Increasing efficiency of the breeding pipeline for East African highland bananas

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Cover: A – Cultivar ‘Entukura’ (3*x* female grandparent), B – ‘1438K-1’ (4*x* female parent) and C – ‘NARITA 17’ (3*x* hybrid)
(photo: Michael Batte)

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Abstract

Banana breeding is a technically challenging endeavor due to the low reproductive fertility, low seed germination, long selection cycle period and large space requirement for field evaluation, among others. Innovations in the current breeding program are required to increase the rate of genetic gain delivery to farmers. This study considers five approaches to tackle this challenge; 1) assessment of the available minimum descriptor list for suitability to characterize the East African highland banana (EAHB) germplasm, 2) analysis of crossbreeding data of EAHB for the first 21 years (from 1995 to 2015) at the International Institute of Tropical Agriculture as a basis for designing future interventions, 3) path analysis to determine a breeding ideotype for EAHB, 4) estimation of heterobeltiosis (hybrid vigour) for the NARITA hybrids (mostly secondary triploids ensuing from the 4x × 2x), and 5) phenotyping a diploid banana population ('Calcutta 4' × 'Zebrina GF') for resistance to *Radopholus similis*. Ten out of 31 descriptors studied were stable but had similar scores in EAHB cultivars and therefore are not suitable to distinguish between them. The month of pollination did not result in significantly different (P = 0.501) pollination success, implying that pollination of EAHB can be conducted throughout the year. However, the seed set, and rate of germination were still low. Thus, further research about seed production and germination is required. Twenty-seven NARITAs were selected for further evaluation in the East African region. Path analysis revealed that fruit length, circumference and number, number of hands and plant cycle number had a direct positive effect on the bunch weight (a proxy for edible yield). Significant progressive heterobeltiosis for bunch weight was found in all the NARITAs. Half of the NARITAs had negative heterobeltiosis for stature. The diploid population was found to segregate for resistance to *R. similis*. Results from Dunnett's test grouped the population in three main phenotypic classes, with 75 susceptible, 17 intermediates and 19 resistant genotypes. Chi-square goodness of fit test revealed that this resembles a phenotypic ratio of 9:3:4 suggesting recessive epistasis.

Key words: bunch weight, descriptor, East African highland bananas, heterobeltiosis, ideotype, NARITA, pollination success, Radopholus similis

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Effektivare förädling av East African highland-bananer

Sammanfattning

Bananuppfödning Bananförädling är tekniskt utmanande då de flesta sorterna sätter få frön som har låg grobarhet och förädlingen kräver långa tidscykler (för korsning-testning-urval) och stor areal under fältutvärderingen. Utveckling av det nuvarande förädlingsprogrammet är nödvändig för att öka odlarnas tillgång till nytt, förbättrat växtmaterial. Fem aspekter för att bättre kunna hantera denna utmaning ingår i avhandlingen: 1) utvärdering av lämpligheten hos den tillgängliga sortbedömningslistan för karakterisering av East African highland-bananer (EAHB), 2) analys av data från de första 21 åren (1995-2015) av det korsningsprogram som bedrivs vid International Institute of Tropical Agriculture för att använda detta som underlag inför utformningen av framtida insatser, 3) path analysis för att identifiera en förädlingsidiotyp för EAHB, 4) uppskattning av förekomst av heterobeltios (dvs avkomman presterar bättre än föräldrarna) bland NARITA-hybriderna (framför allt sekundära triploider från 4x × 2x-korsningar), och 5) fenotypning av en diploid bananpopulation (’Calcutta 4 '×' Zebrina GF ') för kartläggning av resistens mot nematoden *Radopholus similis*. Endast 10 av de 31 studerade deskriptorerna i bedömningslistan var stabila, men befanns inte vara lämpliga för särskiljning då flertalet EAHB-sorter fick samma bedömning för de olika deskriptorerna. Pollineringsframgången var oberoende av vilken månad som pollineringen genomfördes, vilket indikerar att pollinering av EAHB kan genomföras närsomhelst under året. Däremot var frösättningen och frögroningsfrekvensen låg, vilket pekar på att ytterligare forskning om sådana egenskaper behövs. Tjugosju NARITAs valdes ut för vidare utvärdering i Östafrika. Path analysis visade att frukternas längd och omkrets, antal frukter och klasar samt vilken skörd i ordningen (per säsong) hade en positiv effekt på bananstockens vikt (ett indirekt mått på avkastning för konsumtion). Alla NARITA-sorterna uppvisade en positiv heterobeltios gällande stockens vikt. Hälften av NARITA-sorterna visade negativ heterobeltios vad gäller växtsätt. Den diploida populationen segregerade för resistens mot *R. similis*. Jämfört med en resistent och en mottaglig kontroll, grupperade Dunnetts test populationen i tre fenotypiska klasser, med 75 mottagliga typer, 17 mellanformer och 19 resistenta typer. Chi^2-test visade att denna fördelning motsvarar en 9:3:4 fördelning, vilket indikerar recessiv epistasis.

Key words: bananstockens vikt, deskriptorerna, East African highland-bananer, heterobeltiosis, förädlingsidiotyp, NARITA, Pollineringsframgången, *Radopholus similis*

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Dedication

To my family for their support and encouragement

“All things work together for good to those who love God, and to those who are the called according to His purpose”

Romans 8: 28

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Batte Michael, Mukiibi Alex, Swennen Rony, Uwimana Brigitte, Pocasangre Luis, Hovmalm Helena Persson, Geleta Mulatu, Ortiz Rodomiro (2018). Suitability of existing *Musa* morphological descriptors to characterize East African highland 'matooke' bananas. *Genetic Resources and Crop evolution* 65, 645–657

II Batte Michael, Swennen Rony, Uwimana Brigitte, Akech Violet, Brown Allan, Tumuhimbise Robooni, Hovmalm Helena Persson, Geleta Mulatu, Ortiz Rodomiro (2019). Crossbreeding East African highland bananas: lessons learnt relevant to the botany of the crop after 21 years of genetic enhancement. *Frontiers in Plant Science* 10,81. Doi: 10.3389/fpls.2019.00081

III Batte Michael, Nyine Moses, Uwimana Brigitte, Swennen Rony, Akech Violet, Brown Allan, Hovmalm Helena Persson, Geleta Mulatu, Ortiz Rodomiro. Significant progressive heterobeltiosis in banana (manuscript)

IV Batte Michael, Swennen Rony, Uwimana Brigitte, Akech Violet, Brown Allan, Hovmalm Helena Persson, Geleta Mulatu, Ortiz Rodomiro. Path analysis defining an ideotype for East African highland banana breeding (manuscript)

V Batte Michael, Habineza Jean Claude, Kisitu Joseph, Uwimana Brigitte, Kisaakye James, Swennen Rony, Brown Allan, Hovmalm Helena Persson, Geleta Mulatu, Ortiz Rodomiro. Analysis of resistance of a segregating diploid banana population to the burrowing nematode *Radopholus similis* suggests a recessive epistasis gene action (manuscript)

The contribution of Michael Batte to the papers included in this thesis was as follows:

I Planned the experiment with the supervisors, performed experimental work with the second co-author, analyzed data and wrote manuscript with input from other co-authors, was the corresponding author for the journal.

II Participated in data entry and cleaning, analyzed data, wrote manuscript with input from other co-authors, was the corresponding author for the journal.

III Planned the experiment with the main supervisor, performed experimental work, analyzed data and wrote manuscript with input from other co-authors.

IV Planned the experiment with the main supervisor, performed experimental work, analyzed data and wrote manuscript with input from other co-authors.

V Planned the experiment with the supervisors, participated in performing experimental work with other co-authors, analyzed data and wrote manuscript with input from other co-authors.

Related work that **Michael Batte** has been part of but not included in this thesis

I Moses Nyine, Brigitte Uwimana, Rony Swennen, **Michael Batte**, Allan Brown, Pavla Christelová, Eva Hřibová, Jim Lorenzen, Jaroslav Doležel. (2017). Trait variation and genetic diversity in a banana genomic selection training population. *PLoS ONE* 12(6): e0178734. https://doi.org/10.1371/journal.pone.0178734

II Moses Nyine, Brigitte Uwimana, Nicolas Blavet, Eva Hřibová, Helena Vanrespaille, **Michael Batte**, Violet Akech, Allan Brown, Jim Lorenzen, Rony Swennen, Jaroslav Doležel. (2018). Genomic prediction in a multiploid crop: genotype by environment interaction and allele dosage effects on predictive ability in banana. *The Plant Genome*, 11(2).

Abbreviations

AYT	Advanced yield trial
BW	Bunch weight
DH	Days to harvest
DTF	Days to flowering
DTFF	Days to fruit filling
DTM	Days to maturity
EAHB	East African Highland Banana
ECA	East and Central Africa
EET	Early Evaluation Trial
FHIA	Fundación Hondureña de Investigación Agrícola
FC	Fruit circumference
FL	Fruit length
HD	Harvest date
IITA	International Institute of Tropical Agriculture
INSL	Index of non-spotted leaves
ITC	International Transit Centre
MLT	Multilocational trial
NARITA	Banana hybrids jointly produced by NARO and IITA
NARO	National Agricultural Research Organization (Uganda)
NOFB	Number of fruits on the bunch
NOHOB	Number of hands on a bunch
NSL	Number of standing leaves
PYT	Preliminary Yield Trial
SSR	Simple sequence repeats
YLD	Yield potential
YLS	Youngest leaf spotted

1 Introduction

Banana (*Musa* spp.) is an important staple food as well as a favorite fruit crop throughout the world. Bananas are grown in more than 135 countries mainly in the tropical and subtropical regions of the world (Brown *et al*., 2017). In sub-Saharan Africa, bananas are grown mainly by smallholder farmers who eat the fruit and sell the excess to local rural and urban markets (Ochola *et al*., 2013). The Great Lakes region of Africa including countries like Uganda, Kenya, Tanzania, Rwanda, Burundi and the eastern part of the Democratic Republic of Congo (DR Congo) is the home of the East African highland bananas (Karamura *et al*., 2012). In Africa, Uganda is the largest producer and consumer of bananas, with an estimated per capita consumption between 400 to 600 kg per year (Karamura *et al*., 2008), which is ranked as the highest in the world. In Uganda, bananas are grown by 75% of the farmers and cover an estimate of 38% of land under crops (Nowakunda and Tushemereirwe, 2004). Here, the East African highland bananas (EAHB) constitute at least 85% of the bananas grown in the region. Within the East African highlands, banana production requires rainfall amounting between 1,200–1,300 mm year-1 for optimum productivity (van Wesemael *et al*., 2019; van Asten *et al*., 2011). The EAHB fruits are usually boiled or steamed and pounded before consumption. The cooking cultivars in Uganda are called ‘matooke’ and the meal ‘tooke’. However, banana production in the East and Central Africa (ECA) has been declining since the 1970s (van Asten *et al*., 2005). Pests and diseases have been a substantial component of the problem and pose a particularly great threat to the future sustainability of banana production, with a potential for further destabilizing both food security and household incomes across this region. Breeding for host plant resistance to production constraints is the sustainable remedy for the production constraints of this crop (Tushemereirwe *et al*., 2015). However, the process of banana improvement through crossbreeding is slow and less efficient in delivering genetic gains to farmers, taking about two decades to produce a cultivar.

This thesis aimed at finding innovations that can contribute to the improvement of the efficiency of the banana breeding program. The results of this study contribute towards fulfilling the sustainable development goals (Agenda 2030) 1 and 2 which are about ending poverty in all forms everywhere and zero hunger, respectively. This is because the bananas are both food and cash crops to the smallholder farmers in sub-Saharan Africa where poverty and hunger are highest. Therefore, efficient production of new banana cultivars which are high yielding and resistant to production constraints will boost the farmers' livelihoods.

To achieve the aim of the study, five approaches were considered; 1) assessing the suitability of existing *Musa* morphological descriptors to characterise the EAHB germplasm- since variation within the available germplasm is the basis for breeding, 2) analysing banana crossbreeding data for the first 21 years at the International Institute of Tropical Agriculture (IITA) to determine what worked and what did not work, as a basis for designing future interventions, 3) estimating heterobeltiosis of the EAHB-generated hybrids in comparison with their female grandparents so as to rate the performance of the hybrids and also to indirectly select parents with combinations that are likely to result in hybrids with heterobeltiotic performance for the desired traits, 4) determining an ideotype for EAHB breeding program, to help in focusing all the breeding efforts towards a clearly defined goal, and 5) phenotyping a diploid population segregating for resistance to burrowing nematode (*Radopholus similis*), which can be used to develop DNA markers to be further employed in marker-aided breeding.

2 Background

2.1 Importance of bananas

Bananas are important food and cash crops for millions of subsistence farmers in developing countries. The majority of bananas are produced for local consumption. Bananas, including plantain, are good sources of carbohydrates, fiber, potassium, phosphorus, calcium, and vitamins A, B6 and C (Ploetz, 2003; Amah *et al*., 2018). The cultivation of bananas has become woven into the socioeconomic life of the communities in Eastern Africa. The crop is used for medicinal purposes, for celebrating marriages and other rituals. Virtually all parts of the plant are used in the homesteads, and many domestic industries which produce products like baskets, carpets, shoes and indoor decorations (Price, 1995). Fresh leaves can be used as plates for eating or for wrapping food parcels for steaming, and dried leaves as strips for weaving of various articles and for roofing shelters. The absence of seasonality in the production ensures food security as farmers can harvest fruits all year around. Bananas also guarantee a regular source of income to the farmers who sell banana-based products. In addition, farmers in the tropics can intercrop bananas with legumes and can feed animals on the by-products (peels and pseudo-stems) of the crops. Environmentally, the banana plantation behaves like “a tropical forest” because once established, it enters into a phase of continuous growth. The highland bananas are principal sources of mulch for maintaining and improving soil fertility and preventing soil erosion. The leaves and stems rot, resulting in organic matter, which enhances good aeration of the soil. In Uganda, banana fruits can be utilized in various ways. They can be steamed and consumed with sauce as a main meal, roasted when ripe, allowed to ripen and then eaten as dessert or squeezed to produce juice and alcoholic drinks. Recently, value addition and innovations based on bananas have been initiated and this has led to banana crisps, banana flour and subsequent products like banana cakes and

bread. There is a high potential for the banana industrial development in Uganda and this will create jobs and improve production and utilization of bananas.

2.2 Banana production constraints

From the 1970s, Uganda started to witness drastic yield declines in traditional banana growing areas of the East and Central regions. This decline led to the replacement of cooking bananas with exotic beer bananas (ABB) and annual crops such as cassava and sweet potato, which tend to be more resilient to harsh conditions (Gold *et al*., 1993). A preliminary assessment of primary banana production constraints suggested that diseases, pests (weevils and nematodes), declining soil fertility, population pressure, shifted attention to other activities, reduced labour supply and other socioeconomic considerations have all contributed to declining of banana cultivation in central Uganda (Gold *et al*., 1993). The major diseases of bananas include: black leaf streak (formerly known as black Sigatoka leaf spot) caused by *Pseudocercospora fijiensis* (Alakonya *et al*., 2018), fusarium wilt or Panama disease caused by *Fusarium oxysporum* f. sp. *cubense* (Viljoen *et al*., 2017), and banana bacterial wilt caused by *Xanthomonas campestris* pv. *musacearum* (Nakato *et al*., 2018). The main banana pests are: banana weevil (*Cosmopolites sordidus*) and burrowing nematodes (*Radopholus similis*).

2.3 Origin and distribution of bananas

Bananas (*Musa* spp.) originated in Southeast Asia and Indochina (Simmonds, 1962). This is where earliest domestication of bananas is considered to have occurred and from there the crop was introduced to other tropical and subtropical regions of the world. Simmonds (1962) further suggested that edible bananas originated from two wild seed forming species –*Musa acuminata* Colla ($2n = 2x = 22$) and *Musa balbisiana* Colla ($2n = 22$), which are the sources of the "A" and "B" genomes respectively. Inter- and intra-specific hybridisation between *M. acuminata* (AA) and *M. balbisiana* (BB) gave rise to diploid ($2x$), triploid ($3x$) and tetraploid ($4x$) bananas with the following putative genome groups: AA, BB, AB, AAB, ABB, AAA, AAAA, AABB and AAAB, respectively. It is postulated that the domestication of a range of AA and AAA bananas originally occurred in Malaysia, followed by their spread through Southeast Asia (Simmonds, 1962). Their spread to areas where *M. balbisiana* occurred (mainly the Indian subcontinent and Philippines) led to hybridization of the two groups resulting in AB, ABB and AAB cultivars.

According to evidence from culture, linguistics, archaeology, combined with genetic results, it was elucidated that the EAHB were brought from the Southeast Asia by the Austronesian-speaking people, who settled along the Indian Ocean islands like Madagascar, and also the East African coast (Perrier *et al*., 2018). Somatic mutations gave rise to the large variability in EAHB cultivars, making East Africa the secondary center of diversity for this banana group. Němečková *et al*. (2018) reported that the EAHB cultivars originated from a single hybrid clone with *M. acuminata* ssp. *zebrina* and ssp. *banksii* being its most probable parents. They further pointed out that, *M. schizocarpa* also is likely to have contributed to the formation of EAHB group.

2.4 Classification of bananas

Bananas (*Musa* spp.) belong to the genus *Musa*, in family Musaceae under order Zingiberales (Karamura, 1998). The genus *Musa* is divided into four sections, *Eumusa*, *Rhodochlamys*, *Callimusa* and *Australimusa*. All the sections have both seeded and non-seeded members. *Eumusa* and *Rhodochlamys* have species with basic chromosome number of 11 ($2n = 22$), while *Callimusa* and *Australimusa* have species with basic chromosome number of 10 ($2n = 20$) (Daniells *et al*., 2001). Section *Australimusa* is a poorly understood group of bananas. The parthenocarpic edible bananas of this group are also known as Fe'i cultivars. These can be distinguished from other cultivated bananas by their erect fruit bunches and generally red sap. Fe'i bananas are believed to have been originally distributed from the Molluccas to Hawaii and Tahiti islands in the Pacific. They are a good source of food, fibre for making ropes and weaving, and a dark red dye could be extracted from the pseudostems of these bananas. The most likely ancestors for this group have been speculated to be *M. maclayi*, *M. peekelii* and *M. lolodensis*. This section has wild species /groups; *M. maclayi*, *M. peekelii* and *M. lolodensis*, *M. textilis*, *M. jackeyi* and *M. bukensis*, while the cultivated species/group is Fe'i. Section *Callimusa* has wild species/groups; *M. coccinea*, *M. violascens*, *M. gracilis*, *M. borneensis*, *M. beccarii*, *M. salaccensis*; while Section *Rhodochlamys* has wild species/groups; *M. laterita*, *M. ornata*, *M. sanguinea* and *M. velutina.*

Section *Eumusa* is the biggest and most geographically widespread with species being found throughout Southeast Asia from India to the Pacific Islands. Most cultivated bananas arose from this section. The section contains 11 species; *M. acuminata*, *M. balbisiana*, *M. schizocarpa*, *M. basjoo*, *M. itinerans*, *M. flaviflora*, *M. sikkimensis*, *M. cheesmani*, *M. nagensium*, *M. halabanensis* and *M. ochracea*.

Most cultivars are derived from *M. acuminata* and *M. balbisiana*. However, some cultivars derived from hybridizations with *M. schizocarpa* (S genome) as reported by Daniells *et al*. (2001). Likewise, ancient hybridization between *M. balbisiana* and *M. textilis* (T genome) led to a Philippine clone named 'Butuhan'.

Musa acuminata is the most widespread of the *Eumusa* species, with nine wild subspecies/subgroups; *banksii*, *burmannica*, *burmannicoides*, *malaccensis*, *microcarpa*, *truncata*, *siamea*, *zebrina* and *errans.* The cultivated *M. acuminata* diploid (AA) subspecies include; Sucrier, Pisang Jari Buaya, Pisang lilin, Inarnibal and Lakatan**.** The cultivated *M. acuminata* triploid (AAA) subspecies include; Gros Michel, Cavendish, Red, Ambon, Ibota, Mutika/Lujugira, Orotava and Rio.

The EAHB cultivars belong to Mutika subgroup (Perrier *et al*., 2018), which is further subdivided into five clone sets according to their morphological characteristics (Karamura and Pickersgill, 1999). These clones set groupings are named as Nakabululu, Nfuuka, Nakitembe, Musakala and Mbidde. The first four clone sets contain cultivars used for cooking, while the last clone set has cultivars used for making juice/bear.

2.5 Banana descriptors

Morphological descriptors are commonly used for characterizing plant genetic resources. These descriptors should be distinctly identifiable, stable and heritable. They should also show consistence in expression and easy to distinguish by human eye. Simmonds and Shepherd (1955), used 15 morphological characters for the first time to characterize bananas.

The International Plant Genetic Resources Institute - renamed as Bioversity International– developed a list of 74 morphological descriptors for bananas (IPGRI, 1996), of which 25 were related to the inflorescence, 25 cluster-related and 24 about the general plant appearance.

The Taxonomic Advisory Group (2010), basing on the Global Conservation Strategy for *Musa* species defined a list of a minimum set of descriptors (32) for characterization and documentation of bananas. Recently, the process of developing a minimum set of morphological descriptors for each of the different banana groups, instead of a blanket set of descriptors, began. MusaNet published a minimum list of descriptors (33) for Plantains (TAG, 2016), and a new minimum list of descriptors for EAHB which is composed of 18 descriptors, derived from the flower, fruit and vegetative parts of the banana plant (TAG, 2017).

2.6 Banana crossbreeding

Crossbreeding bananas is a technically challenging endeavour due to several reasons, which include low female and male fertility, low seed germination rates, large field space requirement for evaluation (6 to 9 m^2 per plant) and long selection cycle period (about 10 years). Most of the cultivated and commercial bananas are triploids, a condition which makes the process of cell division irregular resulting in the formation of unreduced gametes (2*n* eggs). The identification of female fertile triploid bananas was a major breakthrough in banana breeding. The triploid landraces were crossed with 'Calcutta 4', which is a wild diploid with multiple resistances to pathogens and pests. The hybrid progeny generated were of mixed ploidy levels, but the majority were tetraploids. These primary tetraploids were more fertile than their triploid parents and therefore were further crossed with improved diploids to produce secondary triploids. The secondary triploids are preferred for release to farmers because they are less fertile compared to the primary tetraploids, hence there is reduced risk of setting seeds on farmers' plantations. The banana breeding pipeline comprises; germplasm characterization; selection of parents with desired traits; pollination; harvesting of pollinated bunches; ripening of the harvested bunches; seed extraction from ripened bunches; seed cracking to remove embryo; *in vitro* embryo germination, proliferation; rooting; weaning/growth of plantlets in humidity chamber; growth of plantlets in screen house for hardening; evaluation of plants in early evaluation trial (EET); evaluation of plants in preliminary yield trial (PYT); evaluation of plants in advanced yield trial (AYT); evaluation of plants in multilocational trials (MLT); evaluation of plants on farmers' fields under farmer management practices, and finally release of the best performing cultivars. Sensory evaluations can be conducted from the EET level onwards, while ploidy determination, screening for resistance to pests and diseases can be carried out from hardening stage onwards.

2.7 Improving efficiency and increasing genetic gains

Breeding for host plant resistance was identified as the most feasible solution to the banana production constraints. However, crossbreeding is slow and very tedious. It takes about two decades to release a cultivar. Therefore, there is a need for innovations in the breeding pipeline to quicken the breeding process. The ability to select the best parents that can combine well to produce high quality cultivars within a short time is very crucial. This can be achieved by first defining an ideotype or product profile for the breeding program that will help direct all efforts towards a clear and achievable goal. Likewise, by capitalizing

on heterosis or heterobeltiosis the time taken to produce superior cultivars may be shortened. This can be achieved when parents that produce hybrids with high levels of heterosis/heterobeltiosis for desired traits are used in crossing blocks. It is important for the breeding program to have a mechanism of identifying clearly defined products produced in the program and be able to distinguish the new cultivars from those already existing. A suitable list of descriptors is therefore essential to be able to describe effectively the cultivars produced by the breeding program. It also helps the breeder to quickly and clearly identify the plants with the traits of interest in the field which makes the selection for both parents and hybrids easy. Likewise, molecular tools like DNA markers can speed up the breeding process and also cut the costs for breeding. This can be achieved when the offspring of the hybrids are genotyped and screened for presence of traits of interest at the nursery level before planting in the field. This reduces costs for field preparation and maintenance, at the same time avails results of progeny performance much faster, thereby reducing the selection cycle period and increasing genetic gains. However, developing molecular markers is also a process that often requires selection of parents having contrasting alleles for the trait of interest, followed by developing a segregating mapping population for such a trait by crossing the selected parents, then phenotyping and genotyping the mapping population, constructing a genetic map and finally identifying markers linked to the trait of interest using biometrics-led analysis (Collard *et al*., 2005; Sehgal *et al*., 2016).

Further research on pollination conditions and optimisation of embryo culture protocols are also necessary in order to achieve an efficient breeding program that can deliver increased genetic gains. Use of chromosome doubling of diploids to produce autotetraploids can also help fix some traits as well as shorten the breeding cycle as reported by do Amaral *et al*. (2015). They observed that the autotetraploids produced after chromosome doubling of Pisang Lilin performed better than the original diploid Pisang Lilin in terms of bunch weight, and resistance to yellow Sigatoka. However, for the EAHB, use of this method requires to first develop a diploid cultivar with good 'matooke' qualities which can be doubled to produce an autotetraploid that can be crossed with an improved diploid to generate secondary triploids. Employing genomic selection/prediction is another way of increasing genetic gains mainly through the reduction of the selection cycle time. The best parents for further crossing and hybrids with superior or inferior traits can be predicted using prediction models (Nyine *et al*., 2018). However, the available models in bananas still require more optimization of model parameters and validation. Biotechnological tools like genetic transformation can be employed to complement crossbreeding

especially when the source of trait of interest is from a different organism or when most of the desired traits have been fixed through crossbreeding and an additional simple trait is required to be added to the produced hybrid (Tripathi *et al.*, 2010; Tripathi *et al.*, 2019). This can also shorten the selection cycle. However, in most countries including Uganda, the laws governing transgenic materials are not yet in place which can hamper release and adoption of the new cultivar. Ramstein *et al.* (2019) indicated that plant breeding has undergone four major phases. The first phase represents the first 10,000 years of crop improvement. This was characterized by unconscious selection and conscious mass selection. The second phase occurred between early and mid-twentieth century and was characterized by making crosses basing on Mendelian genetics and pedigree selection. The third phase is estimated to have begun about 30 years back, and this is characterized by use of marker-assisted breeding, considering both phenotype data from agronomic traits and genotype data from DNA information. The fourth phase is considered to have begun in the 1990s, and is characterized by use of genetic transformation, genomic selection and genome editing techniques. Crossbreeding of bananas follows a recurrent selection method of breeding. This study contributes more to plant breeding phases 2 and 3 above.

3 Aims and Objectives

The major aim of this work was to study different ways of increasing the efficiency of the East African highland banana breeding pipeline, considering the practices that have been used for the first 21 years of breeding this crop in Uganda by the International Institute of Tropical Agriculture.

The specific objectives of the thesis were to:

- Assessing the suitability of available banana descriptors for characterizing East African highland bananas
- Review the breeding of East African highland bananas for the first twenty-one years: 1995–2015
- Determining grandparent heterobeltiosis of NARITA hybrids
- Identify traits for banana ideotype by path analysis
- Phenotype a banana population segregating for resistance to the banana burrowing nematode (*Radopholus similis*) for use in mapping host plant resistance.

4 Materials and methods

All the trials (both field and screenhouse) were conducted at the International Institute of Tropical Agriculture research station located at Sendusu/Namulonge in Uganda (00º31'47″ N and 32º36′9″ E). The plant spacing for the field trials was 3 m between rows and 2 m among plants within a row, thereby having a plant density of 1667 plants ha^{-1}.

4.1 Morphological characterization of bananas

Eleven female fertile East African Highland banana cultivars from two different clone sets, with 5 to 20 plants per cultivar were evaluated using the minimum descriptors for Musa (Taxonomic Advisory Group, 2010), and according to guidelines by Channeliére *et al*. (2011). Thirty-one descriptors were used to record the morpho-taxonomic characters, 28 of which were qualitative, while three were quantitative. The quantitative descriptors were: fruit length (of the middle fruit of the third hand), number of hands per bunch and number of fruits on mid hand of the bunch. The qualitative descriptors were: sap colour, edge of petiole margin, colour of cigar leaf dorsal surface, bract behaviour before falling, lobe colour of compound tepal, pseudostem height, predominant underlying colour of pseudostem, blotches at the petiole base, petiole canal leaf III, petiole margins, petiole margins colour, bunch position, bunch shape, rachis position, rachis appearance, male bud shape, bract apex shape, bract imbrication, colour of the bract external face, colour of bract internal face, compound tepal basic colour, anther colour, dominant colour of male flower, fruit shape, fruit apex, remains of flower relicts at fruit apex, fruit pedicel length and fusion of pedicels. Size of male bud at harvest, which is the 32nd descriptor according to the minimum descriptor list, was not used in this study because the male buds were removed from plants before harvest to control the spread of banana bacterial wilt. The descriptors related to colour were examined using standard colour

charts developed by the Taxonomy Advisory Group (2010). All descriptor characters were recorded using scores ranging from 1 to 10, in a categorical manner, except the 3 quantitative descriptors which were measured and recorded directly. Data were analysed using R-software version 3.2.0 (R Core Team, 2015). Categorical variables were first converted to binary scale by calculating mode of the data set. The mode scores were given a value of 0 while the non-mode scores were given a value of 1. The data were then analysed by binomial test at 95% confidence level, the null hypothesis being that ''the probability of getting a mode score is equal to the probability of getting a non-mode score ($P = 0.5$)'', while the alternative hypothesis was ''the probability of getting a mode score is greater than 0.5 ($P > 0.5$)''. The means and standard deviations for the quantitative data were calculated. One-way analysis of variance was done for the quantitative data. The stable (monomorphic) descriptors identified in this study were used to compare the 11 'matooke' cultivars with banana cultivars from other groups. Consequently, seven dessert (AAA) bananas, five Asian cooking (ABB) banana cultivars and 15 East African Highland banana cultivars belonging to five clone sets were compared using the identified stable qualitative descriptors. The data for these three additional banana groups were obtained from Musalogue, which is an international catalogue for *Musa* germplasm (Daniells *et al.*, 2001). The data were first converted to binary scale using the mode. Then data were used to cluster the banana groups using Ward's hierarchical agglomerative clustering method (Murtagh and Legendre, 2014).

4.2 Crossbreeding of bananas

Fourty-one EAHB cultivars belonging to five different clone sets and 10 tetraploid hybrids derived from the EAHB were crossed with wild and improved diploid male parents from IITA and the Fundación Hondureña de Investigación Agrícola (FHIA, Honduras) breeding programs and later with some diploids developed after hybridization between the EAHB and wild or improved diploids. Further intercrossing was also done between diploids and diploids, tetraploids and triploids. Pollen from male parents were always collected around 07.00 a.m. from flowers previously covered with cotton bags at anthesis. This was done to prevent pollen contamination from other pollen sources due to insects, bats or birds. Likewise, emerging inflorescences of female parents were bagged with transparent plastic bags, to avoid natural cross pollination with alien pollen until the last flower was pollinated. Hand pollinations were performed daily between 07.30 a.m. and 10.30 a.m. on freshly exposed female flowers by rubbing a cluster of male flowers containing pollen onto the stigma of female flowers. Pollinated bunches were labelled with tags. Bunches were harvested at maturity – when the

first fruit started turning yellow – and ripened in a store room until all fruits became yellow and the pulp was soft. Seeds were extracted subsequently and immediately sent to the tissue culture laboratory for embryo culture. This was done because the seeds produced by bananas and plantains are reported to have a high degree of dormancy such that direct germination in the soil is unfeasible (Ortiz and Vuylsteke, 1995). Embryos were excised and germinated *in vitro* according to Vuylsteke *et al*. (1990). *In vitro* seedlings were transferred to the greenhouse nursery for weaning, and ploidy level was determined for each genotype by flow cytometry. Aneuploids or hyperploids (pentaploids and above) were discarded because these hybrids exhibited gross abnormal foliage or stunted growth. The following data were taken from April 1995 to December 2015 and used in the analyses: number of pollination/crossing, ploidy of the female parent, ploidy of male parent, date of pollination, harvest date, date of seed extraction, total number of seeds, number of good seeds (with black hard integuments), number of bad seeds (with brown soft integuments), date of embryo extraction, number of extracted embryos, number of germinated embryos after two months, contaminations, number of weaned plants, and number of hardened plants ready for planting in the field. The total number of seeds per cultivar, total number of bunches of a cultivar pollinated but without seeds, highest number of seeds per pollinated bunch, mean number of seeds per bunch and standard errors, and pollination success for triploid and tetraploid cultivars were calculated. Likewise, the total number of seeds per diploid male used to pollinate cultivars, total number of bunches of cultivars pollinated by a particular diploid male but did not set seeds, highest number of seeds per bunch when pollinated by a particular diploid male, mean number of seeds per bunch pollinated by particular diploid male and standard errors, and pollination success for 29 diploid males were calculated. The pollination success throughout the 21 years under study was computed as:

$$Pollination\ success\ = \frac{Number\ of\ pollinated\ bunches\ with\ seeds}{Total\ number\ of\ pollinated\ bunches} x100$$

A one-way analysis of variance (ANOVA) at 95% confidence level was done to study the effect of month on pollination success using R-software version 3.4.1 (R Core Team, 2017). The mean number of seeds per cross type and standard errors, mean embryos extracted and standard errors, mean embryos germinated and standard errors were calculated, and seed embryo germination success per cross type over a period of 11 years (from 2005 to 2015) was obtained as:

$$Seed\ embryo\ germination\ success = \frac{Number\ of\ germinated\ embryos}{Total\ number\ of\ extracted\ embryos} x100$$

4.3 Field evaluation of bananas

Fifty-six banana genotypes comprising hybrids, their parents and grandparents were planted in a 7 × 8 rectangular lattice design with two replications. Data were recorded over three cycles on planting date, date of flowering, height of plant at flowering, number of standing leaves at flowering (NSL), youngest leaf with at least 10 necrotic spots at flowering (youngest leaf spotted or YLS), plant girth at 100 cm from the ground, harvest date (HD), bunch weight (BW), number of hands on a bunch (NOHOB), number of fruits on a bunch (NOFB), fruit length (FL) and fruit circumference (FC). The plant stature (STATURE) was computed as the ratio of plant girth at 100 cm mark to plant height at flowering. The number of days to flowering (DTF) was obtained by counting the number of days from planting or sucker emergence (for ratoons) to the appearance of inflorescence. Number of days to maturity (DTM) or length of growth cycle were obtained by counting the number of days from planting or sucker emergence (for ratoons) to harvest of bunch. The days to fruit filling (DTFF) were obtained by counting the number of days from flowering to harvest. The planting dates for second and third cycles were obtained by recording the dates of sucker emergence from the soil. The index of non-spotted leaves (INSL) –which indirectly measured host plant resistance to black leaf streak caused by the fungus *Pseudocercospora fijiensis*– was computed using the formula below:

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

When the YLS was 0, the above formula was modified in such a way that YLS = NSL+1

Yield potential (t ha $^{-1}$ yr $^{-1}$) was calculated as:

$$YLD = BW \times 365 \times 1667 / (DH \times 1000)$$

where, YLD is yield potential (t ha $^{-1}$ yr $^{-1}$), BW is bunch weight (kg) and DH is days to harvest. The mean bunch weights and standard errors were calculated and used to determine heterobeltiosis using the formula:

Heterobeltiosis (%) = [("NARITA" mean bunch weight - "3*x* Grandmother" mean bunch weight)/ "3*x* Grandmother" mean bunch weight] x 100

Likewise, the mean plant stature and standard errors were calculated and used to determine heterobeltiosis using the formula:

Heterobeltiosis (%) = [("NARITA" mean plant stature - "3*x* Grandmother" mean plant stature)/"3*x* Grandmother" mean plant stature] x 100

Means of grandmothers were used to calculate heterobeltiosis of hybrids instead of their parents because the direct parents are not suitable for consumption and therefore not ideal for comparison, hence the type of heterobeltiosis calculated is grandparent heterobeltiosis. Broad sense heritability for yield, bunch weight, and plant stature, were estimated. Genotyping of this population was done using 19 informative *Musa* SSR primers following the protocol of Christelová *et al.* (2011). Squared Euclidean distances between genotypes were calculated using R software v3.4 (R Core Team, 2018). Hierarchical clustering was done with the function hclust based on ward. D2 method (Ward, 1963; Murtagh and Legendre, 2014).

Pearson's correlation coefficients between grandparent heterobeltiosis for bunch weight and the genetic distances of parents of NARITAs, genetic distance between NARITAs and their female parent's mother, yield, bunch weight and plant stature were calculated. Also, Pearson's correlation coefficients between grandparent heterobeltiosis for plant stature and the genetic distances of parents of NARITAs, genetic distance between NARITAs and their female parent's mother, yield, bunch weight, plant stature and grandparent heterobeltiosis for bunch weight were calculated using R software v3.4 (R Core Team, 2018).

Path analysis was performed using IBM SPSS version 23 (IBM Corporation, 2015) on the agronomic and yield parameters to investigate the traits that contributed significantly to bunch weight. Bunch weight was the dependent variable while, plant cycle, number of hands on a bunch (NOHOB), number of fruits on the bunch (NOFB), fruit length (FL), fruit circumference (FC), plant stature (STATURE), number of days to flowering (DTF), number of days to maturity (DTM), number of days to fruit filling (DTFF) and index of non-spotted leaves (INSL) were the independent variables.

Path coefficients for direct effects and for indirect effects were calculated as follows: (a) the first step was to run Pearson's correlations of all variables together with bunch weight, with the aim of identifying variables to include in the path analysis. Only variables with a significant correlation ($P \leq 0.05$) with bunch weight were selected for path analysis; (b) the second step was to test for

multicollinearity – a phenomenon where two independent variables are highly correlated. This was done by running Pearson's correlation using only the independent variables that had significant correlation ($P \leq 0.05$) with bunch weight. A correlation coefficient of 0.7 and above between two independent variables was regarded to indicate multicollinearity. In order to correct this effect, one of the two highly correlated variables had to be eliminated from the linear regression model. The selection of which variable to eliminate was based on the *P*-value after a linear regression model was run with all variables included. The variable with a higher *P*-value was eliminated. Then a linear regression of only the independent variables with no multicollinearity, on bunch weight was run; (c) regression analysis on bunch weight was run with only variables that significantly ($P \leq 0.05$) contributed to bunch weight, to get the direct effects; (d) the indirect effects on bunch weight were obtained by running regression models using variables that did not contribute to direct effects (DTM, DTFF and INSL) on the variables with direct effects on bunch weight namely: number of fruits on bunch, plant cycle, fruit length and number of hands on the bunch. Error variance for the dependent variable was also calculated.

A regression of selection cycle means on selection cycle number (C_0 for landraces, C_1 for their derived primary tetraploid hybrids and C_2 for secondary triploid bred-germplasm) was calculated to estimate genetic gain per cycle for bunch weight, growth length (or days to harvest, DH) and yield potential.

4.4 Screenhouse evaluation of bananas for resistance to *Radopholus similis*

An F_1 diploid banana population was generated by crossing 'Calcutta 4' (resistant female parent) and 'Zebrina GF' (susceptible male parent). The ploidy levels of 111 genotypes from 'Calcutta 4' × 'Zebrina GF' population were determined by flow cytometry using a Partec ploidy analyzer (Partec GmbH, Munster, Germany). Seven experiments were carried out in series to screen the 111 F_1 population, with a resistant check ('Yangambi Km5'), and susceptible check ('Valery') plus the parents also included in each of the experiments. The experiments were conducted in a screenhouse. In each of the experiments, F_1 genotypes, parents and the checks were planted in a complete randomized block design with one plant of each genotype in a block. There were three blocks per experiment each made of a wooden box containing sterilized sawdust (Fig. 1). Healthy sword suckers were harvested from the field, their corms were pared to remove roots and treated in hot water (boiling water for 5 seconds) - a modification from the method used by Hauser (2007). These were planted in the sawdust and watered daily to keep the saw dust moist. The nematode inoculum

(*R. similis)* was cultured and maintained on carrot discs (*Daucus carota*) at IITA nematology laboratory at Sendusu according to IITA protocol for *in vitro* culturing of lesion nematodes (Coyne *et al*., 2014). After eight weeks from planting, four to six well developed primary roots were identified after removing the top sawdust and selected from each sucker for inoculation. Each selected root was put in a small plastic container containing sterilised and moist sand and placed at 5 cm from the corm. These plastic containers were modified by cutting a small portion in opposite sides to allow roots to pass through for growth. The inoculation was done by pipetting 0.2 ml aqueous suspension estimated to contain 50 *R. similis* nematodes (female and juvenile) and applied directly on the portion of root surface. The root was then covered with moist sterilised sand and the entire corm and root system covered with sawdust. The experiments were managed by regular watering. After eight weeks the experiments were terminated, and data collected. The plastic cups were carefully excavated with root segments of about 8 cm. These were removed and rinsed gently with tap water. The characteristics scored were; percentage root necrosis and total nematode counts. Data on root necrosis were recorded by cutting each root segment lengthwise and determine the percentage of visible necrotic cortical tissue. Then the harvested roots of 8 cm were individually cut into small pieces of about 0.5 cm, which were macerated in waring laboratory blender twice for 10 seconds, the two periods being separated by an interval of 5 seconds. The individually macerated roots were placed in a modified Baermann tray for nematode extraction for 48 hours. Extracted nematodes were put into a beaker and the volume was adjusted to 25 ml. Final nematodes population density per root was obtained by counting nematodes from 3 samples of 2 ml aliquots of each 25 ml aqueous suspension. An average was calculated for all the roots of a genotype.

The data were subjected to statistical analysis using R software v3.4 (R Core Team, 2018). A Pearson's correlation was run for the two traits. As the study was conducted in a series of experiments (7), at different times during the year, the interaction between genotype performance and experiment was tested by running an analysis of variance using resistant check, susceptible check, female parent and male parent for both total nematode counts and percentage root necrosis, since these four genotypes were present in all the seven experiments. Also, one-way analysis of variance was conducted for F_1 population genotypes using total nematodes counts to test if the genotypes performed significantly different from each other for total nematode counts at ($P = 0.05$). The means of total nematode counts for F_1 genotype were compared with the means of resistant and susceptible checks using Dunnett's test to generate phenotypic classes.

Broad-sense heritability was estimated using both total nematode counts and percentage root necrosis data. In order to assess the proposed inheritance of host plant resistance to *R. similis* in diploid bananas, a chi-square goodness of fit test was used to compare the observed and expected phenotypic ratios.

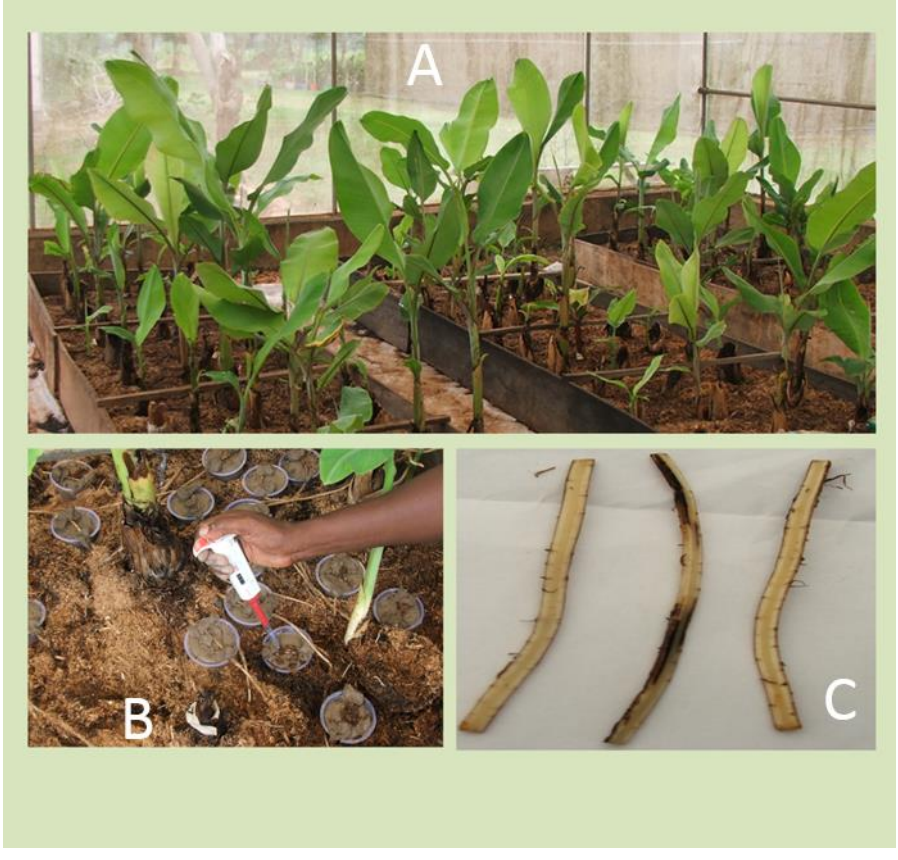

Figure 1. (A) plants randomised in a box trial, (B) inoculation of roots with *Radopholus similis*, and (C) a necrotic root between two healthy roots

5 Summary of Results and Discussion

5.1 Morphological characterization of bananas (Paper I)

The cultivars were significantly different for the quantitative traits. Within each cultivar, stability of qualitative descriptors varied, and hence grouped into highly stable ('***'), moderately stable ('**'), fairly stable ('*'), while others were not stable (NS). This is similar to what Javed *et al*. (2002) observed when they characterized 16 populations of Malaysian wild *M. acuminata* using 46 morphological characters. However, in their study, petiole sheath colour, rachis position and pseudostem colour, were found to be useful characters to distinguish the *M. acuminata* populations, contrary to the findings in this study. Ten descriptors, six of which being flowering related, were stable across all the 11 'matooke' cultivars. However, these stable descriptors had the same mode score across the cultivars. For example, sap colour had a mode of 2 representing milky sap, edge of petiole margin had a mode of 2 representing red–purple color or brown when dried, colour of cigar leaf dorsal surface had a mode of 3 representing medium green color, bract behaviour before falling had a mode of 1 representing revolute (rolling) behaviour, lobe colour of compound tepal had a mode of 2 representing yellow color, bract imbrication had a mode of 1 representing old bracts overlap at apex of bud (no imbrication), compound tepal basic colour had a mode of 2 representing cream color, anther colour had a mode of 6 representing pink/pink-purple, dominant colour of male flower had a mode of 2 representing cream, and fruit shape had a mode of 1 representing straight or slightly curved. This implies that these stable descriptors are not suitable to discriminate between EAHB cultivars. However, they can be used to distinguish the East African highland bananas as a group from other groups of bananas. Only the cultivar 'Tereza' had unique characters which could be used to distinguish it from other cultivars (Fig. 2). These characters are; light green margins with

purple stripes on the bract external face, and the yellow-green bract internal face that turns gradually to orange-red towards the apex. The ten stable descriptors were able to discriminate the EAHB group from other banana groups (Cladogram in Paper 1). Two clusters were formed by the EAHB Cultivars, with Musakala and Nakitembe (except cultivar Mbwazirume) clone sets forming the minor cluster, while the Nakabululu, Nfuuka and the Mbidde clone sets formed a major cluster. This is in agreement with the observation form SSR markers assessment of the genetic variation within and between 53 banana groups by Karamura *et al.* (2016). Several studies about assessing the variation and relationships within and among different banana groups have been conducted using molecular markers. Creste *et al*. (2003) reported that phenetic analysis based on the Jaccard similarity index was in strong agreement with the morphological classification, when they used SSR to analyze 35 polyploid banana cultivars grown in Brazil. Christelová *et al*. (2017) found that 84% of the ITC accessions kept at the global Musa germplasm collection analyzed, using simple sequence repeats (SSR) markers, agreed with the previous morphologically based classification, while the rest did not. However, Kitavi *et al.* (2016), observed no association between genetic diversity classification according to SSR markers and morphological based classification for EAHB germplasm from Kenya and Uganda. Recently, the TAG (2017), introduced a list of minimum descriptors for the EAHB group. This list comprises some descriptors from the former descriptor list and a few new descriptors. Two of the ten stable descriptors identified in this study (bract imbrication and fruit shape/position) were included in the new list of descriptors for EAHB, while three new descriptors (suckers with tubular leaves, position of suckers from parent plant and leaf tip appearance) were introduced. There is a need to further assess this new list of minimum descriptors for suitability to characterize the EAHB. Some of the qualities of a good morphological descriptor are; stability, heritability, distinctiveness, identifiable, easily distinguishable by the human eye, consistence in expression and ability to clearly distinguish the individuals of interest. A minimum set of high-throughput dense DNA markers should be defined for an improved assessment of diversity in *Musa* germplasm (Nunes de Jesus *et al*. 2009), not as a replacement but as a complement to the morphological characterization.

Figure 2. (A) Bunch of cultivar 'Tereza', (B) Male bud of cultivar 'Tereza' showing the light green with purple stripes of bract external face, (C) Bract internal face of cultivar 'Tereza' showing yellow-green with orange-red towards the bract apex.

5.2 Crossbreeding of bananas (Paper II)

Total annual pollination success was better during some years than other years; 2010 being the best with 37.2%, while 1995 was the worst with 5.9%. The month of pollination during a year did not result in significantly different (P = 0.501) pollination success over the 21-year period under this study, according to the results from the analysis of variance at 95% confidence level (Fig. 3). This occurrence implies that pollination of EAHB may be done continuously throughout the year as long as the flowers and pollen are available. However, contrasting results were reported by Ortiz and Vuylsteke (1995) when they crossed triploid plantains at Onne in Nigeria. They observed that the highest seed set was attained at the rain peak in September, while seeds with tetraploid embryos were most likely obtained after pollinations performed between January and mid-March - a period characterized by high temperature, high solar radiation, and low relative humidity. A seed range of 0 to 305 was observed among cultivars after crossing EAHB with wild or improved diploids. Similar results were observed by Vuylsteke *et al.* (1993), when the plantain cultivar 'Bobby Tannap' was crossed with 'Calcutta 4,' where the number of seeds per bunch ranged from 0 to 219. The Nakabululu clone set had cultivars with the highest pollination success (16.9%) followed by the Nfuuka clone set (10%), then the Nakitembe clone set (1.8%) and the Musakala clone set (0.7%), while the Mbidde clone set had zero pollination success. Earlier work by Ssebuliba *et al.* (2005) also revealed that cultivars in the Nakabululu clone set had the highest pollination success (49.2%), followed by the Nfuuka clone set (45.1%), in line with our study. However, they differed from our results in that the Mbidde clone set came third with 8.6% pollination success, then the Musakala clone set (3.9%) and lastly the Nakitembe clone set (1.3%). In our study, the Nakitembe clone

set was in the third place, followed by the Musakala clone set and lastly the Mbidde clone set. Based on these findings, it is advisable for banana breeders to select female parents with good attributes from the Nakabululu and Nfuuka clone sets in order to increase chances of pollination success. Cultivars 'Nakabululu' and 'Nakawere', which had the highest pollination success (34.3% and 31.6% respectively), are missing in the current crossing scheme at IITA for breeding EAHB. Hence, the need to add these cultivars to the triploid banana crossing block. The EAHB derived tetraploids produced, on average, more seeds per pollinated bunch than their triploid parents, with the seed range being 0 to 2279. The highest pollination success for tetraploid females was observed in '1201K-1'(48.4%) with an average of 29 seeds per pollinated bunch, followed by '917K-2' (48.2%) with 39.2 seeds per pollinated bunch, '660K-1' (43.5%) with 14.8 seeds per pollinated bunch, '222K-1' (40.9%) with 16.6 seeds per pollinated bunch and '1438K-1' (37.8%) with 19.3 seeds per pollinated bunch. These tetraploid hybrids, which were the most female fertile are all derived from the female parents from Nfuuka clone set. There was variation in pollination success when diploid males were crossed with females of different ploidy levels. Highest pollination success was obtained when diploid males were crossed with tetraploid females, followed by diploid males crossed with diploid females and lastly when diploid males were crossed with triploid females. The highest pollination success was exhibited by *M. acuminata* subsp. *malaccensis* accession 250 (66.8%) followed by the cultivar 'Rose' (66.6%), which is also of *malaccensis* origin. The above two diploids outperformed 'Calcutta 4' (39.5%), which for a longtime has been known to be the most male fertile diploid and the most used male parent when screening EAHB for female fertility. However, these two accessions result in hybrids with sub-horizontal bunches, which makes them of low breeding value. These accessions can however be useful in screening for female fertility. Seeds from 2*x* × 4*x* crosses exhibited the highest percentage of germination (36%) followed by seeds from 2*x* × 2*x* crosses (22.8%), while seeds from 3*x* × 4*x* crosses had the lowest percentage of germination (6%) (Fig. 4). The percentage seed germination for all crosses combined was (9.6%). Vuylsteke *et al*. (1993) and Talengera *et al*. (1996) also reported that tissue culture could boost rate of seed germination in bananas from 1% (when seeds are planted directly in soil) to a range of 3–10%. The seeds from 3*x* × 2*x* crosses had a higher rate of germination than seeds from 4*x* × 2*x* crosses, yet in field evaluation trials it was observed that there were more hybrids from 4*x* × 2*x* crosses than 3*x* × 2*x*. This is because the 3*x* × 2*x* crosses produce fewer seeds than 4*x* × 2*x* crosses, such that even with the higher percentage of germination of the 3*x* × 2*x* seeds, there are still fewer hybrids compared to the 4*x* × 2*x* derived hybrids. However, these rates of germination are still low

especially for seeds from 4*x* × 2*x* crosses which generate secondary triploid hybrids targeted for release to farmers. Hence the need to optimize the protocol for embryo *in vitro* culture to boost the embryo germination. A total of 7887 crosses were made during the 21 years and a total of 217,599 seeds were produced from these crosses.

The plantlets from the germinated embryos were weaned, hardened in nursery and planted in an Early Evaluation Trial (EET). Twenty EETs were planted during the first two decades, each having an average of 600 genotypes. The most promising hybrids from each EET were selected for further testing in preliminary yield trials (PYT), for growth, bunch, and fruit characteristics during at least two crop cycles (mother plant and first ratoon). At this stage, the sensory attributes of these selected hybrids were also analyzed. The outstanding hybrids were advanced to multisite trials, where their performance was assessed in different agro-ecological zones within Uganda in partnership with NARO. Thereafter the highest performing hybrids were advanced by NARO to on-farm evaluation, where they were planted in farmers' fields and cultivated under farmers' management. This is the final stage of East African highland banana hybrid evaluation before their release as new cultivars. The selection of NARITA cultivars was based on having bigger bunches than their parents or grandparents, host plant resistance or tolerance to black sigatoka, and good culinary attributes. Hence, all NARITA cultivars show bunches bigger than their parents and grandparents and are less affected by black sigatoka. These were therefore regarded as the main output of the banana breeding program. From this study, it was revealed that '917K-2' was the female parent that generated the highest number of NARITAs (9) irrespective of the males, while '9128-3' (bred by IITA in Onne, Nigeria) was the male parent that generated the highest number of NARITAs (8) irrespective of the females. However, the female - male combination that gave the highest number of NARITAs was '917K-2' × 'SH3217', which generated 4 NARITA cultivars namely; 'NARITA 5', 'NARITA 8', 'NARITA 9', and 'NARITA 10'.

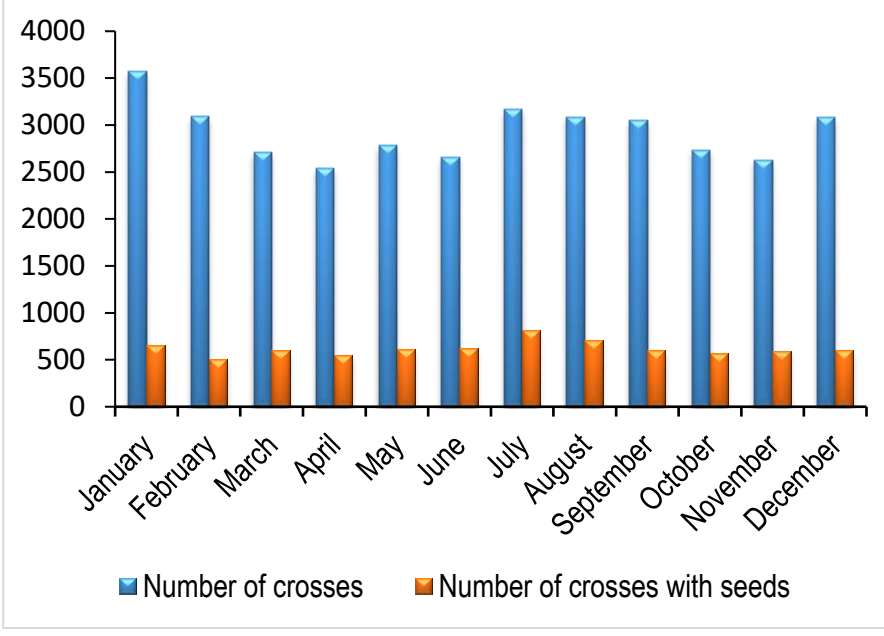

Figure 3. Total number of crosses and crosses with seed per month from 1995 to 2015

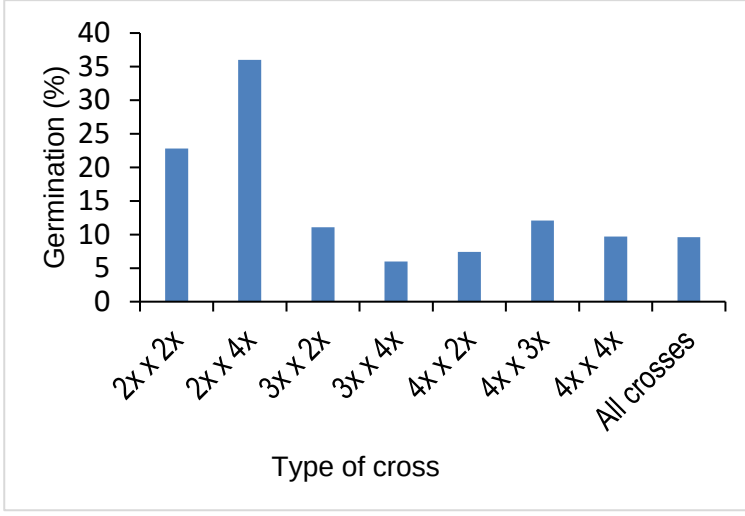

Figure 4. Embryo germination (%) of seeds from different cross types

5.3 Heterobeltiosis and ideotype breeding for improving banana crossbreeding efficiency (Paper III and Paper IV)

Broad sense heritability (H^2) was 0.84 for yield and 0.76 for bunch weight while for plant stature, it was 0.0035. The mean bunch weights for NARITAs ranged from 11.1 kg to 29.3 kg, with 'NARITA 23' having the highest bunch weight while 'NARITA 19' had the lowest bunch weight. However, 'NARITA 17' had the highest yield potential (35.6 t ha^{-1} yr^{-1}), followed by 'NARITA 23' (35.0 t ha^{-1} yr^{-1}) and 'NARITA 18' (34.4 t ha^{-1} yr^{-1}) whereas 'NARITA 19' had the least yield with 14.7 t ha^{-1} yr^{-1}. Similarly, 'NARITA 17' had the highest grandparent heterobeltiosis for bunch weight (248.7%), followed by '26666S-1' (229.3%), and 'NARITA 9' (201.2%) while 'NARITA 19' had the lowest (1.2%). 'NARITA 7', the only released NARITA hybrid cultivar in Uganda so far, had a heterobeltiosis of 77.2%. All the 31 NARITAs available at Sendusu with known pedigrees showed heterobeltiosis for bunch weight, compared to their grandmothers (3*x* 'Matooke'). Plant stature ranged from 0.16 to 0.21. 'NARITA 20' had the highest positive grandparent heterobeltiosis for stature (30.5%), followed by '29285S-20' (27.2%), and 'NARITA 17' (26.9%) while 'NARITA 1' (-18.4%) had the lowest and negative grandparent heterobeltiosis. Half of the NARITAs had negative grandparent heterobeltiosis for plant stature. According to Lamkey and Edwards (1999) and Alam *et al*. (2004), a positive heterosis is desired in the selection for yield and its components, whereas negative heterosis is desired for early cycling and short plant height. However, in our case for plant stature, a positive heterobeltiosis is desirable if we need short plants with robust pseudostems. This is because plant stature as it is used in this study, is a ratio of plant girth at 100 cm mark from the ground to the total height of the plant taken at flowering (girth/height). A short plant with big girth therefore will have a higher value for stature than the tall plant. The results indicate that half of the NARITA cultivars are taller than their grandmothers, which is not desirable for bananas.

There was a positive significant correlation (at 95% confidence level) between grandparent heterobeltiosis for bunch weight and genetic distance for NARITA parents (r = 0.39, $P = 0.036$), bunch weight (r = 0.7, $P < 0.001$), plant stature (r = 0.38, $P = 0.033$) and yield (r = 0.59, $P = 0.0004$). Beche *et al*. (2013) suggested using heterobeltiosis for indirect selection of a trait having significant positive correlation with the heterobeltiosis. In our study, bunch weight showed significant positive correlation with heterobeltiosis (r = 0.7; $P < 0.001$). Hence, these results can be used to select parents that are likely to produce superior hybrids. For example, these could be the parents of 'NARITA 17' ('1438K-1' × '9719-7'), '26666S-1' ('917K-2' × 'SH 3362'), 'NARITA 9' ('917K-2' × 'SH

3217'); NARITA 22 ('917K-2' × '9128-3') and '26874S-5' ('917K-2' × '5610S-1)'. A significant but negative correlation between grandparent heterobeltiosis for plant stature and genetic distance between NARITA parents (r = -0.6, P = 0.0004) was observed. Members of the same known group clustered together, NARITA cultivars, female parents of NARITAs, male parents of NARITAs and female grandparents of NARITAs, except cv. 'Rose' which clustered among the NARITAs between '29285S-20' (which is a progeny having cv. 'Rose' as a male parent) and 'NARITA 5'. There was significant ($P \leq 0.05$) progressive heterobeltiosis for bunch weight in bred 'Matooke' banana hybrids (NARITA) (probably the highest ever documented) when grown together across years with their parents and grandparents in Uganda.

From the regression equation for cycle of selection on yield potential (Y = 13.28 + 5.95X), a unit increase in selection cycle led to an increase in yield potential of six tons per hectare per year. For bunch weight (Y = 10.14 + 4.88X), it indicated that a unit increase in selection cycle resulted in an increase of 4.9 kg of a banana bunch. In both cases above, the increase is significant ($P < 0.001$). The regression of selection cycle on DTM (Y= 472 + 8.5X;) was not significant ($P > 0.05$), indicating that the maturity period of the bananas did not significantly influence selection cycles. The selection cycle time (from seed to seed) was calculated to be 10 years, implying that from available matooke cultivars grown today by Ugandan farmers (C_0) to primary tetraploid hybrids (C_1) took 10 years and from C_1 to secondary triploid bred-germplasm (C_2) it also took another 10 years. The mean bunch weight and yield potential for landraces, primary tetraploid hybrids and secondary triploid bred-germplasm were 17.8 kg and 22.6 tons per hectare per year, respectively. The average genetic gain (from C_0 to C_2) for bunch weight was 1.4% per year [100 × (4.9/17.8)/20], while the average genetic gain per year for yield potential was 1.3% [100 × (6/22.6)/20].

Table 1 provides main features of a matooke product profile and how far the target for each trait was achieved through plant breeding. All the traits evaluated in this study conformed or were very close to the standards of the matooke product profile, except days to maturity (Table 1). Hence the breeding program should consider using parents having shorter maturity periods among other traits, in the crossing block.

Plant stature was not significantly correlated with bunch weight (r = 0.003, P = 0.917) and therefore was removed from the variables for path analysis. DTM and DTF had a high correlation coefficient of 0.86. DTF was eliminated from the model on grounds that its tolerance value was below 0.1, which is indicative of multicollinearity. FL, FC, NOHOB, NOFB and plant cycle were found to have a significant ($P < 0.01$) direct effect on bunch weight with FL and NOFB having the highest direct effects of 0.489 and 0.373, respectively.

DTFF, DTM and INSL had significant indirect effects on bunch weight. DTFF had an indirect effect on bunch weight (0.124) through NOFB and through plant cycle (-0.087). DTM had an indirect effect on bunch weight (0.279) through NOFB, plant cycle (0.339), FL (0.204) and NOHOB (0.121). INSL had an indirect effect on bunch weight (0.108) through NOFB, plant cycle (0.062), and FL (0.075). Hence, an ideotype of EAHB derived secondary triploid hybrid should have many large fruits in each of the many hands. This is in agreement with the results from ideotype determination for False Horn plantains by Ortiz and Langie (1997).

Table 1. Banana product profile with NARITA ranges (based on results used for this PhD thesis)

Banana PRODUCT PROFILE: Matooke					
Region/ Market segment	Trait (economic, sustainability, livelihood) and value	Target trait level	Market Priority	Selection Objective	NARITA ranges (Source Michael Batte PhD Thesis 2019)
Highlands of East and Central Africa					
Fresh market and processing	Yield	25% greater bunch weight than East African highland banana cultivar (e.g. 'Mbwazirume') across a range of soil and management conditions	1	Maximize	Average bunch weight in percentage above East African highland banana grandmothers **(78%)**
	Table quality	A general acceptability score of at least 4 (on a hedonic scale of 1 to 6), using 'Mbwazirume' as a check (acceptability is tested after cooking as taste, aroma, colour, texture/mouth-feel)	1	Reach threshold	TBD [1]
	Earliness: planting to harvest or between ratoon harvests	300 to 390 days	2	Minimize	Range based on average of ratoon cycles **(493 days)**
	Plant stature (girth at 1m/height ratio)	A ratio of at least 0.15	2	Maximize	Average of ratoon cycles **(0.18)**
	Plant height	Less than 350 cm	2	Minimize	Average of ratoon cycles **(315 cm)**
	Suckering behaviour	75% follower sucker growth at harvest	2	Maximize	Average follower sucker growth at harvest in percentage **(68%)**

	Resistance to black leaf streak *Paracercospora fijiensis*	INSL at flowering of 70% and above	2	Reach threshold	Average INSL at flowering in percentage **(79.3%)**
	Resistance to banana weevil *Cosmopolites sordidus*	Resistance higher than that of the susceptible check (Kibuzi)	2	Maximize	TBD
	Resistance to burrowing nematode *Radopholus similis*	Resistance higher than that of the susceptible check (Valery)	2	Maximize	TBD
	Resistance to bacterial *Xanthomonas* wilt of bananas	Sources of resistance to be identified	2	Opportunistic	TBD
	Bunch orientation	Pendulous score of 1 or 2	1	Opportunistic	**Pendulous**
	Drought tolerance (water productivity)	Tools to be developed	3	Reach threshold	TBD
	High ProVitA content	Average -Carotene (µg/100 g) higher than 150	1	Opportunistic	TBD

[1] TBD = to be determined, green colour = target achieved, orange colour = target nearly achieved, red colour = target not achieved.

5.4 Phenotyping bananas for host plant resistance to *Radopholus similis* (Paper V)

The Pearson's correlation coefficient between the total nematode counts and percentage of root necrosis was significantly positive (0.56 and $P < 0.001$). This is in agreement with Dochez *et al*. (2013), Roderick *et al*. (2012), and Moens *et al*. (2001). However, Marin *et al.* (2000) and Hartman *et al*. (2010) reported results contrary to our findings. The analysis of variance for the checks and parents revealed that; for total nematode counts, the effect of genotype was highly significant with $P < 0.001$ while the interaction between genotypes and experiment was not significant, whereas for percentage root necrosis, the effect of genotype was highly significant with $P < 0.001$ but the interaction between genotypes and experiment was significant ($P = 0.037$). Because of this interaction between genotypes and experiment while using percentage of root necrosis, total nematode counts data were used for subsequent analysis to determine the phenotypic classes. Results from one-way analysis of variance of

the F_1 population genotypes using total nematode counts indicated that the genotypes were significantly different in performance regarding resistance to *Radopholus similis* ($P < 0.001$).

Dunnett's test comparing the F_1 population genotypes with resistant ('Yangambi Km5'), and susceptible ('Valery') checks, revealed 19 resistant, 75 susceptible, 10 partially resistant genotypes while the results for seven genotypes were inconclusive. This led to 3 main phenotypic classes: 75 susceptible, 17 intermediate and 19 resistant. The checks performed as expected with 'Yangambi Km5' (resistant check) but significantly different from 'Valery' (susceptible check). A chi-square goodness of fit test estimated that the observed phenotypic values corresponded to a phenotypic ratio of 9:3:4, which indicates recessive epistasis gene action. Dochez *et al.* (2009) reported that resistance of *Musa* against *R. similis* is controlled by two dominant genes "*A*" and "*B*" with interactive and additive effects whereby recessive "*bb"* suppresses dominant *A*, in support of our work. The broad sense heritability was 58% and 41% for total nematode counts and percentage root necrosis, respectively, implying that resistance to *R. similis* can be fixed in bananas through crossbreeding.

6 Conclusions and Future Perspectives

The minimum set of descriptors developed for banana consists of not many (32%) stable descriptors for the characterization of the EAHB, and even the stable ones had the same scores across the tested cultivars making it hard for their use to distinguish between the EAHB cultivars. Thus, the attempt to address this challenge needs to be seriously considered at least for EAHB group, as previously indicated (TAG, 2017). Likewise, a set of genome-wide DNA markers suitable for high-throughput analyses should be established for an improved assessment of genetic diversity in the *Musa* germplasm, which will complement the morphological characterization. A similar kind of research should be initiated on all *Musa* subgroups to find out whether the current minimum set of descriptors are indeed stable or not.

Pollinations in the EAHB breeding program can be performed all year-round whenever flowers and pollen are available. The diploids *M. acuminata* subsp. *malaccensis* accession 250 and cultivar 'Rose' appear to be the most ideal male parents to screen for female fertility. Considering the low female fertility rates of EAHB and their derived tetraploids and the occasional high seed set as observed in 'Nakasabira' (305) and '917K-2' (2279), research is needed to determine factors influencing fertility, so that pollination conditions can be improved to boost seed set. Likewise, the big discrepancy between seeds harvested and embryos germinated (on average, only embryos of 6.8% of seeds germinated) necessitates more research on factors influencing fertilization, seed development and seed germination. This is of particular value for key crosses in EAHB breeding like $3x \times 2x$ and $4x \times 2x$ crosses, which produce the primary tetraploid and secondary triploid hybrids, respectively. The seed fertile EAHB cultivars 'Nakabululu' and 'Nakawere', with the highest pollination success, are not currently in use and hence may need to be considered by the EAHB breeding program. Having only one out of the 27 NARITAs released to farmers in a period of two decades is a clear demonstration that breeding bananas is a challenging endeavor, starting from near sterile cultivars. Therefore, overcoming sterility

barriers and investigating other breeding approaches are required to improve the efficiency of banana breeding programs.

Heterobeltiosis in high yielding banana hybrids was kept after two crossing generations, thus suggesting progressive heterobeltiosis. Since bananas are vegetatively propagated, the effect of heterobeltiosis is easily fixed in the hybrids and will not be lost over time after release and further commercialization of these hybrids. The factors behind heterobeltiosis in banana are yet to be defined. Nonetheless, heterobeltiosis shows the potential to produce high yielding banana hybrids and keep it even after a crossbreeding cycle. The parental combinations that produced hybrids with high levels of grandparent heterobeltiosis can be indirectly selected and given high priority when making crosses.

There were significant increases of bunch weight (kg plant) and yield potential (t ha^{-1} $year^{-1}$) from the cultigen matooke (C_0), their derived primary tetraploid hybrids (C_1) and secondary triploid bred-germplasm (C_2), with genetic gains of 1.4% and 1.3%, respectively. These genetic gains are reasonable but can still be improved. Targeting hybrids with a short maturity period is one approach to further increase the genetic gains since according to the results from the studies, the landrace germplasm, the derived primary tetraploid hybrids and secondary triploid bred-germplasm were not significantly different in terms of maturity period. Related to the above, an ideotype of secondary triploid hybrid produced from the EAHB breeding program that is targeted for release to farmers should have many large fruits in each of the many hands to increase bunch weight.

The 19 diploid genotypes resistant to *R. similis* can be incorporated into the banana breeding scheme after being evaluated for other important traits, as a source of resistance against this nematode. There was a highly positive relation between the total nematode counts and the percentage root necrosis ($r = 0.56$). Therefore, either parameter can be used for assessing the nematode resistance depending on availability of time and resources. The population used for this study segregates for resistance to *R. similis* and therefore can be used for mapping quantitative trait loci associated with the resistance.

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Popular science summary

Banana is an important staple food as well as a favorite fruit crop throughout the world. Bananas are grown in more than 135 countries mainly in the tropical and subtropical regions of the world. The East and Central Africa (ECA) region has over 50% of its cropping area under banana cultivation, which represents around half of the total area under banana cultivation across Africa. In sub-Saharan Africa, bananas are grown mainly by smallholder farmers who eat the fruit and sell the excess to local rural and urban markets. In Africa, Uganda is the largest producer and consumer of bananas, with an estimated per capita consumption between 400 to 600 kg per year, which is ranked as the highest in the world. In Uganda, bananas are grown by 75% of the farmers and cover an estimate of 38% of land under crops. Here, the East African highland bananas (EAHB) constitute at least 85% of the bananas grown in the region. The cultivation of bananas has become woven into the socioeconomic life of the communities in Eastern Africa. The crop is used for medicinal purposes, for celebrating marriages and in other rituals. Virtually all parts of the plant are used in the homesteads, and many domestic industries produce products like baskets, carpets, shoes and indoor decorations. Fresh leaves can be used as plates for eating or for wrapping food parcels for steaming, and dried leaves as strips for weaving of various articles and for roofing shelters. The absence of seasonality in the production ensures food security as farmers can harvest fruits all year around. Bananas also guarantee a regular source of income to the farmers who sell banana-based products. Environmentally, the banana plantation behaves like “a tropical forest” because once established, it enters into a phase of continuous growth. The bananas are principal sources of mulch for maintaining and improving soil fertility and preventing soil erosion. The leaves and stems rot, resulting in organic matter, which enhances good aeration of the soil. Banana and plantain are good sources of carbohydrates, fiber, potassium, phosphorus, calcium, and vitamins A, B6 and C. In Uganda, banana fruits can be utilized in various ways.

They can be steamed and consumed with sauce as a main meal, roasted when ripe, eaten as dessert or squeezed to produce juice and alcoholic drinks. Recently, innovations based on bananas have been initiated and this has led to banana crisps, banana flour and subsequent products like banana cakes and bread. The cooked cultivars in Uganda are called "matooke" and the meal 'tooke'. There is a high potential for the banana industrial development in Uganda which will create jobs and improve production and utilization of bananas. However, banana production in ECA has been declining since the 1970s. Pests and diseases have been a substantial component of the problem and pose a particularly great threat to the future sustainability of banana production, with the potential of further destabilizing both food security and household incomes across this region. Chemicals to kill the pests and to control most diseases are available but they are not affordable to the small-scale farmers who constitute the majority of the banana farmers in ECA. Secondly, the chemicals pollute the environment. The development of cultivars which are resistant to pests and diseases is a sustainable solution. However, the process of developing new banana cultivars is slow and tedious, and it takes about two decades to produce a cultivar.

This thesis aimed at finding innovations that can help speeding up the process of developing new banana cultivars that can be distributed to farmers. This was done through engaging in five activities: 1) assessment of the available minimum descriptor list for suitability to characterize the East African highland banana (EAHB) germplasm, 2) analysis of crossbreeding data of EAHB for the first 21 years (from 1995 to 2015) at the International Institute of Tropical Agriculture as a basis for designing future interventions, 3) analysing hybrids to see whether they outperform their parents, 4) create a picture of the type of banana cultivar that is desirable to the farmer, and 5) evaluate seedlings from crosses in order to find genotypes resistant to the burrowing nematodes.

Only 10 out of 31 descriptors studied were stable but since they had similar scores they are not suitable to distinguish between EAHB cultivars. Hence, the list of descriptors needs to be improved. The month of pollination did not result in significantly different pollination success, implying that pollination of EAHB can be conducted throughout the year as long as the flowers and pollen are available. However, the number of seeds produced and the germination level during the first 21 years of breeding bananas were low. Thus, further research on these aspects is required in order to speed up the production of new cultivars. Two male parents, *Musa acuminata* subsp. *malaccensis* accession 250 and the cultivar 'Rose', outperformed 'Calcutta 4', which up till now has been used to screen for female fertility. Therefore, these two new male parents can be used to test different banana groups for female fertility. Of the twenty-seven banana

hybrids (NARITAs) that were selected for further evaluation in the East African region, one was officially released to farmers in Uganda. The hybrids evaluated had bigger bunches than both their parents and grandparents. The parents which produced hybrids with very big bunches were selected for further crosses to generate more superior hybrids. The traits that according to the farmers were important to include in a new cultivar were, many hands and many large fruits per hand for an increased bunch weight, and resistance to pests and diseases. Out of the one hundred and eleven plants evaluated for resistance to the burrowing nematode, nineteen were found to be resistant, seventeen were of intermediate type, while seventy-five were susceptible. Since this population segregates for resistance to the nematode it can be used for mapping quantitative trait loci associated with resistance.

The results of this thesis contribute towards fulfilling the sustainable development goals 1 and 2 of the Agenda 2030 which are about ending poverty in all forms everywhere and zero hunger. Bananas are both food and cash crops to the small holder farmers in sub-Saharan Africa where poverty and hunger are huge problems. Therefore, efficient production of new banana cultivars which are high yielding and resistant to production constraints will improve quality of life in these communities.

Populärvetenskaplig sammanfattning

Bananer är en viktig basföda och en favoritfrukt världen över. Bananer odlas i över 135 länder, främst i världens tropiska och subtropiska regioner. I Öst- och Centralafrika odlas bananer på mer än 50% av den uppodlade marken, vilket utgör ungefär hälften av det totala arealen bananodling i hela Afrika. I Afrika söder om Sahara odlas bananer främst av småbönder som äter frukten, samt säljer den på lokala marknader på landsbygden och i städerna. I Afrika är Uganda den största producenten av bananer, men här konsumerar man också mycket bananer. Den beräknade konsumtionen per capita ligger på mellan 400 och 600 kg per år, vilket innebär att konsumtionen i Uganda är den högsta i världen. I Uganda odlas bananer av 75% av bönderna och odlingarna täcker ca 38% av den odlade marken. East African highland-bananer (EAHB) utgör minst 85% av de odlade bananerna i regionen. Bananodling är samhällsekonomiskt sett en viktig del av livet i samhällen i östra Afrika. Grödan används för medicinska ändamål, för att fira giftermål och för en rad andra ritualer. Nästan alla delar av växten används i hemmen och många hushåll får en inkomst genom att göra korgar, mattor, skor och dekorationer för inomhusbruk. Färska bananblad kan användas som mattallrikar eller för att göra matpaket för ångkokning. Torkade blad rivs i remsor och används vid vävning av olika föremål och som takläggningsmaterial. Bananer produceras oberoende av säsong vilket garanterar tillgång till födan året runt. Detta medför också en regelbunden inkomstkälla för de bönder som säljer sina bananbaserade produkter. Miljömässigt sett kan bananodlingen ses som en ”tropisk skog” eftersom den går in i en fas av kontinuerlig tillväxt då den en gång har etablerats. Material från bananer används som marktäckning för att bibehålla och förbättra jordmånen samt förhindra erosion. Blad och stjälkar bryts ned och ger organiskt material som förbättrar luftning av jorden. Banan och kokbanan är bra källor vad gäller kolhydrater, fibrer, kalium, fosfor, kalcium och vitamin A, B6 och C. I Uganda tillagas bananer på många olika sätt.

De kan ångkokas och konsumeras med en sås som huvudmåltid, rostas när de är fullmogna, ätas som efterrätt eller pressas för att producera juice och

alkoholhaltiga drycker. Nyligen har man börjar utveckla olika typer av innovationer baserade på bananer och detta har lett till framställning av produkter som bananchips, bananmjöl, kakor och bröd. I Uganda kallas kokbanan för "matooke" och maten som tillagas på dessa bananer "tooke". Det finns en stor potential vad gäller utvecklingen av bananindustrin i Uganda vilket skulle kunna leda till fler jobb, samt förbättra produktionen och öka utnyttjandet av bananer. Bananproduktionen i Öst- och Centralafrika har dock minskat sedan 1970-talet. Skadedjur och sjukdomar har utgjort en väsentlig del av problemet och är ett stort hot mot den framtida hållbara bananproduktionen, och därmed också ett hot mot livsmedelssäkerheten och hushållens inkomster i hela denna region. Bekämpningsmedel mot skadedjur och sjukdomar är tillgängliga, men till ett pris som inte är överkomligt för de småskaliga bönder som utgör majoriteten av bananodlarna i Öst- och Centralafrika. Dessutom förorenar dessa kemikalier miljön. Utveckling av sorter som är resistenta mot skadedjur och sjukdomar är däremot en hållbar lösning. Det är dock en långsam process att utveckla banansorter och det tar ungefär två decennier att ta fram en ny sort.

Syftet med denna avhandling var att identifiera faktorer som kan påskynda utvecklingen av banansorter så att odlarna får tillgång till nytt, förbättrat växtmaterial. Avhandlingens delar omfattade: 1) utvärdering av lämpligheten hos den tillgängliga sortbedömningslistan för karakterisering av East African highland-bananer (EAHB), 2) analys av data från de första 21 åren (1995-2015) av det korsningsprogram som bedrivs vid International Institute of Tropical Agriculture för att använda detta som underlag inför utformningen av framtida insatser, 3) analys av bananhybrider för att se om de presterar bättre än sina föräldrar, 4) definition av den banantyp som odlarna efterfrågar, 5) utvärdering av fröplantor från olika korsningar för att hitta genotyper som är resistenta mot nematoden *Radopholus similis*.

Endast 10 av de 31 studerade deskriptorerna i bedömningslistan var stabila, men befanns inte vara lämpliga för särskiljning då flertalet EAHB-sorter fick samma bedömning för de olika deskriptorerna. Följaktligen måste bedömningslistan utvecklas och förbättras. Pollineringsframgången var oberoende av vilken månad som pollineringen genomfördes, vilket indikerar att pollinering av EAHB kan genomföras närsomhelst under året förutsatt att plantorna blommar och att det finns tillgång till pollen. Däremot var frösättningen och frögroningsfrekvensen låg, vilket pekar på att ytterligare forskning om sådana egenskaper behövs för att kunna snabba upp produktionen av nya sorter. Två (han)sorter, *Musa acuminata* subsp. *malaccensis* accession 250, och sorten ”Rose” presterade mycket bättre än ”Calcutta 4”, vilken hittills har använts för att screena för fertilitet hos (hon)plantor. Följaktligen kan dessa två sorter användas för att undersöka honlig fertilitet i olika grupper av bananer.

Av de tjugosju bananhybrider (NARITAs) om valdes ut för ytterligare utvärdering i den östafrikanska regionen, släpptes officiellt en sort för odling i Uganda. De utvärderade hybriderna hade betydligt större stockar än föräldrar och mor-/farföräldrar. De sorter som gav hybrider med mycket stora stockar valdes ut för ytterligare korsningar för att på så sätt få fram fler överlägsna hybrider. Den banantyp som odlarna efterfrågade bör ha många klasar och många stora bananer per klase för att öka stockens vikt, men ska också ha motståndskraft mot skadedjur och sjukdomar. Av de 111 plantorna som utvärderas för resistens mot nematoden *R. similis* var 19 resistenta, 17 mellanformer och 75 mottagliga. Eftersom populationen segregerade för nematodresistens kan den användas för att identifiera sk quantitiative trait loci kopplade till resistens.

Resultaten från denna avhandling bidrar till att uppfylla mål 1 och 2 i Agenda 2030 för hållbar utveckling. Dessa mål handlar om att avskaffa fattigdom i alla dess former samt att avskaffa hunger. Bananer utgör både mat och inkomst för de småskaliga bönderna i Afrika söder om Sahara där fattigdom och hunger är stora problem. Effektiv utveckling och distribution av nya banansorter som är högproducerande och motståndskraftiga mot skadedjur och sjukdomar skulle kunna bidra till att förbättra levnadsvillkoren för dessa bönder.

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