University of Florida, Entomology and Nematology Department.

#### 1.4.2 Life cycle and damage

*R. similis* is a migratory endoparasite and completes its life cycle in 20-25 days in the roots and corm of banana plants. The optimum reproduction temperatures for *R. similis* is around 25-30°C; it cannot reproduce below 16°C or above 33°C (Sarah *et al.*, 1996). Its reproduction is by amphimixis, however parthenogenesis does occur. For about 7-8 days, eggs are laid in infected tissues at an average of four eggs per day (Brooks, 2008). Males have a degenerate stylet and counted as non-parasitic while adult females and juvenile are the active mobile

forms and are infectious (Sarah *et al.*, 1996). *R. similis* migrate inter and intra-cellular, feeding on the cytoplasm of cortical cells, collapsing cell walls, and causes cavities and tunnels which evolve as a necrosis and may extend over the whole cortex and observed as red-black lesions (Sekora and Crow, 2002). Bacterial and fungal infection can increase necrosis of root and corm tissues (Duncan and Moens, 2006). The destruction of root and corm tissues by *R. similis* leads to reduced water and nutrient uptake. This in turn leads to a reduction in plant growth and yield. Furthermore plant anchorage in the soil is affected, resulting in increased toppling or uprooting of plants (Figure 4) especially those bearing fruits (Gowen and Quénéhervé, 1990). Other symptoms include lack of vigour, leaf yellowing, and premature defoliation, reduction in size and number of leaves and susceptibility to wilt.



Figure 4: Banana plant toppled due to nematode damage

### 1.4.3 Control measures

Yield loss due to Nematode infestation has been controlled by taking some measures such as staking of pseudo stem to avoid toppling, application of mulch and manure, agriculture wastes and addition of organic materials to improve microbial activities that act as biological controls (Gowen *et al.*, 2005 and Quénéhervé, 2008).

Preventive measures have been taken, and these include;- (1) Use of tissue culture plantlets and suckers from nematode free fields (2) Paring and hot water treatment of suckers

(Tenkouano *et al.*, 2006) (3) Chemical treatment of planting material before planting (Quénéhervé, 1993b). Cultural methods have also been applied aimed at reducing the number of *R. similis* population and these include; fallowing the land for 5-12 months, intercropping and crop rotation, mulching and fertilizer/manure application (Quénéhervé, 2008).

Biological means have been applied and these include the use of fungi *Paecilomyces lilacinus* to parasitize eggs, juveniles and adults *R. similis*. Bacteria *Pseudomonas fluorescens* inhibit the invasion of banana roots by *R. similis* (Aalten *et al.*, 1998). Arbuscular mycorrhizal fungi plate used as the source of nutrients and reduces penetration and development of *R. similis* in banana (Fogain and Njifenjou, 2003). *Tithonia diversifolia* if used for mulching adds organic matters and reduces nematode damage (Coyne *et al.*, 2005b)

Chemical control has been an effective method to control *R. similis* widely used by growers producing fruits for international export trade (Quénéhervé, 1993b). Nematicides interfere with the nervous system and interrupt with the nematode's ability to hatch from eggs, move, penetrate the roots, feed and reproduce. The products currently registered are: cadusaphos, fosthiazate, ethoprophos, carbofuran and oxamyl (Gowen *et al.*, 2005). However, the use of these nematicides has led to different drawbacks, e.g. soil and water contamination, loss of efficacy through microbial biodegradation (Quénéhervé, 2008).

Above all the efforts that have been applied to reduce nematode damage, use of resistant cultivars is the most cost effective and sustainable solution to combat the effect caused by nematode infestation (Speijer and De Waele, 1997). Use of resistance cultivars assures no toxic residues, no special application techniques or equipment required and no additional cost to farmers, It is an appropriate and long term solution to the nematode problem in smallholder production systems. Therefore breeding for nematode resistance is a key criterion in banana breeding.

## **1.5 Breeding for nematode resistance**

### **1.5.1 Resistant variety**

Use of resistant varieties has been the best possible solution to combat the effect and loss caused by burrowing nematodes, this is because there is less use of Nematicides, low cost, and environment friendly (Speijer and De Waele, 1997). Resistance is typically defined as the plant's ability to inhibit nematodes reproduction relative to susceptible genotype (Trudgill, 1991 and Robert, 2002). Plant genotype can either suppress (resist) or allow (susceptible)



nematode development and reproduction; also can either allow little injury (tolerate) even under heavy nematode infestation or much injury can be inflicted (sensitive) even when nematode levels are relatively low (Trudgill, 1991).

Normally nematode resistant varieties are obtained by selection of plants with reduces nematode reproduction rates even though resistance is subjective to traits and nematode species and sometime haplotype (Starr *et al.*, 2002). The Musa genome has wide range of resistance alleles which are both horizontal and vertical resistance and can be monogenic, oligogenic or polygenic (Van der Planck, 1963). Resistance to nematodes infection is more durable by pyramiding multiple resistance genes because the plant parasitic nematode occurs in multi-species communities with one species usually predominant (Starr *et al.*, 2002). Resistance for multiple species is the durable option for many small scale farmers (Gowen, 1996)

### **1.5.2 Mechanism of resistance**

Several mechanisms have been elucidated to help explain plant resistance to nematodes, these include; structural changes in the plant's cell wall to inhibit nematode penetration, production of toxic or deterrent chemical compounds. Lignin and phenolic compound especially phenyl propanol has been postulated as contributing to the resistance of nematodes in bananas (Valette *et al.*, 1998), lignin plays a role by forming a physical barrier for nematode penetrations in banana. Fogain and Gowen, 1996 observed that lignification in Pisang Jari Buaya and accumulation of phenolics in Yagambi Km 5 this observation indicate that lignin and phenolic compounds might be involved in nematode resistance in banana. When a nematode penetrates a banana plant it localizes food sources with the help of amphids – the sensory chemoreceptors. Through the stylet, the nematode causes mechanical damage to the host tissue and secretes enzymes which dissolves the cell wall enabling the nematode access to the cytoplasm and its contents (Giebel, 1974), after injury, the accumulation of phenols inhibits enzymatic hydrolysis and thus deprives the pathogen nutrients thus limiting pathogen development and also enhances repair of the damaged tissue (Nicholson and Hammerschmidt, 1992). Artificial mechanisms have been adopted in developing transgenic Cavendish banana by adding a gene coding for the protein cystatin which prevents digestion in parasitic nematodes hence suppress nematode growth, and reproduction (Atkinson *et al.*, 2004)

### 1.5.3 Source of resistance

Worldwide scientists have confirmed source of resistance to *R. similis*, Pisang Jari Buaya and Yangambi (Prince 1994). Pisang Jari Buaya gene pool (AA) have been used in breeding program in Fundación Hondureña de Investigación Agrícola (FHIA) result to different product resistance to *R. similis* such as Synthetic hybrid FHIA-01. Yangambi km 5 (AAA) is resistance to *R. similis* and *Pratylenchus goodye* (Fogain and Gowen, 1998) but have not been used in breeding programme. Wild diploid Musa (AA) lines have been reported to have resistance, some varieties of Bluggoe type (ABB) are quite resistance while in subspecies of *M.acuminata* differ in susceptibility, Gros Michahel (AAA) is more resistance than Cavendish (AAA) cultivars. Additional source of resistance has been identified in Fe'i cultivars Rimina and Menei (Stoffelen *et al.*, 1999c)

### 1.5.4 Heritability and nature of inheritance

Heritability is the ratio of genetically caused variation to total variation (including both environmental and genetic variation). Heritability is a measure on a population in a given environment for a given character. It is very important in selection (in genetic improvement). 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); it is primarily additive type of gene action (Acquaah, 2009). In clonally propagated species (e.g., sugarcane, banana) both additive and non-additive gene actions are fixed and transferred from parent to offspring (Acquaah, 2009).

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 (Vuylsteke *et al.*, 1997). Banana weevil resistance is controlled by Gene(s) with incomplete/partial dominance toward resistant parent in the diploid plantain-banana hybrids (Ortiz *et al.* 1995). *Radopholus similis* nematode resistance is controlled by one or more dominant genes (Rowe, 1991). According to Dochez,

(2004) resistance to *R. similis* in a diploid banana population is controlled by two dominant genes A and B with interactive and additive effects whereby recessive bb suppresses dominant A.

#### **1.5.5 Breeding at International Institute of Tropical Agriculture (IITA)**

IITA collaborate with other research institute in Africa i.e. National agriculture research organisation (NARO) towards the genetic improvement of all Musa types important in the food security for Africa small holder farmers. This effort aims to develop improved genotypes with resistance to multiple diseases and pests, high and stable yield, improved agronomic traits and acceptable fruit quality. IITA and NARO have developed a scheme involves crossing triploid cultivar (EAHB) with fertile diploids (wild banana) to produce tetraploids, selection is done among the tetraploid then crossed with improved diploids to produce sterile secondary triploids with superior character (Tushemereirwe *et al.*,2014). This strategy utilizes the diploids to introgress resistance into the edible EAHB while retaining the farmer-preferred traits in EAHB. Inter-diploid crosses are carried out to improve diploids before crossing with tetraploids to reduce the undesirable traits from the wild diploids. This marks the importance of understand the genetics of resistance gene in diploid banana.

The initial step in any breeding scheme is to identify a source of resistance which can be used in the convectional breeding programmes. Wild species and landraces are useful for contributing genes for resistance to the cultivated gene pool (Pinochet, 1988c). Evaluation of nematodes resistance in F<sub>2</sub> diploid will give the opportunity to understand the inheritance of the resistance gene(s) of the host plant resistance by determine its inheritance and correlation of character within banana diploid. An efficient way to study inheritance of resistance is to test progeny of appropriate crosses against nematode populations. F<sub>2</sub> population offers adequate segregation in banana where resistance mechanisms can be easily investigated (Muhammad *et al.*, 2014). Little research has been done, screening F<sub>2</sub> diploid banana to determine their level of host plant resistance to banana nematode is essential. This information is important for further improvement of bananas for nematode resistance.

## **1.6 The aims of the study**

The aim for this study was to understand the nature of inheritance of the resistance gene(s) against *R. similis*.

Specific objectives

1. To evaluate the resistance of banana genotypes against the burrowing nematode in an F<sub>2</sub> diploid population
2. To determine heritability of nematode resistance gene in a diploid banana population

## CHAPTER TWO

### MATERIALS AND METHODS

#### 2.1 Location

This study was conducted at the International Institute of Tropical Agriculture in the Banana Breeding and Nematology units at Sendusu- Namulonge, Uganda,

#### 2.2 Description of the site

Sendusu is located at 28 Km North-West of Kampala Uganda, (328340E, 08320N), 1150 m.a.s.l. Sendusu receives a mean annual rainfall of 1300mm, and average temperature is 16-28°C. The soil type is dark reddish-brown loamy with p<sup>H</sup> range from 5.5 to 6.2 (Jagtap, 1993).

#### 2.3 Planting material

This population was generated from the cross between Kasaska (ITC 0591) and Borneo (ITC 0258) by the Banana Breeding program at the National Agricultural Research Laboratories (NARL, Kawanda, Uganda). Kasaska was used as female while Borneo was used as male to generate F<sub>1</sub>. F<sub>1</sub> was randomly selected and it was selfed to generate 242 F<sub>2</sub> genotypes. From a previous experiment Kasaska proved to be resistance and Borneo susceptible to *R. similis* infection when compared with the controls (not yet published). F<sub>2</sub> population, parent and F<sub>1</sub> are grown at NARL, Kawanda.

#### 2.4 Culturing of *Radopholus similis*

The nematode inoculum (*R. similis* population from Namulonge, Uganda) was maintained at the IITA nematology laboratory at Namulonge on carrot discs. *R. similis* is cultured on carrot (*Daucus carota*) discs according to the technique described by O'Bannon and Taylor (1968) and Pinochet et al. (1995).

The nematode populations were sub-cultured every 5 to 7 weeks. The nematodes were collected in a test tube by rinsing the Petri dishes containing the carrot discs with distilled water. The nematodes were surface sterilised with streptomycin sulphate (2,000 ppm) for 4 hours followed by three rinses with distilled water. Carrots were surface sterilised with 96% ethanol and peeled two times. The carrots were then cut into discs of about 5 mm and placed in sterile 35 mm diameter Petri dishes. About 50 nematodes were placed on each carrot disc. The Petri dishes were sealed with parafilm and incubated at 28°C in the dark in an incubator.

## 2.5 Experiment

The experiment was conducted in two series at different times. This was because the 242 genotypes of the population cannot fit in one screen house. Moreover, even if they could, the work load of counting the nematodes at the termination of the experiment would be too cumbersome to handle. This study reports on two series of 66 genotypes. In each of the experimental series, 33 genotypes were collected from NARL, Kawanda. Collected genotypes included 28 F<sub>2</sub> genotypes, two parents (Kasaska and Borneo), F<sub>1</sub> and two control varieties: Yagambi Km5 as the resistant check and Valery as the susceptible check. Three suckers of each genotype were collected, making 99 suckers in total. Suckers were selected for absence of weevil damage, pared to remove all roots and corm tissue that showed any signs of nematode or weevil damage. After paring, the suckers were treated in boiling water for 2-4 seconds.

Hot water treated suckers were planted in 3 wooden boxes (1m×4m ×0.2m) containing steam sterilised sawdust. The genotypes were planted in three replications in a completely randomized design (appendix 1). The plants were maintained in the screen house and were watered to keep the saw dust moist but not so wet.

Eight weeks after planting, four to six well developed primary roots were selected from each sucker. On each of selected primary root a small plastic container (8cm diameter, 5 cm height) filled with steam sterilised sand was placed at a distance of approximately 5 cm from the corm. The plastic cup was modified by cutting a portion out of opposite sides down to at least 1.5cm to enable a root to pass through the cup below the surface and enable the root to be fully covered by sand (Figure 5). The roots were inoculated by pouring a 0.2 ml aqueous suspension containing 50 *R. similis* female nematodes directly onto the 8 cm long root segment, then covered with the sterilizes sand.



Figure 5: Individual root inoculation of a primary banana root with 50 *Radopholus similis* females

## 2.6 Data collection

Each experimental series was terminated at 8 weeks after inoculation with nematodes and nematode assessments conducted on root segments. The 8 cm root segment passing through the cup was cut out, removed and rinsed gently with tap water. Root necrosis was recorded by cutting each root segment longitudinally and the percentage of visible necrotic cortical tissue was scored. For every individual root, necrosis was scored out of 20 and the score was multiplied by five to get the percentage of the damaged root area (Speijer and De Waele, 1997).

Each 8 cm long root segment was cut to approximately 0.5 cm pieces, macerated using Waring laboratory blender for 2×10 sec-period separated by 5 sec interval. Each of the macerated roots was individually placed on a modified Baermann tray for nematode extraction for 48 hrs. The extract was collect into a beaker and modified to 25 ml volume. The number of nematodes per sample was determined by counting the female, male and juvenile nematodes from three 2 ml aliquots taken from the 25 ml sample.

## 2.7 Data analysis

Data analysis was done on two traits: total nematode count and percentage necrosis. Collected data were cleaned to remove outlier (extremely low and high values of nematode count) and genotypes with low number of roots to maintain at least 3 to 5 roots for each tested genotype. Statistical analysis of the results was done with the software package GenStat 12th (Payne *et al*, 2009). Total nematode count was transformed (square root) prior to analysis to

normalize the data and attain the assumptions for the analysis of variance (Gomez and Gomez, 1984). The significance of the different terms was determined by the analysis of variance, fitting different models based on the aim of the analysis.

### ***Correlation***

Phenotypic correlation among the two traits was determined using the two-sided test of the Pearson's coefficients of Genstat12.

### ***Effect of the experiment***

Because the experiment was conducted in two series at different times of the year, it was important to test whether there was a significant effect of the experimental series (experiment number) on the damage caused by the nematodes. This was done by analysing those genotypes repeated in all the series, namely the parents and checks. The model used was:

$$\text{Response} = \text{General mean} + \text{Genotype} + \text{Replication} + \text{Experiment number} + \text{Genotype*Replication} + \text{Genotype*Experiment number} + \text{Error} \quad (1)$$

Where the term Response represents the two traits (total number of nematodes and percentage of necrosis), and Genotype represents parents, F<sub>1</sub> and checks.

### ***Effect of all genotypes***

The performance of the genotypes was tested by fitting the following model:

$$\text{Response} = \text{General mean} + \text{Genotype} + \text{Replication} + \text{Genotype*Replication} + \text{Error} \quad (2)$$

Where the term Response represents the two traits (total number of nematodes and percentage of necrosis), Genotype represents F<sub>2</sub> genotypes, parents, F<sub>1</sub> and checks., From this model the expected mean and standard error deviation was used to make a graph to present variability among all the genotype.

### ***Broad sense heritability***

For both traits heritability was estimated for each trait separately as the proportion of the total variance accounted for by the genetic variance using the formula (Chahal and Gosal 2002):

$$H^2 = V_g (F_2) / (V_g (F_2) + V_e/r) \quad (3)$$



Where  $V_g (F_2)$  is the genetic variance among  $F_2$  genotype,  $V_e$  is the environmental variance, and  $r$  is the number of replication.  $V_g (F_2)$  was obtained from the Restricted Maximum Likelihood (REML) analysis in GenStat fitting the mixed model:

$$\text{Response} = \text{General mean} + \underline{F_2 \text{ genotype}} + \text{Replication} + \underline{F_2 \text{ genotype} * \text{Replication}} + \text{Error} \quad (4)$$

Where the term Response represents the two traits (total number of nematodes and percentage of necrosis), the terms  $F_2$  genotype and  $F_2$  genotype \*replication were random and the rest were fixed.  $V_e$  was the error variance determined from model (1).

## CHAPTER THREE

### RESULTS AND DISCUSSION

#### 3. RESULTS

For the two traits, total nematode count and percentage necrosis, the checks performed as expected, with Km5 significantly different from Valery, showing the success of the experiment. The two traits were positively correlated, with a correlation coefficient of 0.65, and  $P < 0.001$ ,  $n=340$ .

#### Effect of the experiment:

There was no significant interaction between genotype and experiment number for total nematode count ( $P = 0.44$ , Table 3). The main effect of the experiment number for the same trait was also not significant ( $P = 0.90$ ). On the other hand, the interaction between genotype and experiment numbers was significant for percentage root necrosis at a  $P$  value of 0.005.

Table 3: Analysis of variance for the two traits (parents and checks)

source of variation	Total nematode count			Percentage root necrosis		
	d.f.	m.s.	F pr.	d.f.	m.s.	F pr.
Replication	2	158.14	0.057	2	2325.7	0.002
Genotype	4	5235.4	<.001	4	12857.1	<.001
Replication × Genotype	5	299.21	<.001	5	173.1	0.733
Exp. number	1	0.84	0.899	1	777.9	0.121
Genotype x exp. number	1	31.87	0.437	1	1834	0.005
Residual	46	51.84		42	311.4	
Total	59	426.63		55	1359.8	

#### Effect of genotypes:

Replication had no significant effect on the two traits, neither as in interaction with the genotypes ( $P = 0.17$  and  $0.11$  for total nematode count and percentage necrosis respectively), nor as main effect ( $P = 69$  and  $0.19$  for total nematode count and percentage necrosis respectively, Table 4). However, there was significant effect of the genotypes on the two traits, with a  $P$ -value less than 0.001.

Table 4: Analysis of variance for the two traits (all genotypes included)

source of variation	Total nematode count			Percent necrosis		
	d.f.	m.s.	F pr.	d.f.	m.s.	F pr.
Replication	2	65.8	0.691	2	776.7	0.192
Genotype	45	1955.4	<.001	45	4202.4	<.001
Replication × Genotype	62	213.3	0.17	59	595.6	0.106
Residual	242	177.9		232	466.6	
Total	351	411.4		338	988.4	

In Figure 6, considering the total nematode count, the genotypes ranged from as resistant as Km 5 to as susceptible as Valery, revealing a quantitative nature of nematode resistance. There was obvious segregation transgression, as some of the F<sub>2</sub> genotypes performed better and others worse than the best and the worst parent. Interestingly, the F<sub>1</sub> genotype was not significantly different from Borneo, though theoretically it should be close to half-way from the two parents.

### Broad sense heritability

The two considered traits had high broad sense heritability (Table 5). The value for broad sense heritability for the total nematode count was of 91%, and that of root necrosis percentage was 74%.

Table 5: Heritability

Variance component	Total nematode count	Percent necrosis
V <sub>g (F2)</sub>	180.5	395.5
V <sub>e</sub>	51.84	311.4
H <sup>2</sup>	0.9126	0.745

V<sub>g (F2)</sub> = genetic variance F2 genotype, V<sub>e</sub> = environmental variance, H<sup>2</sup> = broad sense heritability

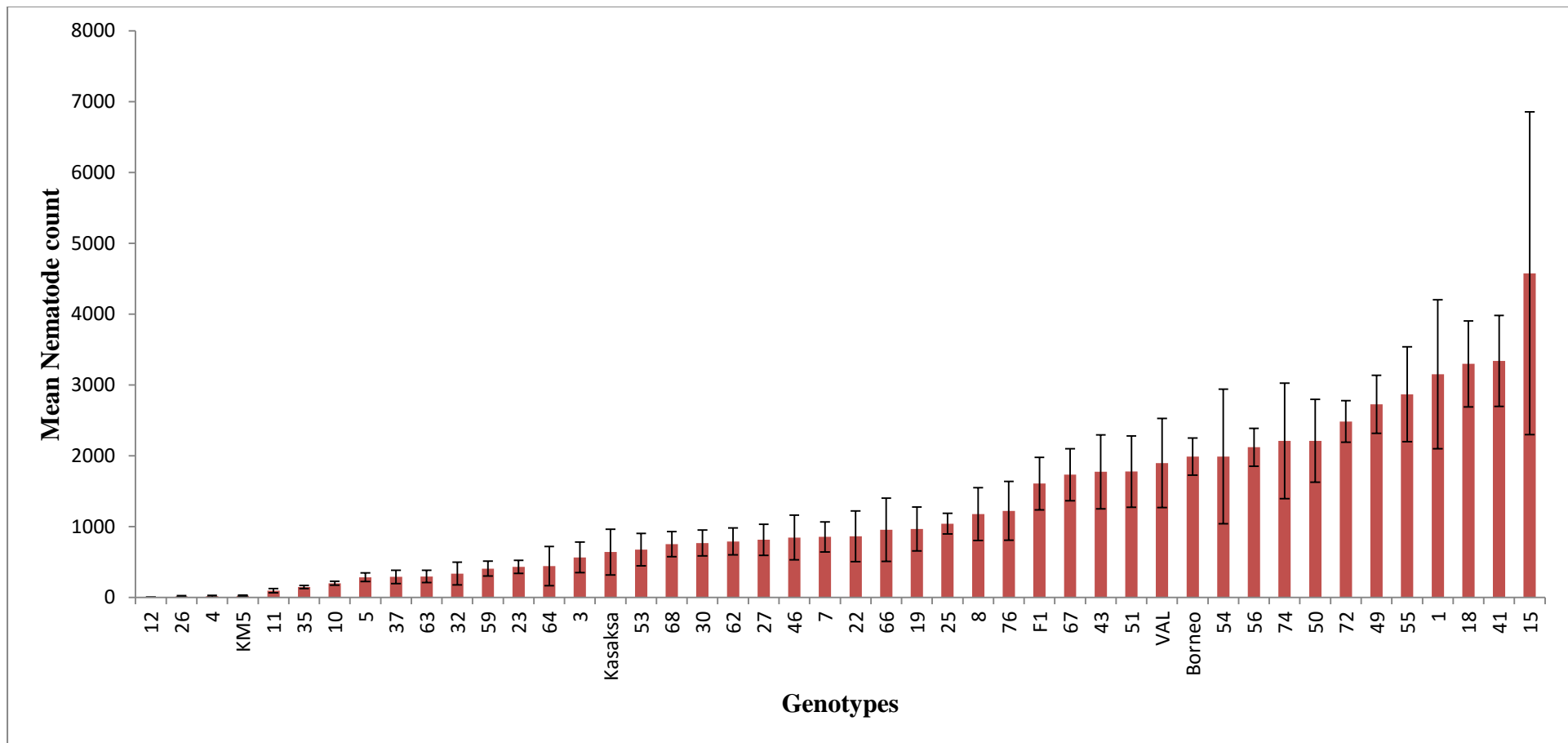


Figure 6: Mean of total nematode count per genotype 8 weeks after inoculation of individual roots with 50 *R. similis* females. The bars indicate the standard error.

### 3.1 DISCUSSION

We studied two traits on the resistance for nematodes in an F<sub>2</sub> population, namely total nematode count and percentage of root necrosis. The two traits had positive correlation indicating that the more the number of nematodes after inoculation the more the damage, the same results was reported by Inamahoro *et al.*, (2011). Regardless the high correlation, total number of nematodes was not affected, the fact that experiment was run in different series, while percentage necrosis was. This reveals the subjective effect of scoring for necrosis. The performance of genotypes was significantly for both traits proof that the population used was segregating and useful for inheritance studies.

The two traits had high heritability in this study. In contrast to Hartman *et al.*, (2010) observed low broad-sense heritability estimated for percentage dead roots, number of large lesions and nematode population density, hence find difficulty to identifying suitable nematode resistance related parameters and breeding for nematode resistance. However, their study was conducted in the field. These values show that there was minimized effect of the experimental or different environmental effects on the traits (Acquaah, 2009). Arinaitwe *et al.*, (2016) observed high heritability value for weevil resistance related traits in the same segregating diploid population. High heritability associated with quantitative segregation of the traits suggest that there few genes or quantitative traits (QTLs) involved in controlling the traits, with one or two of them having a big effect. The results are in line with the findings by Dochez (2004) reported two dominant genes A and B governing nematode resistance in diploid banana, with additive and interactive effects. This implies that selection for these traits during breeding will be effective and fast.

Total nematode count proved to be more objective, more stable (not affected by the experiment), with a higher heritability as compared to scoring root necrosis. This trait should be used in future studies of banana resistance to *R. similis*. This study of a segregating diploid banana population provided valuable quantitative data on the resistance to *R. similis* and gave insight into the possibility of obtaining effective QTLs in the future studies. It has also pinpoint promising genotypes that are highly resistant to be used in future breeding programme.

## CONCLUSION

This study was conducted in a quest to evaluate and determine inheritance of resistance to *R. similis* in an F<sub>2</sub> diploid population. The conclusions drawn from the study are stated below.

- There was obvious segregation of resistance gene
- There was no effect of conducting the experiment in different series, so screening of the entire population can continue as planned.
- The gene(s) controlling total nematode count are high heritable and quantitative hence it might be effective during breeding and selection.
- The data generated are promising in finding QTL associated with *R. similis* resistance in banana.

## WAY FORWARD

This work should be continued to ascertain the response of all the 242 banana accessions towards *R. similis* infestation. The entire population should be genotyped with good quality markers. Resistance to *R. similis* should then be analysed molecularly in order to identify QTL or markers associated with resistance and incorporate them in marker associated selection in banana breeding.

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## APPENDICES

### Appendix 1: LAYOUT- EXPERIMENT 1

<b>Rep1</b>	8	37	Valery	15	36	F1	2	13	39	33	30
	9	3	22	17	10	26	Valery	27	12	1	KM5
	Borneo	28	11	5	32	16	35	34	19	23	4
<b>Rep2</b>	Valery	33	F1	11	1	34	37	3	26	5	36
	39	2	KM5	Valery	13	4	23	17	9	19	15
	27	Borneo	10	35	30	22	16	8	28	12	32
<b>Rep3</b>	11	39	36	32	10	15	33	26	F1	19	4
	22	KM5	12	28	37	8	Valery	34	Valery	2	30
	Borneo	23	3	1	16	17	5	35	27	9	13

### LAYOUT- EXPERIMENT 2

<b>Rep1</b>	63	18	74	72	22	8	67	Borneo	53	25	56
	61	F1	76	54	51	58	62	30	66	KM5	46
	43	68	50	64	27	7	49	Valery	55	Kasaska	59
<b>Rep2</b>	27	50	62	55	56	8	30	64	74	54	63
	59	49	76	22	Kasaska	72	51	Valery	18	61	46
	58	66	25	43	68	7	F1	67	53	Borneo	KM5
<b>Rep3</b>	Kasaska	50	8	43	25	30	22	61	7	46	51
	56	66	55	54	27	58	76	18	64	42	67
	KM5	F1	53	63	59	72	Borneo	68	Valery	49	74



## Appendix 2: Residual plots for the two traits

