ENHANCING BANANA (*Musa acuminata*) BREEDING EFFICIENCY: GENOTYPIC POLLEN PERFORMANCE AND THE INFLUENCE OF IRRIGATION AND POTASSIUM ON SEED SET AND VIABILITY

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ABSTRACT

Banana (*Musa spp.*) is a crucial staple food for over 30 million people in Tanzania. Mchare is significant globally as it is one of the ancestors of popular dessert bananas like Cavendish and Gros Michel. However, Mchare is highly susceptible to all potential banana pathogens that cause significant yield losses and threaten food and income security. Developing resistant banana varieties through crossbreeding is essential, with researchers identifying wild banana types as potential sources of resistance. A major challenge in this process is low seed production, which this study aims to address by exploring the effects of pollen quantity, supplemental irrigation, and potassium fertilization on seed set and viability in Mchare banana breeding. The study was conducted at the Nelson Mandela African Institution of Science and Technology (NM-AIST), Tengeru campus, and the World Vegetable Centre in Arusha. Fourteen banana genotypes were analysed for pollen production and viability, selecting the best male and female candidates for crossbreeding under irrigation and rainfed conditions with varying levels of potassium fertilization 0, 150, 300 and 500 g K/mat/year (0, 375, 750 and 1250 kg K/ha/year). Data analysis was performed using the GenStat 21st version. The results showed that Calcutta 4 and Borneo (both wild types) and Huti-White (Mchare sub-group, a landrace) exhibited high pollen production. Calcutta 4 and Borneo produced 31 000 pollen grains per anther, while Huti-White produced 8120 pollen grains per anther. Pollen viability was 74.2% for Calcutta 4 and Borneo and 59.3% for Huti-White, indicating their potential as suitable male parents for breeding programs. It was found that higher temperatures of 25 to 27°C (the temperature range was 18 to 29°C), rainfall 250 to 300 mm per month, and irrigation improved pollen quantity and viability, with the best pollination occurring between 6:00 AM and 10:00 AM. Supplemental irrigation and potassium levels of 300-500 g K/mat/year (750 and 1250 kg K/ha/year) positively influenced seed viability. The study concludes that supplemental irrigation and potassium application are critical for improving pollen and seed quality, recommending 300 g K/mat/year (705 kg K/ha/year) for optimal results as it give significant result like 500 g K/mat/year (1250 kg K/ha/year) so for proper resources immobilization 300 gK/mat/year (705 kg K/ha/year) is recommended. Further research is needed to understand factors affecting pollen viability and seed production, as well as to explore more male parent varieties with superior pollen traits for enhancing breeding success.

DECLARATION

I, Stanley John Bayo, declare to the Senate of Nelson Mandela African Institution of Science and Technology that this dissertation is my original work and has neither been submitted nor concurrently submitted for degree award in any other institution.

Stanley John Bayo Date

The above declaration is confirmed by:

Dr. Pavithravani Venkataramana Date

Prof. Rony Swennen Date

Dr. Allan Brown Date

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance and approval by the Senate of the Nelson Mandela African Institution of Science and Technology a dissertation titled "*Enhancing Banana (Musa Acuminata) Breeding Efficiency: Genotypic Pollen Performance and the Influence of Irrigation and Potassium on Seed Set and Viability*" in partial fulfilment for the requirements for the Degree of Master of Science in Sustainable Agriculture of the Nelson Mandela African Institution of Science and Technology, Arusha Tanzania.

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DEDICATION

With great joy, I dedicate this work to my Wife Ms. Levina Fadhili Moshy, my dad, Mr. John D. Qamara and my mother, Ms. Hilder W. Gunda

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LIST OF ABBREVIATIONS AND SYMBOLS

AA	Diploid Banana with Musa acuminata genome
AAA	Triploid Banana with Musa acuminata genome
AAB	Triploid Banana with two Musa acuminata genomes and one Musa balbisiana genome
AB	Diploid Banana with one Musa acuminata genome and one Musa balbisiana
ABA	Abscisic Acid
ABB	Triploid Banana with one Musa acuminata genome and two Musa balbisiana genomes
ABBB	Tetraploid Banana with one Musa acuminata genome and three Musa balbisiana genomes
ANOVA	Analysis of Variance
ATP	Adenosine triphosphate
BTracT	Banana tracking tool
BW	Bunch weight
CARBAP	African Research Centre on Banana and Plantain
CIRAD	Centre de Coopération Internationale en Recherche Agronomique pour le Développement
CV	Coefficient of Variance
DF	Degree of freedom
EAHB	East African Highland Cooking Bananas
EMBRAPA-CNPMF	Empresa Brasileira de Pesquisa Agropecuária
F PR	F-Probability
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Statistics
FHIA	Fundación Honduras de Investigación Agrícola
FL	Finger length
HCL	Hydrochloric Acid
ID	Identification
IITA	International Institute of Tropical Agriculture
IPNI	International Plant Names Index

IR	Full irrigation
ITC	International Musa Germplasm Transit Centre
JPEG	Joint Photographic Experts Group
K	Potassium
KJ	Kilojoules
LSD	Least significance difference
MS	Means of squire
N	Nitrogen
NaOCL	Sodium Hypochlorite
NARO	National Agricultural Research Organization in Uganda
NFB	Number of fingers per bunch
NHB	Number of fruits per bunch
NM-AIST	Nelson Mandela African Institution of Science and Technology
NPK	Nitrogen: Phosphorus: Potassium
NRCB	National Research Centre for Banana
NSB	Number of seeds per bunch
P	Phosphorus
QR	Quick Response code
RF	Rainfed
RH	Relative Humidity
SD	Standard deviation
SS	Sum of squire
t	Tons
t ha-1 year-1	Ton per Hectare per Year
TNAU	Tamil Nadu Agricultural University
TR4	Tropical Race 4
TTC	Triphenyl Tetrazolium Chloride
WAP	Week after Planting

CHAPTER ONE

INTRODUCTION

1.1 Background of the problem

Banana is one of the world's most important fruit crops and among the ten most important staple crops providing a crucial source of nutrition and income for millions of people (Petsakos *et al.,* 2019; Brown *et al.,* 2017; Wairegi *et al.,* 2014). It is also an important source of income for millions of people in tropical and subtropical regions of the world and has particular importance to the Great Lakes region of Africa (Ndabamenye *et al.,* 2013; Nyine & Pillay, 2007; Taulya, 2013). The East African Highland cooking bananas (EAHB) are a daily staple for over 20 million people in the region (Dotto *et al.,* 2018; Batte, 2019; Mbabazi *et al.,* 2020; Madalla *et al.,* 2023).

In Tanzania, banana is an essential food and commercial crop and a significant source of raw materials for beverage and handcraft industries (Luzi-Kihupi *et al.,* 2015). Despite its recognized importance for food security, banana production is low due to the susceptibility of currently utilized genotypes (landraces) to insects, nematodes, and diseases (Nyine & Pillay, 2007) and low soil fertility (Amah *et al.,* 2020; Meya *et al.,* 2023). These constraints include black Sigatoka (*Mycosphaerella fijiensis*), Fusarium wilt (*Fusarium oxysporum f. sp. cubense*), and bacterial wilt (*Xanthomonas spp*.) (Okole *et al.,* 2000; Vishnevetsky *et al.,* 2011).

Viral diseases include banana bunchy top (Jain & Priyadarshan, 2009; Perrier *et al.,* 2011; Shimwela *et al.,* 2022) and Banana leaf streak (James *et al.,* 2011; Karanja *et al.,* 2008; Harper *et al.,* 2005). These bananas are also susceptible to pests such as weevils and nematodes (Ssali *et al.,* 2012; Ssebuliba *et al.,* 2008). The decline in production has led to food security issues, particularly for farmers in rural areas who depend on bananas as their principal source of food and an essential source of supplemental income (Nyine & Pillay, 2007).

Banana reproduces primarily through clonal propagation, using suckers that emerge from banana corm. However, conventional breeding methods, such as crossbreeding and selection have attracted attention to improving banana cultivars by incorporating desirable traits such as disease resistance, higher yield, and improved fruit quality (Batte *et al.,* 2021; Hibler & Hardy, 1994; Ortiz, 2013; Ssebuliba *et al.,* 2008).

Crossbreeding in bananas involves the controlled pollination of two different banana varieties, typically a female parent (mainly cultivated bananas with good agronomic traits but lacking

traits like resistance to diseases) and a male parent (usually a wild banana species or another cultivar with desired traits) (Hibler & Hardy, 1994; Ortiz, 2013; Persley *et al.,* 1987; Ssebuliba *et al.,* 2006; Ssebuliba *et al.,* 2008). Successful hybridisation depends on various factors, including environmental conditions like temperature, relative humidity, and plant nutrition (Ssebuliba *et al.,* 2006). Among these factors, pollen quantity, potassium (K) fertilisation and supplemental irrigation have garnered attention as critical components for enhancing seed set and seed viability in banana crossbreeding.

Since the 1920s, various programs have been engaged in the breeding of bananas (Jain & Priyadarshan, 2009). The EMBRAPA-CNPMF in Brazil, India Council of Agriculture - National Research Center for Banana (ICAR-NRCB) in India all strive to breed indigenous varieties of dessert and cooking bananas, whereas FHIA in Honduras breeds bananas for export as well as "cooking" varieties. The CARBAP in Cameroon and IITA are conducting research regarding the breeding of plantains and bananas in Africa (Jain & Priyadarshan, 2009; Ortiz *et al.,* 1995) and Centre de Cooperation Internationale en Recherche Agronomique pour le Développement- CIRAD breeds dessert bananas in Guadeloupe (Bakry *et al.,* 2009). Most cultivated bananas are triploids (2n = 3x = 33), which complicates breeding efforts and requires intensive resource allocations to develop superior varieties (Brown *et al.,* 2017; Ortiz *et al.,* 1995). The initial steps in conventional crossbreeding of bananas are hybridizing and selecting superior diploid recombinants to introduce desired traits (Aguilar, 2013; Ortiz, 1996).

Improved, disease-resistant, and pollen-fertile diploids are subsequently crossed with preferred triploid (3x) varieties to produce tetraploid plants (4x), which, in turn, are crossed by improved diploids in a second cycle to produce secondary triploids (Adeleke *et al.,* 2002). In this crossing scheme, poor seed set is the principal bottleneck, with fertility varying considerably among genotypes. While there are a considerable number of genetic and cytogenetic factors (i.e., parthenocarpy, low seed viability, irregular meiotic behaviour, and diverse genomic configurations) that likely also contribute to poor seed set in banana (Ortiz, 2013; Batte *et al.,* 2019; Amah *et al.,* 2020; Waniale *et al.,* 2021; Němečková *et al.,* 2018), limited pollen viability has been noted as a critical limitation.

Environmental factors affect fertility by influencing seasonal changes and causing variations in moisture, temperature, humidity, and day length (photoperiod) (Ortiz & Vuylsteke, 1995a; Brown *et al.,* 2017). High temperatures, solar radiation, low rainfall, and high evapotranspiration reduce the size of the bunch and fruits (Lufu *et al.,* 2020; Waniale *et al.,* 2020; Rajatiya *et al.,* 2018; Dhillon & Gill, 2011). This can affect the seed set in *Musa spp*. as

there is a correlation between a reduced fruit circumference of Gros Michel and a reduced seed set, which may indicate a reduction in parthenocarpy (Dzoyem *et al.,* 2024; Shepherd, 1954). Increased seed set in Matooke is correlated with high temperatures, solar radiation, and low rainfall (Ssebuliba *et al.,* 2009). The mechanisms through which these environmental factors impact seed sets are not well understood, but distinct seasonal effects have been noted (Ortiz & Crouch, 1997; Ortiz & Vuylsteke, 1995a).

Many wild (seeded) diploid bananas produce abundant pollen, which generally has a higher degree of viable pollen when compared to cultivated (seedless) bananas (Fortescue & Turner, 2004, Bayo *et al.,* 2024). The quantity and viability of pollen are important considerations in selecting male parents (Ssebuliba *et al.,* 2008) and are indispensable in the efficient genetic improvement of bananas (Fortescue & Turner, 2005). Wild diploid bananas such as ITC0249-Calcutta 4 and ITC0253-Borneo produce abundant pollen and have been used extensively as donors of disease resistance alleles by multiple breeding programs (Pillay *et al.,* 2012). Unfortunately, they also contribute unfavourably to several important agronomic traits such as bunch and fruit size, reduced shelf life and consumer acceptance. Mchare also called Mlali or Muraru bananas, grown extensively in certain highland regions of East Africa (Arusha, Kilimanjaro, southern part of Kenya and some East African Islands) as cooking bananas, are currently being bred for improvement for multiple disease resistance, including Fusarium (race 1 and Foc TR4), which threatens banana production worldwide (Brown *et al.,* 2017).

Mchare is recognized as the unreduced gamete source for many of the most economically important dessert bananas globally, including Cavendish, Gros Michel, Pome (or Prata), and Silk bananas, contributing 2/3rd of the genomic complement to each of these triploid dessert banana types (Jeensae *et al.,* 2021; Martin *et al.,* 2020; Jeridi *et al.,* 2012; Perrier *et al.,* 2011; Raboin *et al.,* 2005). Mchare (formerly Mlali) subgroup was revealed as the closest 2n gamete donor for the Cavendish and Gros Michel subgroups (Jeensae *et al.,* 2021; Martin *et al.,* 2020; Martin *et al.,* 2017; Perrier *et al.,* 2011). Thus, improved diploid Mchare could efficiently introduce disease resistance into related and economically important dessert bananas. Therefore, the improvement of these bananas is of immediate importance to food security in Africa, but it also holds promise as an essential donor source for the conventional improvement of dessert bananas worldwide.

Despite the importance of these cultivated (parthenocarpic) diploid (2n) bananas to farmers in Tanzania, there is a need for more information on pollen production and viability in Mchare genotypes to support the development of improved varieties through conventional breeding

methods.The only published data on these bananas focused on a single Mchare (syn. Mlali) genotype Chicame from the Comoros islands. This cultivar produced 40% pollen viability in the diploid state, and after chromosome doubling for use as a tetraploid in the breeding scheme, the percentage of viable pollen was observed to increase to 61.3% (Goigoux *et al.,* 2013).

Potassium is an essential macronutrient required for plant growth and development (Wu *et al.,* 2023). It plays a pivotal role in various physiological processes, including enzyme activation, photosynthesis, and the transport of water and nutrients within the plant. In banana plants, K deficiency can lead to reduced fruit yield, poor fruit quality, and limited seed development (Aba & Baiyeri, 2015; Zheng *et al.,* 2022). Adequate K levels are essential for the overall health of the banana plant and, consequently, for the success of crossbreeding efforts. Several studies have demonstrated the positive impact of K fertilization on banana plant development and fruit yield (Hau *et al.,* 2019; Silva *et al.,* 2020). However, the specific effects of K on seed set and seed viability in banana crossbreeding require further investigation. The impact of this macronutrient on seed production and viability is evaluated in this study.

Soil moisture availability for plants is another crucial factor influencing banana plant growth and reproduction (Carr, 2009; Holder & Gumbs, 1982). Bananas are typically grown in regions with high rainfall, but even in these areas, fluctuations in precipitation can occur. Banana is a non-seasonal crop with a reproductive cycle lasting over 12 months. During its growth, it goes through various seasons—long rains, short rains, dry periods, and cooler temperatures. To maintain consistent soil moisture, especially during flowering and fruiting, supplemental irrigation is often needed. Adequate soil moisture is essential for developing healthy flowers, successful pollination, and forming viable seeds. Inadequate moisture during these stages can result in poor seed set and low seed viability (Bramley *et al.,* 2007; Holder & Gumbs, 1982; Santos *et al.,* 2018). Previous research has explored the impact of irrigation on banana crop yield and quality (Wong *et al.,* 2018; Zuo *et al.,* 2021). However, more focused investigations are needed to determine the specific effects of supplemental irrigation on seed set and seed viability in banana breeding.

In conclusion, this research aims to assess both the quantity and viability of banana pollen. It explores differences between wild-type and Mchare genotypes to enhance breeding efficiency. The study also examines the effects of potassium nutrition and supplemental irrigation on seed set and viability. Understanding these factors is key to improving banana breeding and maintaining high-quality cultivars.

1.2 Statement of the problem

Banana breeding programs aim to develop improved varieties that exhibit desirable traits such as high yield, disease resistance, and environmental adaptability to changing climate to ensure food security and livelihoods sustainability. However, achieving these goals requires a comprehensive understanding of factors influencing banana reproduction and seed viability. Despite advancements in banana breeding, there is a gap in knowledge regarding pollen amount, pollen viability, supplemental irrigation effects, and K fertilization impacts on seed set and viability, particularly within the context of Mchare banana crossbreeding.

The substantial obstacle to effective banana improvement is the inherent limitation in seed production (Brown *et al.,* 2017; Ssebuliba *et al.,* 2009a). The IITA 2020 Annual Report reveals that an average of 2 to 5 seeds per bunch is produced annually. The number of seeds obtained is low, and so is their viability (Manassés *et al.,* 2018). Field experience shows that the number of seeds obtained per bunch is less than 25, and only 20 to 50% of these seeds germinate. Low seed set in bananas has been a severe problem that slows down breeding activities (Pillay & Tripathi, 2007). Unlike many other crops, cultivated bananas reproduce asexually through vegetative propagation, resulting in minimal genetic diversity and hindering the development of improved varieties. As a result, breeding a new banana variety typically takes 15 to 20 years, significantly delaying genetic improvements. This stumbling block curtails the introduction of beneficial traits that could mitigate challenges posed by diseases, pests, and changing environmental conditions. This study aims to develop strategies to shorten the lengthy breeding cycle and accelerate banana improvement.

Therefore, there is a need to accurately quantify pollen variability in Mchare and wildtype banana germplasm, as this directly influences successful pollination and subsequent seed formation. Furthermore, the impact of supplemental irrigation on banana seed set and viability in Mchare crossbreeding remains unclear, posing challenges in optimizing water management practices for enhanced reproductive outcomes. Additionally, the effects of K fertilization on banana seed set and viability in the Mchare crossbreeding hypothesis have not been sufficiently explored, hindering the development of tailored fertilization strategies to improve seed yield and quality.

Addressing these knowledge gaps is essential for advancing banana breeding efforts and ultimately contributing to food security and livelihoods in regions reliant on banana cultivation. By elucidating the factors influencing seed set and viability in Mchare crossbreeding,

researchers can develop targeted interventions and breeding strategies to enhance banana productivity, resilience, and sustainability.

1.3 Rationale of the study

Bananas (*Musa spp.*) play a critical role in global food security and livelihoods, particularly in Eastern Africa where they are stapled crops. Addressing the challenges of low seed set and seed viability in banana production is crucial for the rapid development of improved varieties with desirable agronomic traits, as well as resistance to diseases and pests, thereby sustain agricultural productivity and genetic diversity. This study aims to investigate the influence of pollen amount, supplemental irrigation, and potassium levels on Mchare banana seed set and viability, drawing from existing research on moisture and nutrient stress effects on seed production. Numerous studies have explored the impact of moisture and potassium levels on seed sets and viability in various crops. Pervez *et al.* (2004) reported significant increases in seed production with the application of potassium, highlighting the potential role of this essential nutrient in promoting reproductive success. Similarly, Alqudah *et al.* (2011) demonstrated that water stress significantly affects seed production and quality, underscoring the importance of moisture availability in reproductive processes.

The adaptation mechanisms of plants under stress have been extensively studied. It is well-documented that plants respond to drought stress by increasing the number of seeds as a survival strategy for ensuring propagation in subsequent seasons (Norton *et al.,* 2016; Bandurska, 2022; Seleiman *et al.,* 2021; Alqudah *et al.,* 2011; McDowell *et al.,* 2008). This phenomenon is corroborated by the findings of Behboudian *et al.* (2001), who observed enhanced seed nutritive value under water stress conditions. These adaptations may involve increased accumulation of soluble sugars, amino acids, and proteins, contributing to seed development and viability. Despite the existing body of research on the effects of soil moisture and nutrient stress on seed production, there is a notable knowledge gap regarding Mchare banana seed setting and viability in Tanzania. The dearth of studies investigating these critical aspects of banana reproductive biology in the region underscores the urgency of research in this area (Kole, 2020).

Additionally, studies have indicated that limited seed production is observed in East African Highland Banana (EAHB) varieties, with successful pollination resulting in only a few seeds (Ssebuliba *et al.,* 2009b). Nevertheless, the significance of increased seed count for subsequent breeding efforts cannot be overstated. A higher number of seeds increases the potential for

selecting desired traits in offspring and marks a significant advancement in banana breeding programs. This research intends to bridge this knowledge gap by exploring the influence of supplemental irrigation and K levels on the Mchare banana seed set and viability. The outcomes of this study are expected to provide insights that can contribute to enhancing the efficiency of banana breeding efforts, particularly for diploidy edible Mchare banana varieties. By improving seed production and viability, this research ultimately strives to enhance food security among smallholder farmers who heavily rely on banana cultivation.

These gaps represent a fundamental aspect that our study aims to address, as understanding the reasons behind these variations can have profound implications for banana breeding programs. By addressing these gaps, our research has the potential to enhance the efficiency and success of banana breeding programs, ultimately contributing to improved crop quality and agricultural sustainability.

1.4 Research objectives

1.4.1 General objective

Evaluating the effects of pollen quantity, supplemental irrigation, and potassium fertilization on seed set and seed viability in Mchare banana crossbreeding. The information gathered from this research will help to increase the efficiency of banana breeding and result in direct benefits to farmers in the form of new and improved varieties.

1.4.2 Specific objectives

(i) To assess and compare pollen amount and viability between Mchare and wildtype banana varieties, aiming to identify suitable parental candidates for optimizing seed production in banana breeding.

(ii) To assess the impact of supplemental irrigation and potassium fertilization on banana seed set and viability in Mchare breeding.

1.5 Research hypothesis

1.5.1 Hypothesis for the First objective

Ho: There is no significant difference in pollen quantity and viability among Mchare and wildtype banana varieties.

Ha: There is a significant difference in pollen quantity and viability among Mchare and wildtype banana varieties, influencing parental selection for optimized seed production in banana breeding.

1.5.2 Hypothesis for the Second objective

Ho: The application of supplemental irrigation and potassium fertilization has no significant impact on banana seed set and viability in Mchare crossbreeding.

Ha: The application of supplemental irrigation and potassium fertilization significantly impacts banana seed set and viability in Mchare crossbreeding, leading to improved reproductive outcomes.

1.6 Significance of the study

Since seed production has been a big challenge in banana breeding, understanding the mechanism of this issue is an important first step to finding a solution. Many theories have been proposed to understand seed-setting problems in bananas, including irregular meiosis, changes in chromosomal structure, or inter (sub)species hybridity, which have been implicated in gametic sterility (Huang, 2019). Morphological and physiological differences among bananas have been described before (multiple archesporial) or after gametogenesis (failure of embryo sac formation) (Fortescue, 2002; Turner *et al.,* 2007; Huang, 2019). It has been suggested that infertility may also be caused by subsequent flaws such as pollen tube growth inhibition in the style or ovary or lack of embryo development after fertilization. It is also possible that specific female sterility genes are involved (Xue *et al.,* 2007; Huang, 2019). It is also likely that both genetic and environmental conditions contribute to reduced fertility in bananas and this research looks primarily at two environmental factors to which little or no research has been directed. To wit, the effect of water stress and K fertilisation on the Mchare banana seed set and viability.

This study will examine the influence of different water regimes and K fertilisation levels on Huti-white Mchare (AA) subgroup seed set and seed viability. This study outcome will add value to breeding efforts by improving seed production, thus speeding up the breeding process. However, this finding will also guide breeders on the proper agronomic practice that will result in high seed productivity and appropriate resource mobilization. Generally, this study contributes to the fight against food insecurity in Africa, Latin America, Asia, and the world.

Seed production remains a formidable challenge in banana breeding, and solving the underlying mechanisms behind this obstacle is paramount for devising practical solutions. The intricate nature of seed setting in bananas involves a complex interplay of genetic, physiological, and environmental factors. While previous studies have offered insights into specific causes of sterility, such as polyploidy, chromosomal anomalies, and hybridity (Pandey, 2007; Tuner *et al.,* 2007; Fortescue *et al.,* 2002; Xue *et al.,* 2007; Huang, 2019), a comprehensive understanding of the impact of supplemental irrigation and K fertilisation on Mchare banana seed set and viability is yet to be explored.

The findings of this research hold several implications for both banana breeding and agricultural practices. Firstly, unveiling seed set mechanisms by understanding the effects of water stress and K fertilisation on seed set and viability is pivotal to resolving the intricate mechanisms underlying banana reproductive processes. This study contributes valuable insights into banana breeding challenges and opportunities by shedding light on the factors contributing to or hindering successful seed formation. Secondly, guiding breeding efforts using the outcomes of this research can significantly impact banana breeding strategies. Improved knowledge of the impact of supplemental irrigation and K applications on seed set and viability can guide breeders in selecting the optimal amount of K as one of the essential nutrients, managing irrigation practices, and enhancing the efficiency of hybridisation efforts. Third, enhancing Seed Production:

The study's results could pave the way for developing targeted agronomic practices that enhance seed production. This research improves the overall efficiency of banana breeding programs by identifying the conditions that promote higher seed sets and viability. Fourth by Accelerating Breeding: Improved seed production translates to accelerated breeding processes. More viable seeds allow breeders to select from a more comprehensive genetic pool, potentially leading to improved banana varieties with desired traits more rapidly. Fifth, by Contributing to Food Security: Bananas are a vital staple crop in many regions across the globe.

By enhancing seed set and viability, this research directly addresses the challenge of food insecurity by enabling the development of improved banana varieties that can withstand pests, and changing climates. Sixth, Global Impact: The findings of this study are relevant not only to the regions where bananas are a dietary staple, such as Africa, Latin America, and Asia but also to global efforts to ensure food security and sustainable agricultural practices.

In conclusion, investigating the effects of supplemental irrigation and K fertilisation on Mchare banana seed set and viability holds immense significance for advancing banana breeding and addressing food security challenges. By contributing to our understanding of the mechanisms behind seed production and viability, this research offers valuable insights for improving the efficiency of banana breeding programs and ultimately benefiting agricultural systems and communities worldwide.

1.7 Delineation of the study

The present study aimed at assessing both the quantity and viability of banana pollen. It explored differences between wild-type and Mchare genotypes to enhance breeding efficiency. The study also examined the effects of potassium nutrition and supplemental irrigation on seed set and viability. The study was conducted at the Nelson Mandela African Institution of Science and Technology (NM-AIST), Tengeru campus, and the World Vegetable Centre in Arusha. Fourteen banana genotypes were analysed for pollen production and viability, selecting the best male and female candidates for crossbreeding under irrigation and rainfed conditions with varying levels of potassium fertilization. Understanding these factors is key to improving banana breeding and maintaining high-quality cultivars.

CHAPTER TWO

LITERATURE REVIEW

2.1 Banana

Banana is one of the most important fruit crops in the world (Dhont *et al.,* 2012; Wilberforce *et al.,* 2015). Banana morphology encompasses a unique set of characteristics that define this tropical fruit (Karamura *et al.,* 2011). Bananas exhibit traits such as large, elongated leaves that grow in a spiralling pattern from a central pseudostem. The plants inflorescence, known as the "banana bunch," contains multiple hands, each comprising individual fruit clusters called "fingers." The fruit is a berry, typically with a curved shape, smooth or ridged skin, and soft, creamy flesh (Karamura *et al.,* 2011). While banana morphology varies among the numerous cultivars and wild species, the basics of the plants structure remain consistent. This distinctive morphology plays a crucial role in both the plants growth and reproduction (Karamura *et al.,* 2011).

2.2 Banana classification, evolution, and domestication

Bananas belong to the genus Musa, a part of the Musaceae family. The genus Musa is divided into two primary sections: Eumusa and Australimusa (Perrier *et al.,* 2011). Banana is a member of the Zingiberales order, which includes three genera of the Musaceae family. Musa is the most significant genus for both consumption and production (Perrier *et al.,* 2011). Most cultivated cultivars are diploid (2 sets of chromosomes: 2n = 22) or triploid (3 sets of chromosomes: 3n = 33) hybrids of the wild progenitors *Musa acuminata* (AA) and *Musa balbisiana* (BB). The letters A or B are used to identify genomes, indicating the origin of the chromosomal set AA, AAA, AB, AAB, ABB, and ABBB are the six primary groupings based on their genetic makeup. Some researchers suggest that the B genomes proportional genomic contribution to hybrids may be correlated with drought stress tolerance in banana (Stevens *et al.,* 2021).

The cultivated bananas fall under the Eumusa section. Among these, the most widely consumed variety is the Cavendish banana (*Musa acuminata* AAA group), recognized by consumers for its sweet taste and creamy texture. The dominant banana type in the Great Lakes region of East Africa is the East African Highland Bananas (EAHBs), commonly known as Matooke (AAA), which is mostly preferred in Uganda (Marimo *et al.,* 2020). The Mchare subgroup (AA), genetically distinct from Matooke, is common in Tanzania (Němečková *et al.,* 2018). Mchare

is mistakenly classified as a dessert banana by the FAO, although in Kenya they are referring Mchare as Mlali and they use it as dessert banana (Bayo *et al.,* 2024). Wild bananas, on the other hand, include species from both the Eumusa and Australimusa sections, with varying levels of edibility (Perrier *et al.,* 2011; Simmonds *et al.,* 1990).

The banana plant is a substantial monocotyledonous herbaceous plant (D'hont *et al.,* 2012; Fortescue *et al.,* 2011). The corm, often known as the "real stem," is an underground stem from which the aerial shoot and suckers emerge. The apical meristem (apex) is found at the corms highest point and remains there during vegetative development. The apex begins leaf primordial, from which leaves emerge sequentially, with older leaves enclosing freshly produced leaves, forming the pseudostem comprising densely compacted and contained leaf bases (Fortescue *et al.,* 2011). Each new leaf is created within the pseudostem and pushed out like a rolled cigar through the core of the pseudostem, where it unfurls and reaches full photosynthetic potential (Perrier *et al.,* 2011).

The plant's apex rises above the leaf bases at "flower initiation," transitioning from the vegetative to the floral stage and forming the inflorescence. At "flower emergence or anthesis," the growing inflorescence is propelled upwards by an aerial stem until it emerges at the top of the pseudostem (Wang, 2007). The term "flowering" is frequently used in plantations to refer to developing "flowers or in the field. The "fruiting phase" begins after flower/bunch emergence and lasts until bunch maturity (Stevens *et al.,* 2021).

The evolution of cultivated bananas involves a complex history of hybridization, polyploidy, and selection (Brown *et al.,* 2017). Genetic evidence suggests that the wild ancestor of the cultivated bananas is likely Musa acuminata, a species native to Southeast Asia (Simmonds *et al.,* 1990). The domestication process, however, involved multiple rounds of hybridization between *M. acuminata* and other wild species. The presence of wild banana relatives in various regions, including Africa, the Pacific, and the Americas, indicates that humans played a pivotal role in their dispersal (Perrier *et al.,* 2011). Human preferences for improved taste, texture, and reduced seed content drove the shift from wild to domesticated bananas (Perrier *et al.,* 2011). This transformation was achieved through selective breeding, with humans choosing desirable plants. Over generations, these selections led to the development of the cultivated varieties we enjoy today (Li *et al.,* 2013). The transition from seed-dispersed wild bananas to sterile, seedless cultivated types contributed to their global spread, as they could be propagated through vegetative means (Perrier *et al.,* 2011).

Banana domestication is a fascinating case study of human-plant interaction (Perrier *et al.,* 2011). Ancient civilizations recognised the potential of bananas and began cultivating them as early as 7000 years ago (Li *et al.,* 2013). Archaeological evidence from Papua New Guinea and the Philippines suggests that bananas were deliberately propagated, indicating an early form of domestication. Over time, humans identified and propagated plants with favourable characteristics, such as higher yields, improved taste, and ease of cultivation (Heslop-Harrison & Schwarzacher, 2007; Li *et al.,* 2013; Perrier *et al.,* 2011). The most significant leap in banana domestication came with the development of parthenocarpy, a trait that allows banana fruit to develop without pollination, resulting in the familiar seedless fruit (Li *et al.,* 2013; Perrier *et al.,* 2011). This trait likely arose through natural mutation or selection, revolutionising banana cultivation. By allowing sterile plants to produce fruit, parthenocarpy made it easier for farmers to propagate desired traits without relying on seeds (Perrier *et al.,* 2011).

The journey of bananas from their wild origins to becoming one of the world's most important staple crops exemplifies the dynamic interplay between human ingenuity and natural evolution (Li *et al.,* 2013). Through careful selection and cultivation, humans have transformed bananas from their original, wild forms into the diverse and widely loved fruits we enjoy today. The storey of banana domestication underscores the significance of plant genetic diversity and human agency in shaping the foods we rely on for sustenance and pleasure (Li *et al.,* 2013).

2.3 Importance of banana

Banana is among the most important fruits in the world, the twelfth most important food crop in production, and the fourth most important in Africa (FAOSTAT, 2019). In 2017, global banana and plantain production was 153 million metric tonnes, cultivated on 5.6 million hectares in over 135 countries. It was also recorded in 2020 that 119.8 million tonnes of bananas and plantains were cultivated in about 130 countries worldwide (FAOSTAT, 2022). India is the leading banana producer worldwide, followed by China, Indonesia, Brazil, Ecuador, the Philippines, Guatemala, Angola, the United Republic of Tanzania, and Costa Rica in the top ten producers worldwide (FAOSTAT, 2022). East African production was 10 million tonnes of bananas, with negligible exports (FAOSTAT, 2018). More than 85% of production is for house consumption and the local market; the remaining 15% is for export (Meya *et al.,* 2023a, 2023b).

Banana is grown in the humid tropics and is prized worldwide for its flavour, nutritional content, and year-round they. Cavendish bananas (dessert type) are usually eaten fresh but may also be fried or mashed and chilled in pies or puddings (Simmonds *et al.,* 1962). It may also be

used to flavour muffins, cakes, and bread. Plantains and East African highland bananas (EAHB) "Matooke-AAA and Mchare-AA" are starchy, not sweet, and are widely farmed as a staple food. They are cooked when unripe or immature. Ripe fruit is rich in dietary fibre, potassium, manganese, and vitamins B6 and C and contains up to 22% carbohydrates (Simmonds *et al.,* 1962). Banana is an essential staple food; 122.85 million tons of bananas and plantains are cultivated in about 130 countries worldwide (FAOSTAT, 2012).

Bananas are one of Tanzania's three main essential staple foods. Its production is 5.1 million tonnes annually; maize and cassava produce 8.2 and 5.7 million tonnes yearly. Bananas are essential as they feed 30% of the population in Tanzania and contribute to 377 KJ/year per capita caloric intake and 280 kg/year per capita consumption (Meya *et al.,* 2020).

2.4 Challenges facing banana production

Banana production experienced a severe drop from 70 t ha^{-1} $year^{-1}$ to 5–30 t ha^{-1} $year^{-1}$; this has been observed from the highest yield levels attained in the 1970s (Viljoen *et al.,* 2017). The area harvested between 1960 and 1990 remained almost constant, with an average of 201 770.6 ha and a production quantity of 524 995.6 tons (t). Then, around 1990, the area and quantity produced almost doubled, with an average of 348 144 ha and 913 202 t, respectively. This increase could be linked to the increased demand for food to feed more people due to the ever-increasing population growth. However, the average yield remained at only 2.6 t ha^{-1} $year^{-1}$. This could be linked to the outbreak of banana weevils (Cosmopolites sordidus) and nematodes (*Radopholus goodeyi*) in the early 1970s, Panama disease in the mid-1980s, and insufficient nutrient inputs due to a decline in the cattle population, an essential source of animal manure (Meya *et al.,* 2023, Rugalema *et al.,* 1994).

The banana production decrease is due to fungal, bacterial, and viral diseases, nematodes and insect pests, poor agronomic practices, and abiotic factors (Ramirez *et al.,* 2011; Waniale *et al.,* 2021). In Tanzania, black Sigatoka, Fusarium wilt, nematodes, bacterial Xanthomonas wilt, Banana Bunchy Top Virus (BBTV) and weevils are the most serious diseases and pests that can cause up to 100% yield losses (Ploetz, 2015). Cultural practises to control these pests and diseases are not feasible for holders' farmers, but the use of resistance cultivars is reported to be a cheap and effective means of pest and disease control (Issue, 1999; Ploetz, 2015; Siamak *et al.,* 2018). Breeding programmes worldwide focus on different constraints to improve the quality of bananas and reduce losses by imparting disease resistance, abiotic stress resistance, and increasing yield. Some of these breeding programmes include Empresa Brasileira de

Pesquisa Agropecuária (EMBRAPA, Brazil), the Fundación Honduras de Investigación Agrícola (FHIA, Honduras), the National Research Centre for Banana (NRCB, India), the International Institute of Tropical Agriculture (IITA, Tanzania, Uganda, and Nigeria), the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD, France) (Amorim *et al.,* 2013), and the National Agricultural Research Organization in Uganda (NARO).

Improvement of banana hybrids through conventional breeding is faced with many challenges related to reduced fertility, that can arise through meiotic abnormalities resulting from polyploidy, changes in chromosomal translocations and inversions, or inter-species incompatibility (Huang, 2019). This is a crucial roadblock to developing hybrids with disease- and pest-resistant traits and improved agronomic features. Efforts have been made to improve this vital crop through breeding practices, which have been going on for several years. Several researchers have reported that factors such as timing of pollination, temperature, solar radiation, moisture levels, nutrient management, and relative humidity can be altered to improve seed production (Meya, 2020; Ramirez *et al.,* 2011; Ravi *et al.,* 2013; Vantyghem *et al.,* 2020; Wilberforce *et al.,* 2014).

2.5 Pollen viability in banana breeding

Most cultivated bananas are triploids (2n = 3x = 33), which complicates breeding efforts and requires intensive resource allocations to develop superior varieties (Brown *et al.,* 2017a). The initial steps in conventional breeding of bananas are hybridizing and selecting superior diploid recombinants to introduce desired traits (Aguilar, 2013; Ortiz, 1996). Improved, disease-resistant, and pollen-fertile diploids are subsequently crossed with preferred triploid (3x) varieties to produce tetraploid plants (4x), which, in turn, are crossed with improved diploids in a second cycle to produce secondary triploids (Adeleke *et al.,* 2002). In this crossing scheme, poor seed set is a bottleneck, with fertility varying considerably among cultivars.

Environmental factors influence fertility, including time of the year, due to variations in moisture, humidity, day length, and temperature (Ortiz & Vuylsteke, 1995a; Brown *et al.,* 2017). In addition to numerous putative genetic, cytogenetic, environmental and cultural contributions to reduced fertility (Ortiz & Crouch, 1997; Ortiz & Vuylsteke, 1995a; Ortiz, 2013; Batte *et al.,* 2019; Amah *et al.,* 2020; Waniale *et al.,* 2021; Němečková *et al.,* 2018), limited pollen quantity and viability have been noted as critical limitations on seed set. This paper focuses on differences in pollen production and viability over seven months (representing

the dry and rainy seasons in East Africa) to determine if there are significant differences among Mchare cultivars that can be exploited for greater breeding efficiency (Stone *et al.,* 1995).

2.6 Pollen behaviour in mchare and wildtype bananas

In banana breeding, pollen counts in both Mchare, and wild-type bananas are important for several crucial reasons. Many wild diploid banana varieties are known for their abundant pollen production, which, in comparison to cultivated seedless bananas, often exhibits a higher percentage of viable pollen (Fortescue & Turner, 2004). The quantity and viability of pollen are not merely academic concerns; they are integral in the selection of male parents (Ssebuliba *et al.,* 2008) and play an indispensable role in the genetic enhancement of banana cultivars (Fortescue & Turner, 2005).

Wild diploid banana varieties like ITC0249-Calcutta 4 and ITC0253-Borneo have been extensively utilized as donors of disease-resistance alleles by multiple breeding programs (Pillay *et al.,* 2012). Their contribution is significant in bolstering disease resistance in cultivated bananas, particularly regarding emerging diseases such as BBTV and TR4 (Brown *et al.,* 2017). However, it's important to note that while these wild varieties offer disease resistance, they may also introduce unfavourable traits such as bunch size and fruit quality. This dilemma underscores the need for a thorough understanding of pollen counts to strike a balance between positive and negative traits in the breeding process.

Mchare bananas, also known as Mlali or Muraru bananas, are extensively cultivated in specific highland regions of East Africa, primarily as cooking bananas. These cultivars are particularly valuable as they are the unreduced gamete source for some of the most economically important dessert bananas globally, including Cavendish, Gros Michel, Pome or Prata, and Silk bananas. Mchare bananas contribute two-thirds of the genomic complement to each of these triploid dessert banana types (Jeridi *et al.,* 2012; Raboin *et al.,* 2005). As a result, improving the diploid Mchare cultivars is not only essential for enhancing food security in Africa but also holds promise as a vital donor source in the conventional improvement of dessert bananas worldwide. The understanding of pollen counts in Mchare bananas is a critical step in this journey toward more disease-resistant and agriculturally viable banana cultivars. Integrating higher fertility rates into potential diploid donors such as Mchare increases the likelihood of producing future dessert bananas with resistance to diseases such as TR4 and BBTV.

A single Mchare (syn. Mlali) cultivar called "Chicame" from the Comoros Islands was found to have 40% viable pollen when it was diploid. When its chromosomes were doubled so that it

could be used as a tetraploid in breeding, the percentage of viable pollen rose to 61.3% (Goigoux *et al.,* 2013). The existing literature on pollen counts in bananas presents critical insights that are closely related to the objectives of our study while also pointing out certain gaps that our research aims to address. The studies reviewed shed light on two fundamental aspects: pollen viability and the number of pollen grains, both of which have direct implications for banana breeding, hybridization, and overall crop improvement. There are other studies on non-Mchare bananas?

One of the key findings in the existing literature is the strong influence of the timing of pollen collection on pollen viability (Althiab-Almasaud *et al.,* 2024; Ejsmond *et al.,* 2011). The present study aims to investigate pollen viability in Mchare cultivars and selected wild-type bananas used as donors of beneficial traits in crop improvement. The literature suggests that maximal pollen viability is observed when pollen is collected in the morning, typically around 0800 h, irrespective of the banana cultivar (Fig. 2) (Bayo *et al.,* 2024; Jayashantha, 2015). Examples are provided in other crops as described by Kaian *et al*. (2016) in the case of maize, emphasising the significance of morning pollen for higher germination rates. Furthermore, Mondo *et al*. (2022) and Soares *et al*. (2015) confirm this trend, underscoring the morning hours as ideal for pollination success due to maximal pollen viability and stigma receptivity. Understanding the optimal timing for pollen collection, particularly in Mchare and wild-type bananas, can significantly enhance the efficiency of the hybridization efforts. However, the existing literature does not describe potential factors underlying pollen viability variations, particularly in the context of these bananas.

The second aspect explored in the literature relates to the number of pollen grains produced, which varies significantly among banana cultivars (Jayashantha, 2015). The studies reveal that wild, seeded banana cultivars tend to produce a higher quantity of pollen with greater viability than cultivated bananas. The research objectives focus on comparing pollen counts among Mchare cultivars and wild-type bananas. Considerable gaps exist in the literature regarding the precise mechanisms influencing pollen production and viability, especially in bananas. The study evaluated some factors that might influence this crucial aspect of breeding but acknowledge that a comprehensive evaluation is beyond the scope of the current study. By addressing gaps in current knowledge, the present research has the potential to enhance the efficiency and success of banana breeding programs, ultimately contributing to improved crop quality and agricultural sustainability.

2.7 Supplemental irrigation impact on plant reproduction

Water is a critical resource for plant growth, playing a fundamental role in numerous physiological processes. Among these, plant reproduction which includes flowering, pollination, seed formation, and fruit development is particularly sensitive to water availability (Bhattacharya & Bhattacharya, 2021; Bykova *et al.,* 2019). In many parts of the world, where rainfall is inconsistent or insufficient to meet crop demands, supplemental irrigation is a key strategy to enhance plant productivity. Scientific research across various crops and agro-climatic conditions underscores the significant impact of well-timed supplemental irrigation on reproductive success (Geert & Raes, 2009; Oweis *et al.,* 2004; Fox & Rockström, 2003). This detailed examination explores how supplemental irrigation influences various aspects of plant reproduction, including increased yields, improved flowering and pollination, enhanced seed set and fruit quality, mitigation of drought stress, and the risks associated with over-irrigation (Geert & Raes, 2009; Oweis *et al.,* 2004; Fox & Rockström, 2003).

Supplemental irrigation is widely recognized for its capacity to increase crop yields, largely due to its positive impact on reproductive processes. Crops are particularly vulnerable to water stress during their reproductive stages, such as flowering, fruit set, and seed filling. These stages are crucial because they directly determine the quantity and quality of the final harvest (Geert & Raes, 2009; Oweis *et al.,* 2004; Fox & Rockström, 2003). Several studies show that providing additional water through irrigation during these critical periods can significantly enhance reproductive success, ultimately boosting yields (Geert & Raes, 2009; Oweis & Hachum, 2006; Oweis *et al.,* 2004; Fox & Rockström, 2003). For instance, in cereal crops like wheat, maize, and barley, irrigation during the flowering and grain-filling stages has been shown to increase the number of grains per ear and the overall grain size.

Grain yield is often constrained by water availability during the reproductive phase, as water stress can limit photosynthesis, reduce carbohydrate production, and inhibit the transport of sugars to the developing grains (Rasool *et al.,* 2020; Gheysari *et al.,* 2017; Amin *et al.,* 2015). Research conducted in Mediterranean and semi-arid regions has demonstrated that even modest supplemental irrigation can mitigate water deficits during flowering and grain filling, resulting in higher yields and improved grain quality (Guelloubi *et al.,* 2005; Torres *et al.,* 2017).

In fruit crops, such as tomatoes, melons, and apples, supplemental irrigation plays an equally crucial role. Studies indicate that providing water during fruit set and development stages can significantly enhance fruit size, improve sugar content, and reduce the occurrence of fruit

defects such as cracking or shriveling (Funes *et al.,* 2021). This is especially important for crops grown in regions where rainfall is scarce or unreliable. In these environments, supplemental irrigation can ensure that crops reach their full reproductive potential, leading to more marketable produce and higher overall yields (Funes *et al.,* 2021; Guelloubi *et al.,* 2005; Torres *et al.,* 2017).

Water stress during flowering can severely hinder the reproductive success of many plant species (Alqudah *et al.,* 2011). One of the most critical impacts of drought or insufficient water is reduced flower formation and retention. Flowers are essential reproductive organs, and their successful development is directly tied to the availability of resources, particularly water. When plants experience water stress, they often produce fewer flowers, and the flowers that do form may be of lower quality or more prone to abortion (Blum & Blum, 2011; Sun *et al.,* 2004). Supplemental irrigation can help alleviate these issues by maintaining optimal plant water status during flowering (Alqudah *et al.,* 2011). For example, in legumes such as beans, soybeans, and lentils, water stress during flowering has been shown to reduce flower production and increase flower drop, leading to lower pod and seed set (Shrestha *et al.,* 2006; Khatun *et al.,* 2021; Ullah *et al.,* 2022). Studies have found that applying irrigation during the flowering period can significantly reduce flower abortion rates, resulting in more flowers transitioning to fruits or seeds. This improved flower retention can directly translate into higher yields (Shrestha *et al.,* 2006; Khatun *et al.,* 2021; Ullah *et al.,* 2022).

In addition to improving flower production, supplemental irrigation also enhances the quality of pollen, which is critical for successful pollination. In many crops, including cereals, fruits, and vegetables, water stress can reduce pollen viability, making it less likely that pollen grains will successfully fertilize ovules (Karmi *et al.,* 2017; Crone *et al.,* 2006). Research has shown that irrigation can improve pollen hydration and viability, increasing the chances of successful pollination. This is particularly important in crops such as maize, where poor pollination due to water stress during silking can lead to substantial yield losses (Karmi *et al.,* 2017; Crone *et al.,* 2006).

The development of seeds and fruits is another critical stage of plant reproduction that is highly sensitive to water availability (Karmi *et al.,* 2017; Crone *et al.,* 2006). After pollination, the plant needs a consistent supply of water to support the growth and development of seeds and fruits. Insufficient water during this period can lead to poor seed development, reduced seed size, and lower fruit quality. Supplemental irrigation ensures that plants have adequate water to support these processes, leading to better reproductive outcomes (Karmi *et al.,* 2017; Crone

et al., 2006). In oilseed crops, such as sunflower, canola, and soybean, irrigation during seed development has been shown to increase seed size and improve oil content (Aiken & Lamm, 2006; Adeleke & Babalola, 2020; Attia *et al.,* 2021) For example, in sunflower, research indicates that supplemental irrigation during the flowering and seed-filling stages increases both the number of seeds per head and the oil content of the seeds, leading to higher overall yields and better-quality oil (Aiken & Lamm, 2006; Adeleke & Babalola, 2020; Attia *et al.,* 2021). Similarly, irrigation during the reproductive phase in canola improves seed set, seed weight, and oil yield (Faraji *et al.,* 2009; Sharghi *et al.,* 2011).

Fruit crops also benefit significantly from supplemental irrigation during the fruit development stage. In crops like tomatoes, cucumbers, and melons, water stress during fruit development can lead to smaller fruits, poor texture, and lower sugar content. Studies have shown that irrigation during fruit growth not only increases fruit size but also enhances fruit sweetness, firmness, and overall marketability. This is particularly important in high-value fruit crops, where even slight improvements in quality can lead to substantial economic benefits for farmers (Hao *et al.,* 2019; Parkash *et al.,* 2021; Fawzy, 2019).

2.8 Banana and water stress

Today's biggest abiotic threat to banana production is water stress, as the crop requires ample water given its large leaf area, evergreen canopy, and shallow rooting system (Stevens *et al.,* 2020). In the tropic and subtropical regions of the world, it has been suggested that this is attributable to weather fluctuations that result from climatic changes (Ravi *et al.,* 2013). In tropical regions, it has been recorded that annual water use ranges between 1112 and 2690 mm, but there are severe yield losses of up to 65% when annual rainfall drops below 1100 mm. Since bananas are long-cycle crops (10–14 months), they cut across different climatic conditions to complete the cycle.

Water stress can be experienced at different growth stages along the cycle. When soil water drops below a critical threshold, leaves close their stomata in response to abscisic acid (ABA) accumulation in the stomata guard cells. As water stress increases, stomata are closed for extended periods, inhibiting transpiration, assimilation, and yield reduction (Stevens *et al.,* 2020). Higher economic yield under water stress is the characteristic feature of drought-tolerant accessions (Aslam *et al.,* 2015). Reproductive success measures water stress resistance: Plants have different ways to attain this, either by having deep roots, conserving and prioritising the use of available water for grain maturation, or modifying the life cycle to adopt favourable conditions. Plant growth and development, plant phenology, grain filling, and translocation of

photo assimilate reserves are essential processes that occur when the plant is exposed to water stress (Aslam *et al.,* 2015). Different studies focus on the effect of water stress on banana production, but no study has been conducted on that effect on seed production. This is because banana seed is only needed for breeding purposes.

Wairegi *et al*. (2010) and Van Asten *et al*. (2010) observed that water stress was the primary yield constraint in a quarter of the studied farmer fields in southwest Uganda. Bananas are water-intensive crops, relying on a consistent water supply for their growth, development, and fruit production. Water stress disrupts various physiological processes within banana plants, affecting their overall health and productivity (Nyombi *et al.,* 2010; Van Asten *et al.,* 2011). One of the primary consequences of a water deficit is a decrease in photosynthetic activity, leading to reduced carbohydrate production. This, in turn, negatively impacts fruit development, yield, and quality. The Musa genus, including dessert and cooking bananas, has distinct responses to water stress. Dessert bananas, such as Cavendish varieties, are particularly susceptible to water scarcity; cooking bananas, like plantains, exhibit better tolerance to short-term drought conditions. Ravi *et al*. (2013) reported that bananas are quite sensitive to water stress.

2.9 Potassium nutrient

2.9.1 Potassium in plant

Potassium (K) is one of the 17 chemical elements required for plant growth and development (Wang, 2007). Due to potassium's osmotic action, cells are physically forced to expand as they grow. The vast central vacuole, which contains about 80–90% of the cell volume, is where newly formed cells store K. The K draws water, which expands the cell to a new, larger size. Plants lacking in potassium can have slow development rates and tiny cells (Hu *et al.,* 2018). It plays a significant role in various vital processes within plants. More than 60 enzymes within the plant depend on potassium for their activation, called "the regulator." Potassium helps plants resist several biotic and abiotic stresses in the environment. Scientists reported that potassium is the most abundant cation in plants, i.e., up to 10% on a dry weight basis (Leigh *et al.,* 1984). With higher concentrations, it is present in the cytosol and chloroplast. It activates many enzymes by stabilising the pH in the range of 7 to 8 with the help of changes made in enzymatic conformation.

Potassium assimilation is the essential pathway for offsetting heavy metal toxicity in plants by forming soluble proteins, soluble carbohydrates, and soluble nitrogen compounds in the cell

sap of the plants (Nieves-Cordones *et al.,* 2016; Wang *et al.,* 2013; Wang, 2007). It also increases the tolerance of plants to disease (Wang *et al.,* 2013). It is reported that potassium aids plants in producing starches controls root growth, and regulates the opening and closing of stomata, which is essential for efficient water use (Wang *et al.,* 2013). All plants require potassium for their growth and development; however, plants with a rich carbohydrate source require more potassium than non-starchy plants. Based on several studies, it is declared that adequate amounts of potassium may promote the growth of long, strong cotton fibres, the enhancement of the self-life of fruits, and overall positive changes in the qualitative and quantitative parameters.

2.9.2 Potassium and plant reproduction

Potassium (K) plays a crucial role in plant reproduction by influencing various physiological and biochemical processes necessary for the successful growth, flowering, and fruiting stages of plants. It is a key macronutrient that significantly impacts photosynthesis, nutrient transport, water regulation, enzyme activation, and stress tolerance, all of which are essential for optimal plant reproductive development. Potassium is involved in regulating the opening and closing of stomata, which control gas exchange and water loss through transpiration (Hasanuzzaman *et al.,* 2018; Sardans & Peñuelas 2021; Wang *et al.,* 2013). This is especially important for photosynthesis, where potassium ensures the efficient uptake of carbon dioxide, enabling the plant to generate the carbohydrates required for growth and reproduction. The transport of these carbohydrates, along with other nutrients, is also facilitated by potassium, ensuring that reproductive organs such as flowers, fruits, and seeds receive adequate nourishment (Wang *et al.,* 2013; Mostofa *et al.,* 2022; Rawat *et al.,* 2022; Hu *et al.,* 2018).

In the context of flowering and fruiting, potassium's role in enzyme activation is particularly critical. It activates enzymes involved in the synthesis of proteins, starches, and sugars, which are vital for the development of reproductive tissues (Khan *et al.,* 2017; Hu *et al.,* 2018). These biochemical products are key components of pollen, ovules, and fruits, making potassium essential for the fertilization process and subsequent seed and fruit formation (Wu *et al.,* 2011). Additionally, potassium improves the structural integrity of plant cells by enhancing the synthesis of cellulose, which strengthens reproductive structures like petals and fruits (Khan *et al.,* 2017; Hu *et al.,* 2018). In crop production, the availability of potassium is closely linked to the quality and yield of fruits and seeds (Pettigrew, 2008). For example, adequate potassium supply has been shown to increase fruit size, sugar content, and overall marketable quality in crops such as tomatoes, strawberries, and melons (Oosterhuis *et al.,* 2014; Lester *et al.,* 2005).

Another significant role of potassium in plant reproduction is its contribution to water regulation and osmotic balance. Potassium ions are involved in maintaining turgor pressure within plant cells, which is critical for cell expansion and the elongation of reproductive tissues (Pandey & Mahiwal, 2020; Khan *et al.,* 2017). This ensures that flowers can fully open and that fruits can grow to their optimal size. Moreover, potassium helps to regulate water uptake and transport through the plant, ensuring that reproductive tissues remain hydrated, especially under conditions of water stress. This is particularly important in the reproductive phase, as water stress during flowering or fruiting can lead to reduced yields and poor fruit quality. By maintaining cellular osmotic balance, potassium supports the efficient use of water, helping plants to withstand periods of drought or inconsistent water availability.

In addition to its role in water regulation, potassium enhances a plant's ability to cope with various environmental stresses, which can severely impact reproductive success. Potassium improves a plant's resistance to drought, salinity, and extreme temperatures by regulating stomatal conductance and reducing the accumulation of harmful reactive oxygen species (ROS) that are often generated under stress conditions. During the reproductive phase, plants are particularly vulnerable to environmental stresses, and a potassium deficiency can lead to poor flower and fruit development, reduced seed viability, and lower crop yields (Hu *et al.,* 2018). Potassium helps mitigate these risks by maintaining cellular homeostasis and supporting the plant's antioxidant defence mechanisms, which protect reproductive tissues from oxidative damage (Pandey & Mahiwal, 2020; Khan *et al.,* 2017).

In terms of nutrient interactions, potassium plays a synergistic role with other essential nutrients like nitrogen and phosphorus in supporting plant reproduction. While nitrogen is crucial for vegetative growth and phosphorus is important for root and flower development, potassium enhances the overall efficiency of these nutrients by optimizing their uptake and utilization. For example, potassium aids in the transport of nitrate and phosphate ions within the plant, ensuring that reproductive tissues receive adequate supplies of these nutrients (Khan *et al.,* 2017). Furthermore, potassium helps to balance the uptake of other cations, such as calcium and magnesium, which are also important for reproductive tissue development. The cation exchange capacity (CEC) of soil or growth media plays a critical role in potassium availability, and maintaining an appropriate potassium level is necessary to ensure that plants can access other essential nutrients (Maathuis & Podar, 2011; Sardans & Peñuelas 2021).

In addition to its direct effects on reproduction, potassium influences the hormonal balance within plants, particularly concerning hormones that regulate reproductive processes.

Potassium is known to affect the synthesis and signalling of plant hormones such as auxins, gibberellins, and abscisic acid (ABA), which play important roles in flowering, fruit development, and seed maturation. For instance, potassium enhances the production of auxins, which promote cell elongation and are vital for the growth of reproductive organs (Khan *et al.,* 2017). It also influences gibberellin activity, which is involved in seed germination and fruit growth. On the other hand, potassium can modulate ABA levels, helping to delay fruit senescence and prolong the reproductive phase, thereby increasing fruit yield and quality (Pandey & Mahiwal, 2020).

In hydroponic systems, where nutrient management is more controlled, the importance of potassium is even more pronounced. Since plants rely entirely on nutrient solutions for their nutrition in such systems, the concentration of potassium must be carefully regulated to ensure optimal reproductive development (Thakur *et al.,* 2023). Potassium deficiencies can lead to severe reproductive issues, including poor flower formation, low fruit set, and decreased seed viability, in cotton it limits reproductive success by altering carbohydrate and protein balance (Hu *et al.,* 2018). Conversely, excess potassium can disrupt the uptake of other essential nutrients, leading to imbalances that affect plant health and reproductive success. Therefore, maintaining the right potassium concentration is crucial for achieving high yields and good-quality produce in hydroponic cultivation.

2.9.3 Potassium in banana

Another big challenge is plant nutrients because bananas require large quantities of NPK. Potassium (K) is essential in catalysing critical reactions such as respiration, photosynthesis, chlorophyll formation, and water regulation. It is crucial for water relations, transport, and the accumulation of sugars in the plant (IPNI, 2015). Bananas have more extensive K requirements than other nutrient elements. Total nutrient uptake per tonne of whole banana bunches (Cavendish) ranged from 4–7 kg N, 0.9–1.6 kg P_2O_5, and 18–30 kg K_2O (IPNI, 2015). Likewise, Akida (2021) reported an export of 1.7 kg N, 0.2 kg P, 6.3 kg K, 0.3 kg Mg, 0.1 kg Ca, 0.1 kg S, 2 g B, 1 g Cu, 10 g Fe, 2 g Mn, and 2 g Zn per tonne of harvested banana bunches in Mchare-Huti Green Bell (AA-EAHB).

Low K supply induces increased dry matter allocation to some plant species' roots while reducing dry matter allocation to roots in other species. Adequate K increases bananas' vigour and disease resistance, improves fruit weight, and increases the number of fingers per bunch (Aba & Baiyeri, 2015). In addition, K stimulates earlier fruit shooting and shortens the time to

fruit maturity. Also, K improves the storage quality of bananas. Its deficiency leads to the deformation of bunches (short, stunted), with fewer fingers per hand and poor fruit filling (Taulya, 2013b). Since K deficiency affects the translocation of sugars and starch, it produces poor fruit quality, but seed formation is part of fruit development.

Since bananas have been pollinated and result in few seeds, the physiology of seed development is affected by a particular factor, either biotic or abiotic. The number would be increased by putting in all the necessary conditions for fruit development. Several studies have shown that K is essential for increasing banana yield and fruit quality. It has been revealed that bunch mass response decreases as K decreases, and clearly shows there is a 65% bunch mass increase as well as several hands and fingers per bunch as K application increases (Taulya, 2013b), but it also was explained that K does not show the effect on cycle duration, but it significantly increases bunch filling; studies show there is a high relationship between K and moisture content (Taulya, 2013b).

2.10 Banana improvement

Improving cultivars that are resistant to diseases such as Fusarium wilt, black Sigatoka, bacterial Xanthomonas wilt, and pests like banana weevil and nematodes, requires addressing abiotic factors like soil nutrient deficiencies and climate changes, alongside seed production. While agronomic and cultural methods have not yielded permanent solutions to reduced banana production, breeding for improved varieties offers promise but faces significant challenges during seed (Ssebuliba *et al.,* 2006; Waniale *et al.,* 2021). It is suggested that weather conditions such as temperature, rainfall, relative humidity, or day length correlate with seed set variation (Ssebuliba *et al.,* 2009). According to Fortescue and Turner (2011), failure to produce seeds in Musa species can potentially occur in the gametophyte, sporophytic, or even during the interaction between male and female gametes. However, it is hypothesised that nutrient and moisture content in the soil might play a role in seed setting and quality.

2.11 Challenges facing banana improvement

Bananas face many challenges that lower their yield. It has been recorded that there is a drastic decrease in banana yield from 70 t ha^{-1} $year^{-1}$ to less than 30 t ha^{-1} $year^{-1}$ due to biotic and abiotic factors (Wilberforce *et al.,* 2014). Different efforts have been made to improve this vital crop with a small quantity of achievement. Breeding practices have occurred for several years (Hibler, 1994; Ortiz, 2013b; Wang *et al.,* 2016). In the 1920s, the first attempt was made to produce Fusarium wilt on Gross Michel cultivar AAA (Huang, 2019); for all years, only a few

improved varieties have been released due to the difficulties of hybridization on this crop. The success of any breeding program depends on various factors, including sufficient genetic variability of the traits of interest in a well-defined population, a breeding scheme suited to the physiological and biological nature of the crop, and the ability to produce recombinant seeds through sexual hybridization.

However, poor seed set significantly prolongs the breeding cycle, making genetic improvement slower and more challenging. Personal observation suggests that there is significant variation in seed sets among Musa cultivars. Successful pollinations can yield from a single seed to more than 100 using the same genotypes and macro environments (Brown, Personal communication) (Waniale *et al.,* 2020) observed variation that ranged from Musa hybridizations in Uganda. Preliminary analysis suggests that seasons of the year influence seed setting fluctuation; there is a correlation between seed set pattern and monthly weather variation, including rainfall, evaporation, air temperature, wind speed, solar radiation, and relative humidity, but also the result indicated that there are months that have high seed set but poor seed quality (Ssebuliba *et al.,* 2009).

Appropriate seed production depends on many crucial reproductive stages in a flowering plant (Tiwari *et al.,* 2023). The crucial reproductive stages in a flowering plant begin with flower bud formation, where the plant transitions from vegetative growth to producing buds under the influence of environmental factors like light and temperature. Following this, the flowering stage exposes the reproductive organs for pollination, which is essential for the transfer of pollen to enable fertilization. Once pollination occurs, fertilization follows as the pollen tube facilitates sperm-egg fusion, leading to seed formation. The seeds then develop and mature, often accompanied by fruit development, with adequate water and nutrients being crucial during this phase for healthy seed and fruit production. To produce viable seeds, pollination and subsequent fertilisation of the ovules in the fruit are required (Huang, 2019).

To breed bananas successfully, you need to know about the specific morphological, physiological, and environmental factors that affect the processes of pollination, fertilization, and seed production, as well as any problems that might get in the way of these processes (Huang, 2019). At the seed and embryo germination levels, breeding programmes have additional obstacles that must be handled. Studies on fruit development show that the active hormone promoting fruit growth significantly disrupts seed development (Simmonds, 1960). The volume of the fruit determines the amount of the hormone auxin that interferes with seed

production. This study hypothesised that there is no relationship between bunch size and seed set.

2.12 Conceptual framework

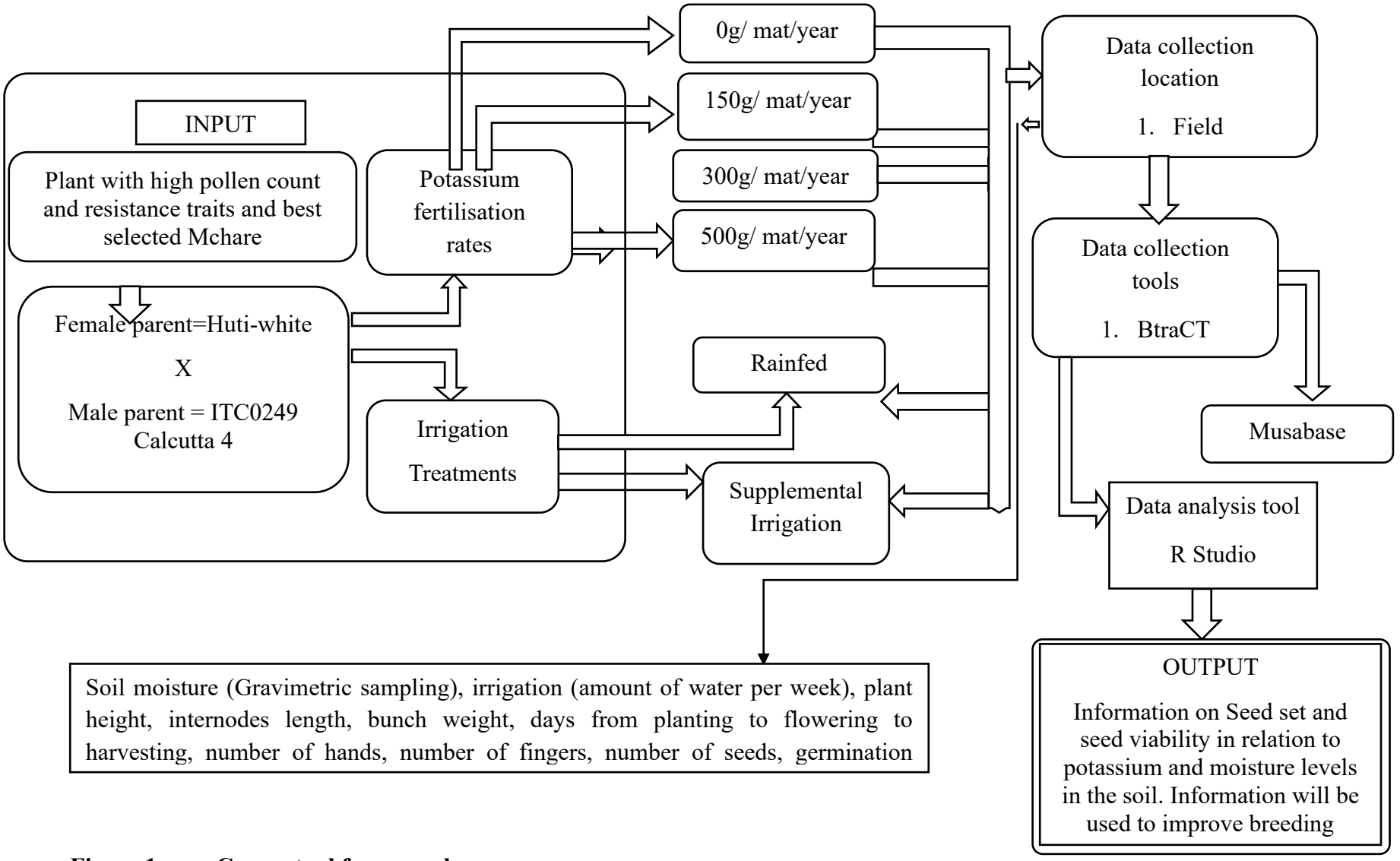


Figure 1: Conceptual framework

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The study was conducted at two sites: The Nelson Mandela African Institution of Science and Technology (NM-AIST), Tengeru campus (3°24'11"S, 36°47'34"E; 1201 m a.s.l.), and the World Vegetable Centre, Arusha (3°22'11"S, 36°52'11"E; 1264 m a.s.l.). Experiments were set up in banana fields managed by the International Institute of Tropical Agriculture (IITA) at both locations.

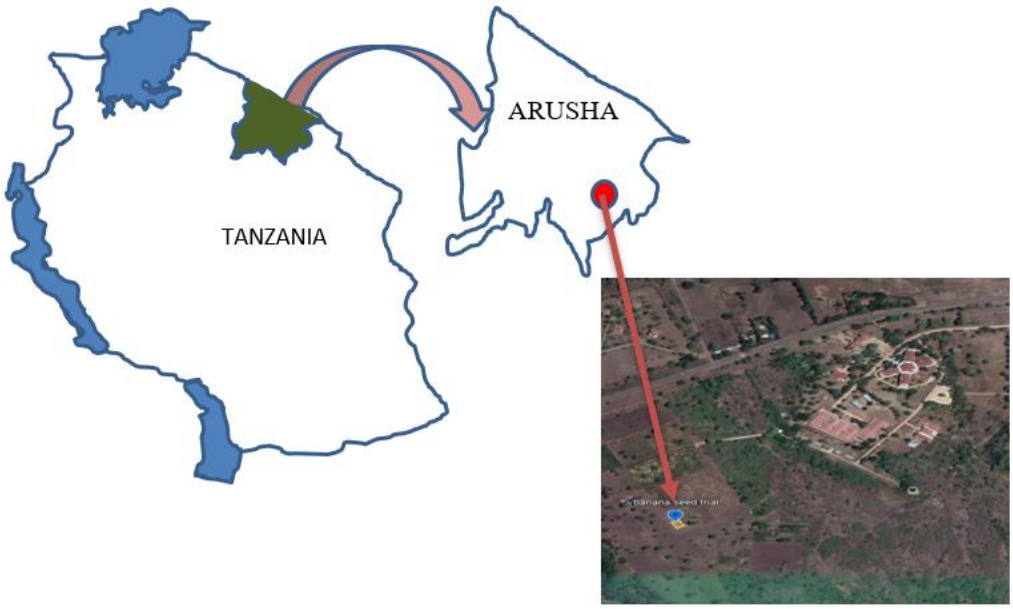


Figure 2: Location of the experiment

3.2 Soil type

The volcanic soil of 1 m overlies an igneous pyroclastic volcanic ash and tuff, gently sloping upward from the alluvium Manyire River bordering the field (Meya *et al.,* 2014).

3.3 Climate

The climate is a tropical highland with a moderately cool thermal zone (FAO, 2012). Rainfall follows a bimodal yearly pattern, with a long rainy season from late March to early June and a short rainy season from October to December, although rainfall varies yearly (Grieser *et al.,* 2006; FAO, 1996).

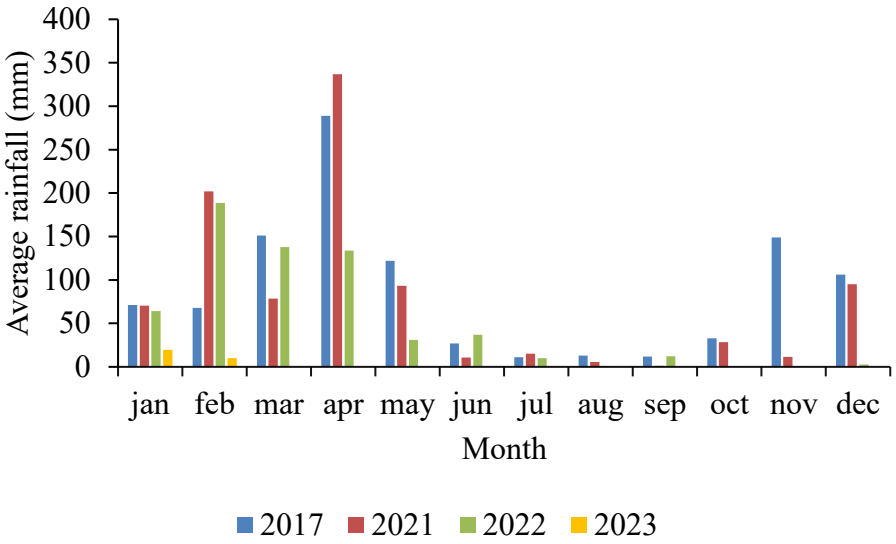


Figure 3: Tengeru rainfall in 2017, 2021 and 2022

Total rainfall during the experiment was 903 mm per year, which is less than the ideal rainfall for banana production, which is between 1100 and 2650 mm per year. The rainfall was also not distributed out evenly, with dry spells lasting more than two months (Fig. 3), which suggests that the bananas need supplemental irrigation (Robinson & Alberts, 1986; Van Asten *et al.,* 2011; Varma & Bebber, 2019).

3.4 Experimental set up

3.4.1 Pollen count and viability

This study evaluated 14 diploid banana accessions as mature plants in their second production cycle (second ratoon). Seven diploid banana accessions represent known variability and consumer preference of the cultivated Mchare (*Musa* AA group, Mchare subgroup) in Tanzania. Five accessions were included for comparison, including wild-type banana diploids used as donor sources for disease resistance and two cultivated (seedless) diploids (Table 1). Plants within blocks were spaced at 3 x 2 m and planted in a randomized complete block design with three replications. All plants were rainfed, with a maximum rainfall of 289 mm in April and a minimum of 11 mm in July; the average rainfall per month for the year 2017 was 88 mm, a maximum temperature of 28ºC was recorded in February, and a minimum temperature 13ºC was recorded in July (Fig. 2). Data was collected once a month over a period of seven months (February to August 2017).

Table 1: Fourteen banana varieties used in pollen variation and viability studies

No	ITC Codes	Varieties	Subspecies	Type
1	ITC0249	Calcutta 4	ssp. *burmannica*	Wild
2	ITC0253	Borneo	ssp. *microcarpa*	Wild
3	ITC0712	CV. Rose	ssp. *malaccensis*	Cultivated
4	ITC1121	Pisang Lilin	ssp. *malaccensis*	Cultivated
5	ITC0609	Pahang	ssp. *malaccensis*	Wild
6	ITC0393	Truncata	ssp. *truncata*	Wild
7	ITC0966	Zebrina (G.F.)	ssp. *zebrina*	Wild
8	ITC1559	Huti green bell	Mchare	Cultivated
9	-	Huti-White	Mchare	Cultivated
10	-	Mchare laini	Mchare	Cultivated
11	ITC1446	Makyughu-II	Mchare	Cultivated
12	ITC1455	Mchare mlelembo	Mchare	Cultivated
13	ITC0281	Akondro mainty	Mchare	Cultivated
14	ITC1460	Ijihu Inkundu	Mchare	Cultivated

3.4.2 Pollen collection

To determine the optimal time for pollen collection, six diploids (Calcutta 4, Borneo, Huti-White, Pisang Lilin, Zebrina G.F. and Akondro mainty) were assayed for quantity and viability in two-hour intervals from 0600 to 1400 h once a week for four weeks. Based on the results, pollen collection was standardized at 0800 h for all subsequent assays.

3.4.3 Quantification of pollen grain

As banana plants flower in a non-seasonal manner, plants were selected based on availability. The third hand of emerging male flowers (subsequent clusters of flowers emerge each day) was sampled, and pollen was collected from 3 anthers of 3 plants per genotype. Plants were selected randomly. Pollen was carefully removed from the anthers using sterilized tweezers and evenly distributed on microscopic slides. One drop of detergent solution (prepared by diluting two drops of commercial dish washing soap in 250 mL of distilled water) was added to the sample to disperse the pollen grains. A Nikon SM275T Zoom stereo microscope with a magnification range of 6.5 to 22.5 X and an attached digital camera were used to take digital images (JPEG format), which were then input for image analysis using Image J software as described by Rasband (2008) which provides reliable counts of pollen grain numbers (Costa & Yang, 2009). Each image was individually processed to sharpen, remove noise, and enhance individual pollen grains for accurate counting.

3.4.4 Pollen viability test

Pollen viability of 14 banana varieties was assayed according to Soares *et al*. (2016). Pollen grains were stained with a 1% Triphenyl Tetrazolium Chloride (TTC) solution diluted in Tris buffer (0.15 M HCl, pH 7.8), followed by a two-hour incubation period at room temperature. Four sub-sample counts were carried out from multiple regions of the slide (25 grains per region) under a stereo microscope. Viable pollen grains stained by TTC appear light to dark red, while non-viable pollen grains remain translucent in the presence of the stain (Soares *et al.,* 2016). The number of viable and non-viable pollen grains was converted to percentage using the below formula

$$(Viable\ pollen/Total\ pollen)\ *100$$

3.5 Effect of soil moisture and K fertilisation on seed set and viability

3.5.1 Plant material

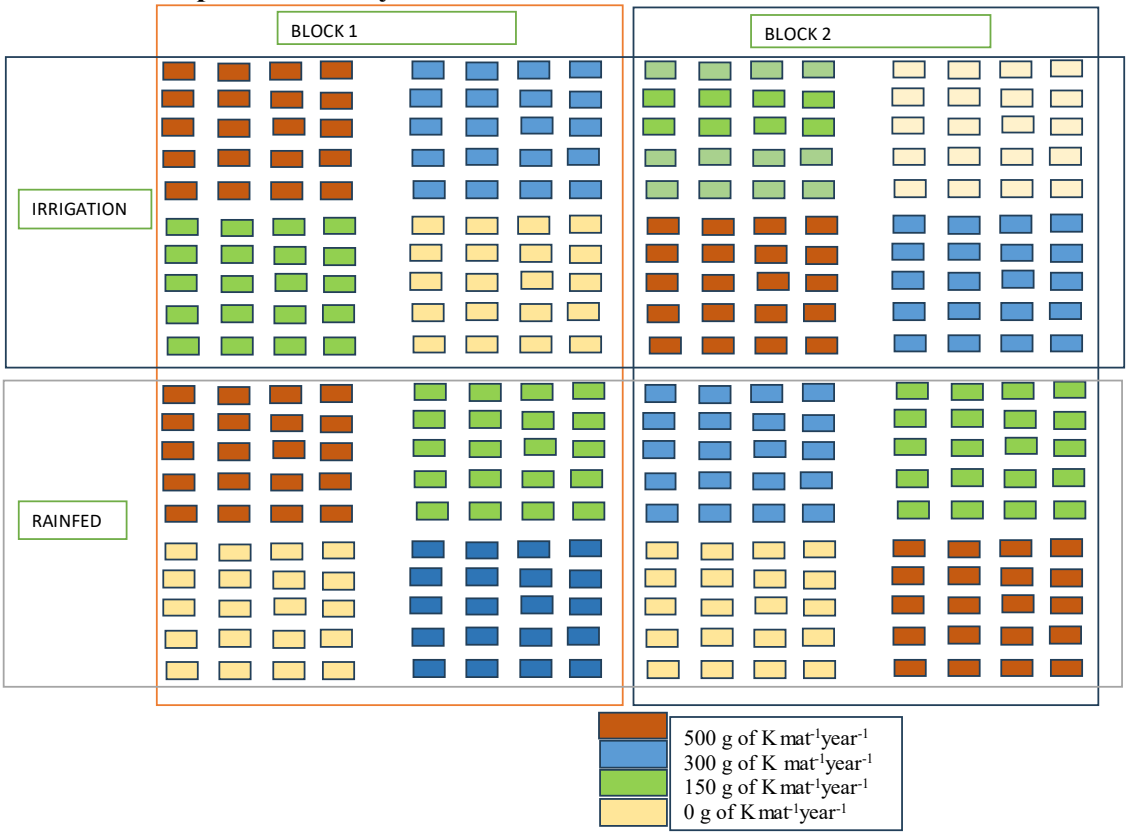
East African Highland banana Huti-white (*Musa* AA group, Mchare subgroup) was selected as the female parent because it is the most fertile compared to other Mchare varieties and also preferred by farmers (Dr. Allan Brown, personal communication). Huti-white suckers of two months were obtained from disease-free existing farm. Suckers were used instead of tissue culture material because the availability of tissue culture material were limited, and the order indicated that I could water for almost a year to get them so the fast option were to use suckers. Suckers within one week after emergence were tagged to ensure the identical age of the planting material. After sucker collection, sword suckers were cleaned to remove any possible contamination by using Jik as a disinfectant (NaOCl) and then transplanted to the prepared planting holes. Suckers of the same size were transplanted to avoid age as the confounding effect and have homogeneous plant performance.

3.5.2 Field layout

The field was planted in June 2021. Planting mat (holes) size were 0.7 m by 0.6 m at a spacing of 2 m (row) by 2 m (line) (8 row*20 column =160 plants) replicated twice (320 mats). One plant in a mat (planting hole) is referred to as reproductive cycle (one harvest). “Mat” is a total composition of different reproductive cycles present in one location (Stevens *et al.,* 2020). In every mat, maximally, two plant cycles are kept, i.e., parent plant or cycle 1 (P), first ratoon or cycle 2 (R1).

Time is noted in weeks after planting (WAP) and month after planting (MAP). Calcutta 4 was grown in a separate trial, receiving fertilizers at a rate of 92: 100: 300 g/mat/year N, P, K split three times at planting, vegetative stage and at flowering, 80 L/mat/week (1040 mm/ha/year) was applied as supplemental irrigation. The experimental design (Table 2) was a split-plot design with four subplots having different potassium levels (0 g K mat $^{-1}$ year $^{-1}$ as a negative control, 300 g K mat $^{-1}$ year $^{-1}$ as a positive control, 150 g K mat $^{-1}$ year $^{-1}$ as low application rate and 500 g K mat $^{-1}$ year $^{-1}$ as high application rate) within the two main plots (whole plots) with varying irrigation schemes (full irrigation "IR" and rainfed "RF"). Every subplot had 20 mats, which gives 80 mats for RF (0RF, 150RF, 300RF and 500RF), and 80 mats for IR treatment (0IR, 150IR, 300IR and 500IR) which are 160 mats replicated twice, totalling 320 mats. Hybridization was performed by crossing Huti-white by Calcutta 4. To avoid border effects, six central plants in each subplot were used for data collection. All accessions were supplied with irrigation for 4 MAP, and at 5 MAP 160 mats were exposed to moisture stress, while the other 160 mats were supplied with 80 L/mat/week (1040 mm/ ha/ year).

Table 2: Experimental layout



3.5.3 Field management

Potassium from muriate of potash was split applied, based on the rain season, where one-third of the respective K levels (0, 150, 300, and 500 g K/mat^{-}/year^{-1}) was applied on 30th June 2021,

17th March 2022, and 23rd June 2022, 100 g P /mat-/year from triple superphosphate and 92 g N /mat /year from Urea were split along the application of K.

Every mat in the irrigation treatment had four drippers each providing four liters per hour. Dripper discharge was examined by recording the time to fill one-liter beaker. This was done along the dripper lines to observe any difference at the beginning of the line, middle, and end. Irrigation was applied every day; water pump was switched on for one hour and 15 minutes to ensure the plant received 20 L per day for four days and made 80 L per week. All plants were treated identically during the first 16 WAP. As of 17 WAP 160 plants were given 80 L per week being fully irrigated (FI) and other 160 plants were rain-fed (RF).

At 4 MAP, plants were de-suckered to allow the growth of one sucker only (single sucker management as is standard practice), whereby suckers of 30 cm height at the western direction of the field were allowed to grow as the first ratoon (second cycle) while others were pruned. This means each banana mat was left with two plants with stages called cycle 1 and cycle 2. Leaves with less than 80% green area were pruned since they were considered as dead leaves. Weeding was done every three months during the dry season since the rate of weed growth is high during this time, but during the rainy season weeding was done as needed.

3.6 Data collection

3.6.1 Data collection tools

Collected data were uploaded to the Banana database (Musabase), from which QR codes for every accession were generated. Then, a crossing plan (crosses between Huti-white and the selected diploid wild banana Calcutta 4) was followed by the Banana tracking tool (BTracT).

3.6.2 Banana growth measurements

Banana plant growth was measured in the central six mats (2×3 mats) twice monthly at the plot level, within all subplots; this is because a banana plant produces on average one new leaf per week. Measured variables and variables calculated from growth measurements are listed in (Table 3). Plant height was measured from the collar (intersection of the first two youngest fully emerged leaves) to the bottom of the plant and recorded in centimetres. Plant girth was measured at 100 cm above the ground. The number of functional leaves was recorded, along with the internode length of the top three successive leaves. Phenological data were recorded to identify potential different growth phases of the crop.

3.6.3 Pollination techniques and procedure

Pollination was done at flowering around 6 am, when pollen from selected males Calcutta 4 and SH-3142 was collected from the male plot that receivesstandard fertilizer application (92: 100: 300 g/mat/year N, P, K) and brushed on Huti-white female flowers. This was repeated for 5 to 7 days until the last hand had opened. After the first pollination, the flower was covered with cotton bags to avoid unwanted pollen from other sources.

3.6.4 Bunch harvesting and ripening

After pollination, the bunches were left to mature until one finger from the first hand started to turn yellow or burst. Bunch weight, number of hands and fingers, and finger dimensions were recorded. From here, bunch parameters (Table 3) were recorded to evaluate the relationship between bunch size and seed set. Digital weighing balances were used for weight measurement and a tape measure were used to measure finger length and circumference. All harvested bunches were sent to the ripening chamber at a temperature of 27 to 35°C to soften the fruits for 5 to 10 days.

3.6.5 Seed extraction

Seeds were extracted manually from ripe bunch with their cross-ID QR code and all extracted seeds sent immediately to the tissue culture laboratory.

3.6.6 Seed germination

In the tissue culture laboratory, good seeds (those with embryos) were separated from bad seeds by soaking all extracted seeds overnight. Seeds with embryos sank, while those without embryos referred to as bad seeds floated on the water surface. Good seeds were cracked, and the embryo were cultured on growth media (Murashige and Skoog) and then placed in the dark room. Cultured embryos were observed weekly for a maximum of eight weeks and germination was recorded.

3.6.7 Soil moisture

To monitor soil moisture, soil was sampled in six positions in each subplot using core samples from 0 to 30 cm depth and 30 to 60 cm depth (Sanchez *et al.,* 2012). Soil samples were collected randomly in every split-plot. Fresh sample weight was measured with a digital balance and then oven-dried at 80ºC until constant weight. This was done monthly from the 5th WAP onwards. The T-RH sensors were installed at every first plant of each split plot at 50 cm from the plant

base. These sensors were set to monitor temperature and relative humidity below the canopy of the experimental layout.

Table 3: Parameters measured include phenological events, vegetative growth, harvesting, and seed extraction and germination data

Parameters for plant growth	Description
Phenology	
Days to flowering (days)	Number of days from planting to flowering
Days to harvesting (days)	Number of days from flowering to harvesting
Vegetative growth data, periodic	
Pseudostem height, H (cm)	Measured until petiole divergence on the top
pseudostem girth at base, $Gbase$ (cm)	Measured at middle
pseudostem girth at mid, $Gmid$ (cm)	Measured at 100 cm from the ground; measured from the ground.
Functional leaves, $functL$ (no.)	Leaves with less than 80% necrotic area
Dead leaves, $deadL$ (no.)	Leaves with more than 80% necrotic area
Harvesting data	
Bunch weight (kg)	Weight total bunch harvested from collar
Number of hands-on bunches, $Nhand$ (no.)	Counted hands on a bunch
Finger length, $Lfinger$ (cm); Finger radius, $rfinger$ (cm)	Average length/radius of individual finger

Table 4: Parameters measured include phenological events, vegetative growth, harvesting, seed extraction and germination data

Parameters for plant growth	Description
Seed extraction data	
Number of good seeds (no.)	Number of seeds from the bunch which are well filled with the endosperm (hard seeds) per bunch
Number of bad seeds (no.)	Number of seeds from the bunch which are not well filled with the endosperm (soft and shrink seeds) per bunch
Germination data	
Number of embryos cultured (no.)	Number of seeds sown on the growth media
Number of embryos germination (no.)	Number germinating embryos
Embryo germination rate	Number of germinating embryos/total number of embryos cultured

3.7 Data analysis

3.7.1 Pollen amount and viability

To evaluate the effects of genotype, month, and their interaction on the measured traits, statistical analysis was performed. An analysis of variance (ANOVA) was conducted using GenStat software (21st Edition, 64-bit) at a significance level of $P \leq 0.05$. The linear model used was:

$$Y_{ijkl} = \bar{Y} + G_i + R_j + M_k + GM_{ik} + e_{ijkl},$$

where $\bar{Y}$ is the grand mean, G_i is the genotype effect, R_j is the replication effect, M_k is the month effect, GM_{ik} is the genotype × month interaction, and e_{ijkl} is the experimental error. All effects were considered fixed, except for the month, which was treated as random. Mean separation was done using Fisher's protected least significant difference (LSD) test at a 5% probability level when F-values were significant. Additionally, Pearson correlation analysis between pollen viability and concentration was performed using the same software.

3.7.2 Effect of Supplemental irrigation and K application on seed set and viability

The seed data was treated as binary, taking the number seeds viability (SV) as proportion of the total number of seeds (TS) and the total number of germinated seeds (NEG). Because of the binary nature of the seed data a Generalized Linear Mixed Model (GLMM) as implemented in GenStat, was fitted, with a logit link function g(μ) and assuming a Binomial distribution for the fixed effects and a Beta distribution for the random effects. The dispersion parameter for the variance of the response was set as "estimate". The GLMM was of the following form (random terms underlined):

$$g(\mu) = \mu_0 + Block_b + Cycle_c + Irrigation_i + K_k + Irrigation{:}\,K_{ik} + \underline{Wplot_{b(w)}} + \underline{Splot_{w(s)}} + \epsilon_{bciksw}$$

Having Block, Cycle, Irrigation, K and the interaction Irrigation: K as fixed terms, and Whole plots nested into Block and Subplots nested into Whole plots as random terms. The overall significances of the fixed effects were assessed by a Wald test for dropping fixed terms, as implemented in GenStat (GenStat for Windows 22nd Edition. VSN International, Hemel Hempstead, UK. Webpage: Genstat.co.uk).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Pollen amount and viability between Mchare and wildtype banana varieties

4.1.1 Results

In the first objective, this study compared the pollen quantity and viability between Mchare and wild-type banana genotypes to identify suitable parental candidates for optimising seed production in banana breeding programs. Pollen quantity and viability are critical factors influencing successful fertilisation and seed set, making them key considerations in breeding programs. By evaluating these traits in Mchare and wild-type genotypes, this study aimed to determine which genotype showed greater potential for enhancing seed production. The following results and discussion present the findings, highlighting the differences between the 14 genotypes and their implications for improving banana breeding strategies.

The study revealed that pollen viability peaked at 0800h for all 14 genotypes and declined dramatically thereafter (Fig. 5). Lower pollen viability was observed independently per banana genotype. Analysis of variance of total pollen production revealed significant differences ($P \leq 0.05$) among the genotypes (Table 5). Average pollen production (pollen grains/anther) over the seven months ranged from 155 to 31 000 pollen grain/anther, with Calcutta 4 and Borneo producing over 31 000 pollen grains/anther, followed by CV. Rose (28 847 grains/anther), Pisang Lilin (27 262 grains/anther), Pahang (25 715 grains/anther), and Truncata (22 259 grains/anther) (Table 5, Fig. 8).

The Mchare genotypes produced significantly less pollen than the wild-type bananas, but there were significant differences among the Mchare genotypes. Huti green bell (8120 grains/anther) and Mchare laini (7875 grains/anther) represented about 20% of the pollen observed in Calcutta 4, while others (Mchare mlelembo and Akondro mainty) produced about 10%. Ijihu Inkundu was completely sterile, producing either no pollen or only trace amounts depending on the month (Table 6). Pollen production varied significantly between months (Table 5) with low production in May (14 519 grains/anther) July (14 451 grains/anther) and August (14 706 grains/anther).

Borneo and Calcutta 4 recorded the highest amount of viable pollen both with (74.2%) and were not significantly different (Fig. 9) but were significantly different from CV. Rose (64.6%)

($P \leq 0.05$), Huti-White (59.3%) and Pahang (57.2%). The lowest pollen viability was observed in the Mchare cultivar Ijihu Inkundu (7.3%) (Table 6).

Table 5: ANOVA for pollen production and viability during seven months

Source of variation	DF	SS (Pollen production)	MS (Pollen production)	SS (Pollen viability)	MS (Pollen viability)
Rep	2	89920000	44960000ns	125.20	62.60 ns
Month	6	213420000	35570000ns	4460.16	743.36 ***
Genotypes	13	38103000000	2931000000***	91750.10	7057.70 ***
Month *Genotypes	76	2122680000	27930000ns	7790.76	102.51 ***
Residual	190	4142000000	21800000	5891.90	31.01
SD			4669.05		5.57
CV (%)			30.30		11.70

There was a significant difference in terms of average pollen viability over the seven months ($P < 0.001$), suggesting that pollen viability was influenced by the weather condition (Table 4). The highest amount of viable pollen (51%) was observed during humid and warm months (250 to 300 mm per month) (April and May) with temperature (25 to 27°C), while the lowest viability was observed from June to August (41-45%) (Table 6), which is typically drier and cooler. There was a significant interaction between month and genotype in pollen viability ($P < 0.001$) suggesting that genotypes may behave differently (Table 4). A positive correlation was observed between the amount of pollen produced per anther and the percentage of pollen viability (r = 0.76) (Fig. 7).

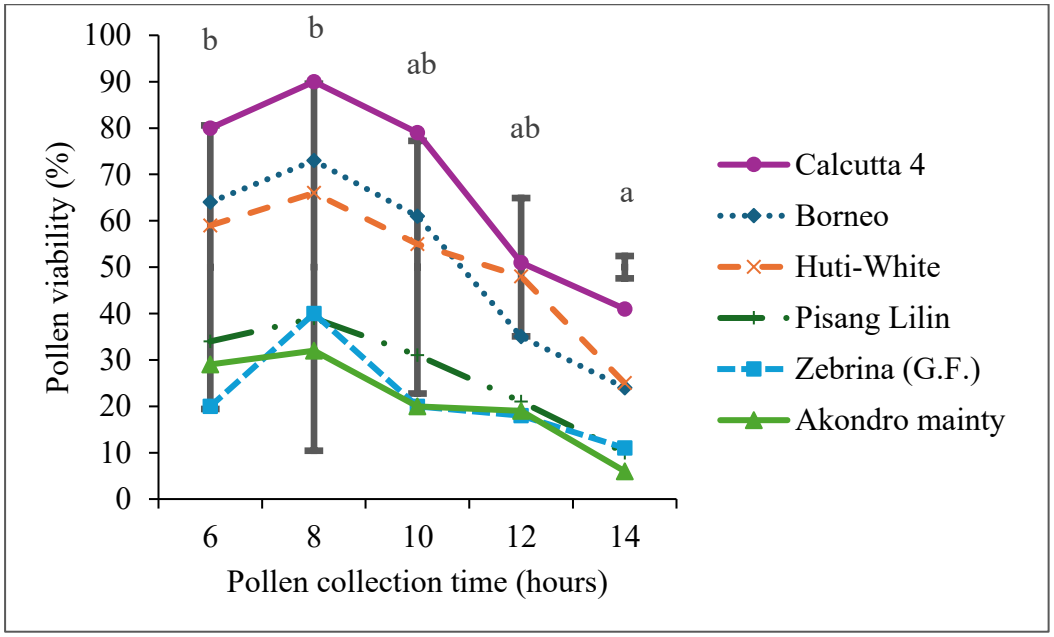


Figure 4: Average pollen viability (%) of six selected banana genotypes collected at different times of the day. The mean of genotypes with similar letters is not significantly different at $P \leq 0.05$

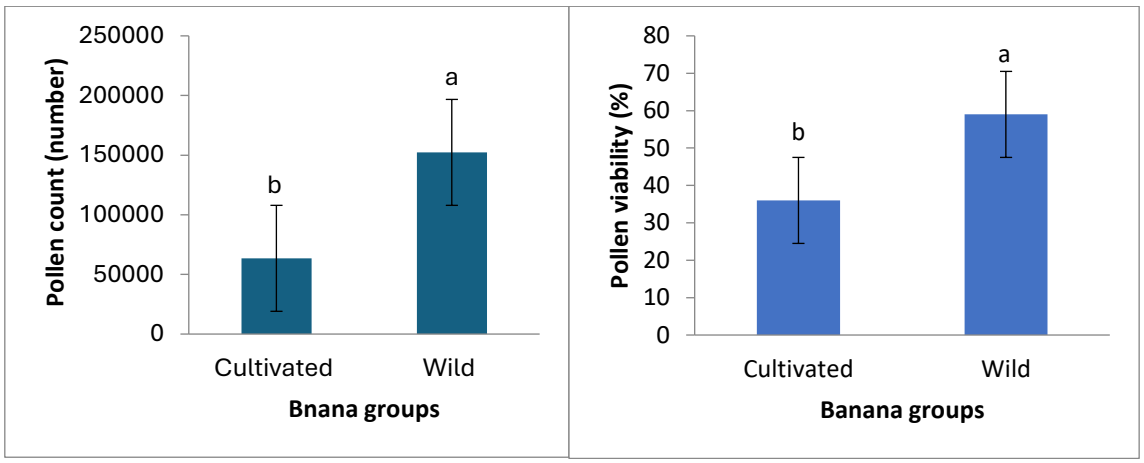


Figure 5: **Comparison of pollen production and viability (%) between cultivated and wild banana genotypes. LSD = Least significant difference; Mean with similar letters are not significantly different at $P \leq 0.05$**

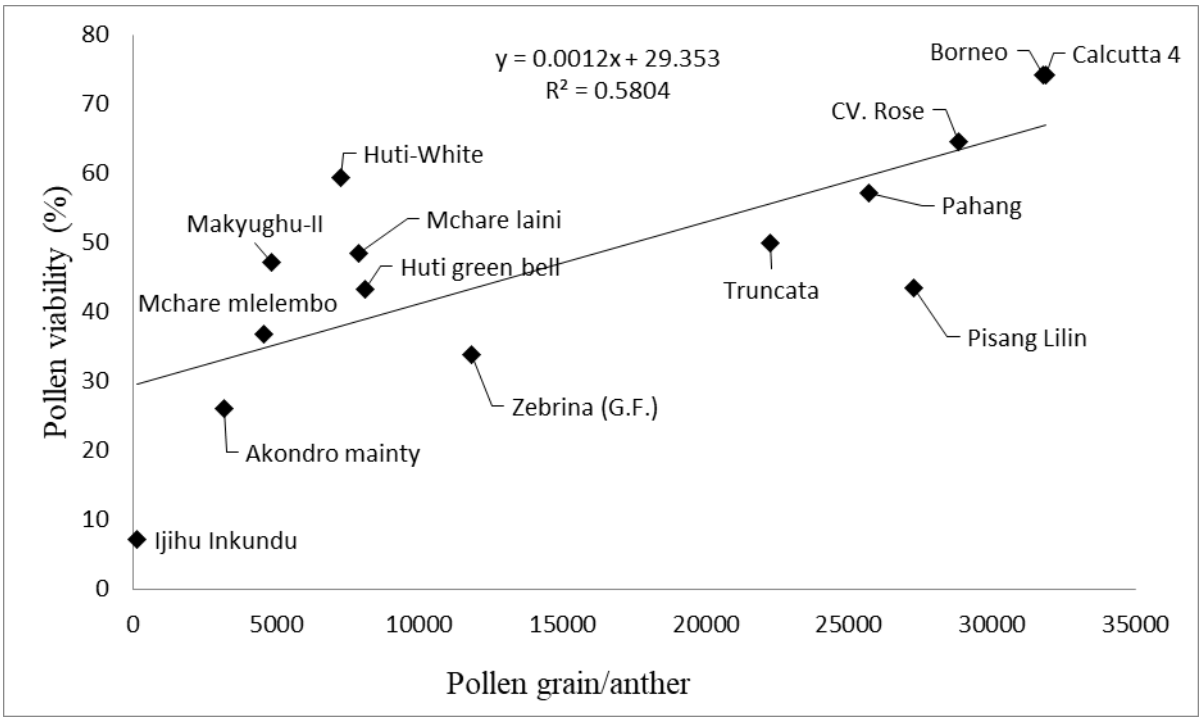


Figure 6: **Association between the amount of pollen and viability (%) in 14 banana genotypes**

Table 6: Average amount of pollen per anther of 14 diploid banana genotypes during seven months in 2017

Genotypes	February	March	April	May	June	July	August	Total	Mean	SD
Calcutta 4	33 550	31 217	31 023	32 793	32 176	30 784	31 499	223 042	31 863a[1]	1018.9
Borneo	32 779	34 510	33 456	29 351	29 483	30 905	32 158	222 642	31 806a	1972.3
CV Rose	29 206	30 162	29 309	28 158	30 037	25 346	29 714	201 932	28 847b	1681.8
Pisang Lilin	27 167	29 343	27 444	27 040	26 899	26 193	26 749	190 835	27 262bc	996.6
Pahang	31 807	28 082	29 726	22 321	17 535	26 264	24 266	180 001	25 715c	4824.2
Truncata	25 494	23 669	23 697	21 556	21 455	21 561	18 378	155 810	22 259d	2280.8
Zebrina (G.F.)	8699	12 728	16 513	9470	11 234	11 751	12 450	82 845	11 835e	2544.8
Huti green bell	4211	3939	8933	5864	22 402	5722	5769	56 840	8120f	6503.4
Mchare laini	5046	5010	6474	5667	22 306	5107	5514	55 124	7875f	6384.1
Huti-White	5840	7022	9920	6900	6662	6591	7876	50 811	7259f	1320.5
Makyughu-II	4748	4577	5794	236	4877	4472	4194	33 898	4842g	532.1
Mchare mlelembo	2676	5106	5168	5497	5059	4477	4006	31 989	4570g	971
Akondro mainty	3028	3347	3369	3219	3245	2913	3068	22 189	3170g	171.1
Ijihu Inkundu	0	0	197	199	222	232	237	1087	155h	107.2
Total	214 251	218 712	231 023	203 271	233 592	202 318	205 878			
Monthly mean	15 304bc	15 622abc	16 502ab	14 519c	16 685a	14 451c	14 706c			

SD = Standard deviation, LSD = Least significant difference, Mean with the similar letter in the same column (Mean) and same row (Monthly mean) are not significantly different at $P \leq 0.05$

Table 7: Average pollen viability (%) per anther of 14 diploid banana genotypes during seven months in 2017

Genotypes	February	March	April	May	June	July	August	Total	Mean	SD
Borneo	79.1	84.3	77.4	77.1	67.7	64.8	68.8	519.2	74.2a	7.1
Calcutta 4	79.1	84.3	77.4	77.1	67.7	64.8	68.8	519.2	74.2a	7.1
CV. Rose	75.6	67	73.4	61.3	56.9	58.3	59.4	452	64.6b	7.5
Huti-White	64.3	61.6	62.1	65.5	56.5	49.2	55.9	415.2	59.3c	5.8
Pisang Pahang	55.2	52	67.4	67.4	59.1	49.2	49.7	400.1	57.2c	7.8
Truncata	66.8	63.9	47.9	56.4	38.9	35.5	40.6	349.9	50.0d	12.5
Mchare laini	58.9	55.1	47.7	56.1	43.9	38.5	39.1	339.4	48.5d	8.4
Makyughu-II	50.6	52.3	53.7	51	42.2	42.2	38.4	330.4	47.2d	6.1
Pisang Lilin	46.8	36	48.9	46.9	45.4	36	44.2	304.4	43.5e	5.3
Huti green bell	37.8	36.2	52.7	48.2	47.2	39.2	42.1	303.4	43.3e	6.2
Mchare mlelembo	41.3	41.5	36.4	41.2	35.8	29.2	32.6	258	36.9f	4.8
Zebrina (G.F.)	26.4	27.9	29.4	37.9	38.5	37.6	39.1	236.7	33.8f	5.6
Akondro mainty	31.5	26.1	29.8	26.1	27.5	20.6	20.8	182.5	26.1g	4.2
Ijihu Inkundu	0	0	11.9	10.2	8.1	10.2	10.1	50.6	7.2h	5.1
Total	713.6	688.2	716.2	722.4	635.6	575.1	609.6			
Monthly mean	51ab	49.2b	51.2ab	51.6a	45.4c	41.1d	43.6c			

DS = Standard deviation, LSD = Least significant difference, Mean with the similar letter in the same column (Mean) and same row (Monthly mean) are not significantly different at $P \leq 0.05$.

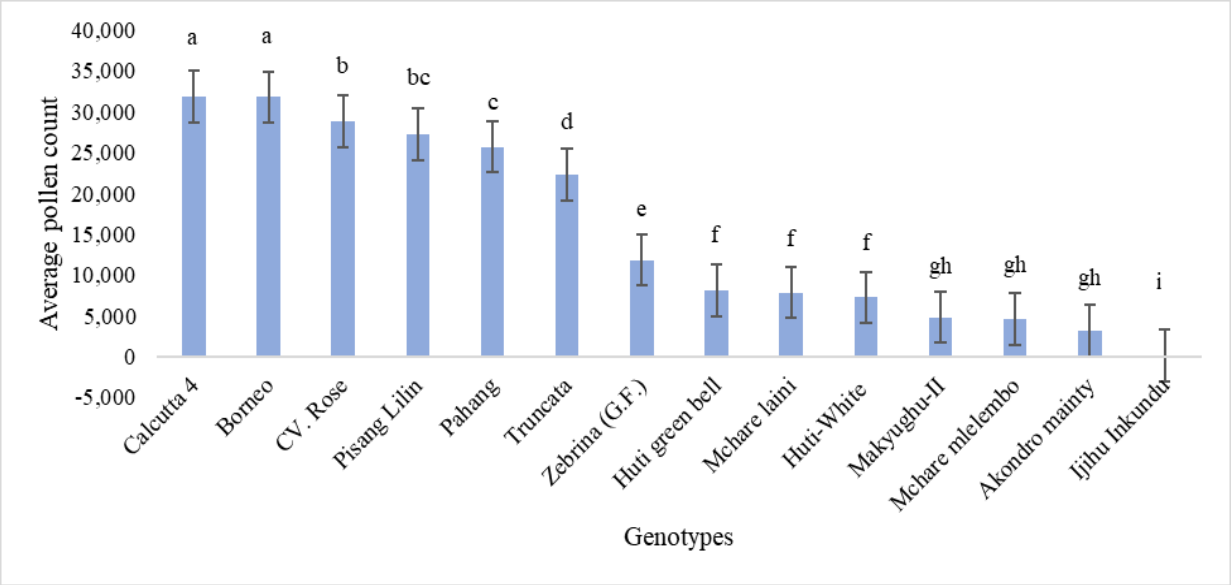


Figure 7: **Average amount of pollen per anther of 14 diploid banana genotypes observed during seven months in 2017. LSD = Least significant difference; Mean with similar letters are not significantly different at $P \leq 0.05$**

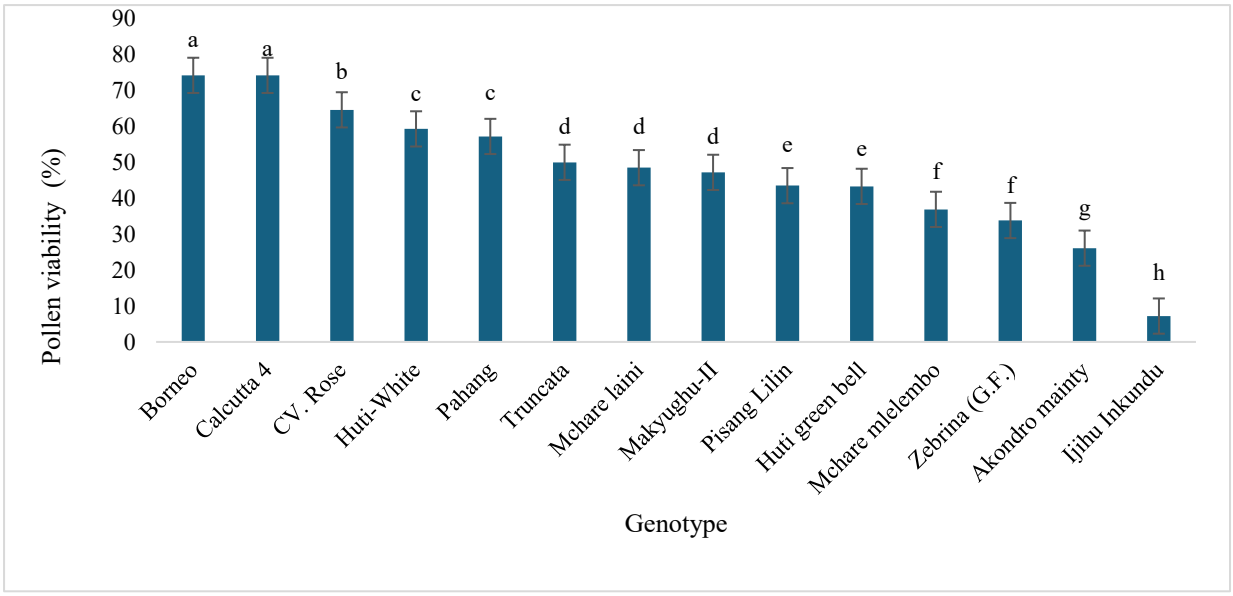


Figure 8: **Pollen viability (%) per anther of 14 diploid banana genotypes during seven months in 2017. LSD = Least significant difference; Mean with similar letters are not significantly different at $P \leq 0.05$**

4.1.2 Discussion

Maximum pollen viability in banana genotypes was observed when pollen was collected in the morning particularly, at 0800h, irrespective of the tested genotype (Fig. 5). Similar results were reported by Kaian *et al*. (2016), who collected pollen from maize (*Zea maize*) at different time points (0900 h, 1400 h and 1600 h) and determined that pollen collected in the morning presented a higher germination rate. Mondo *et al*. (2022) reported that the pollination success rate in yam (*Dioscorea alata*) is more conducive in the morning (0800-1200 h), though the *D.*

rotundata were more conducive in the afternoon (1200-1700 h). The results are also supported by Soares *et al*. (2015), who postulated that banana pollination success should be high in the morning (0700-1000 h) due to maximal pollen viability and stigma receptivity. High pollen viability in the morning could be associated with lower temperatures and/or lower vapour pressure deficit, and some have observed that higher temperatures harm pollen viability in cereals crops (Ge *et al.,* 2011; Rang *et al.,* 2011; Wang *et al.,* 2004). Low pollen viability could also be attributed to additional environmental factors like relative humidity, daylight and wind therefore further work will be required to fully understand the phenomena (Johri & Vasil, 1961; Shivanna & Johri, 1989).

This study showed that the number of pollen grains per anther varied significantly among the tested banana genotypes, with wild genotypes producing the highest amount of pollen (with greater viability) than cultivated bananas. Similar results have been reported by Soares *et al*. (2016). According to Tenkouano *et al*. (1998), the wild diploid (Calcutta 4) produces about 10% higher pollen rates when compared to cultivated diploids. This could be caused by chromosomal heterogeneity in many important cultivated bananas (including Mchare) as they have been demonstrated to be hybrids of two or more subspecies (Martin *et al.,* 2017: Barankova *et al.,* 2024). The production of seeds is generally poor and has been reported to be 0.3 to 21.7 seeds per bunch (Batte *et al.,* 2019; Vuylsteke *et al.,* 1993). Low pollen production in banana cultivars poses a challenge to banana improvement, as it severely limits the production of viable seeds through crossbreeding, which is crucial for a successful breeding programme. It is interesting to note that the *malaccensis* subspecies cultivars (CV. Rose, Pisang Lilin and Pahang) in this study produced significantly less pollen than the representatives of the subspecies *burmannica* (Calcutta 4) and *microcarpa* (Borneo). Further work will be required to determine if these results are characteristics of the subspecies.

Pollen viability percentage was considerably higher in wild-type bananas when compared to cultivated bananas. Adeleke *et al*. (2004), and Dumpe and Ortiz (1996) in their fertility studies in diploids and triploids, found that the wild banana Calcutta 4 had very high pollen viability compared with other diploids. Dumpe and Ortiz (1996) estimated that Calcutta 4 and Borneo had pollen viability of up to 98% in Nigerian environments. This might be attributed to high humidity in Nigeria which is between 75% to 85% during the wet season and 60% to 75% during the dry season. Given that the relative humidity between 60% to 90% is favourable for pollen germination (Bhatnagar & Desha, 2019).

Monthly variations were found to influence pollen viability across banana genotypes. The highest viability occurred between February and May, a period marked by increased rainfall and warmer temperatures. In contrast, the lowest viability was recorded from June to August, coinciding with dry conditions and cooler temperatures. Similar results by Ssebuliba *et al*. (2009) were observed regarding the seed set in Uganda, with two major seed set peaks occurring between March and April and a minor peak in September where the temperature in Uganda is between 21°C and 30°C in March, between 20°C and 28°C in April and between 19°C and 28°C in September. In this study, pollination occurred from February to August. The meteorological data indicated that the maximum temperature was recorded in February at 28°C, followed by 27°C in March and 25°C in April. Precipitation levels were highest in March (151 mm), April (289 mm), May (122 mm), and November (149 mm) (Fig. 1).

The observed temperature and rainfall patterns during the study period align with findings from Tenkouano *et al*. (1998), who reported that higher pollen viability in Nigeria was positively correlated with increased solar radiation and higher temperatures, while it was negatively impacted by high rainfall and high relative humidity. In this study, the relatively high temperatures in February and March, coupled with moderate rainfall, may have created favourable conditions for pollen viability during the initial stages of pollination. Conversely, the high rainfall and lower temperatures observed in July could have contributed to less favourable conditions for pollen survival and germination, consistent with Tenkouano *et al*. (1998).

The Mchare banana subgroup has been recognized as the unreduced gamete donor of some of the most important triploid dessert bananas (Cavendish, Gros Michel, Pome, and Silk subgroups) consumed worldwide (Martin *et al.,* 2023; Jeensae *et al.,* 2021; Martin *et al.,* 2020; Perrier *et al.,* 2011). The improvement of these triploid bananas with wild germplasm has proven problematic because of the offspring's lack good sensory traits (Raboin *et al.,* 2005). Fortunately, some of the highest pollen-producing Mchare genotypes (also with the highest percentages of viable pollen, i.e., Huti-White and Mchare laini) possess the farmer's preferred characteristics. Some of these genotypes (Mchare laini and Mchare mlelembo) are in the highest demand in Tanzanian local fresh markets. Male and female fertility is a prerequisite for any recurrent breeding programme (Ssebuliba *et al.,* 2009). The observed differences in pollen viability among the cultivated Mchare bananas suggest that selecting specific genotypes could be a strategy to improve seed production. Breeders can optimize reproductive success and

enhance seed yield in banana breeding programs by choosing genotypes with higher pollen viability.

4.2 Impact of supplemental irrigation and potassium fertilization on banana seed set and viability in Mchare breeding

4.2.1 Results

The second objective focuses on assessing the impact of supplemental irrigation and K fertilisation on banana seed set and viability following Mchare crossbreeding. Both water and nutrient management are critical factors influencing seed development and quality in bananas, particularly in enhancing seed set and viability. Supplemental irrigation ensures consistent moisture availability, essential for a successful seed set, while K plays a crucial role in reproductive processes, including seed formation and viability. The following results and discussion will explore how these two factors interact to affect seed set and viability, offering insights into their potential to optimise seed production in banana breeding programs.

The results show that irrigation intensities and K levels have an impact on the seed set, while their interaction does not (Table 8). A higher number of seeds per bunch were extracted under rainfed conditions (RF) with 300 and 500 g of K/mat/year (Fig.10) an average of 9 and 10 seeds per bunch, respectively. The results show that cycle by K interaction has a significant impact ($P = 0.015$) and separately irrigation and K levels ($P < 0.001$) and ($P = 0.001$), respectively (Table 9). Cycle two seems to perform better than cycle one. Note that the banana crop cycle refers to the stages of growth a banana plant goes through from planting to harvest, typically spanning 12 to 18 months. It begins with the vegetative stage, where the plant develops leaves and roots.

Following this, the plant enters the flowering stage, producing an inflorescence that will develop into fruit. The fruit grows during the fruit development stage, which lasts about 3 to 6 months, before reaching horticultural maturity and being harvested. After harvest, new suckers originated from the existing plant corm become the next cycle. Environmental factors, banana variety, and farming practices can influence the length and success of each stage, with commercial plantations often having staggered cycles for continuous production. The result shows that block and irrigation have a significant ($P < 0.001$) impact on seed viability (Table 11). Although a large number of seeds were produced under rainfed (RF) conditions, the seeds from plants under supplemental irrigation exhibited higher viability (Fig. 14).

Table 8: **ANOVA on the total number of seeds per bunch (TS) in Mchare banana Huti-White during two crop cycles from 2021 to 2023 as affected by supplemental irrigation and K fretilisation, N = 320**

Fixed effect	Wald statistic	DF	Pr
block	1.06	1	0.304
cycle	3.61	1	0.058
Potassium (K)	14.75	3	0.002
Irrigation	30	1	<0.001
K* Irrigation	0.697	3	0.874

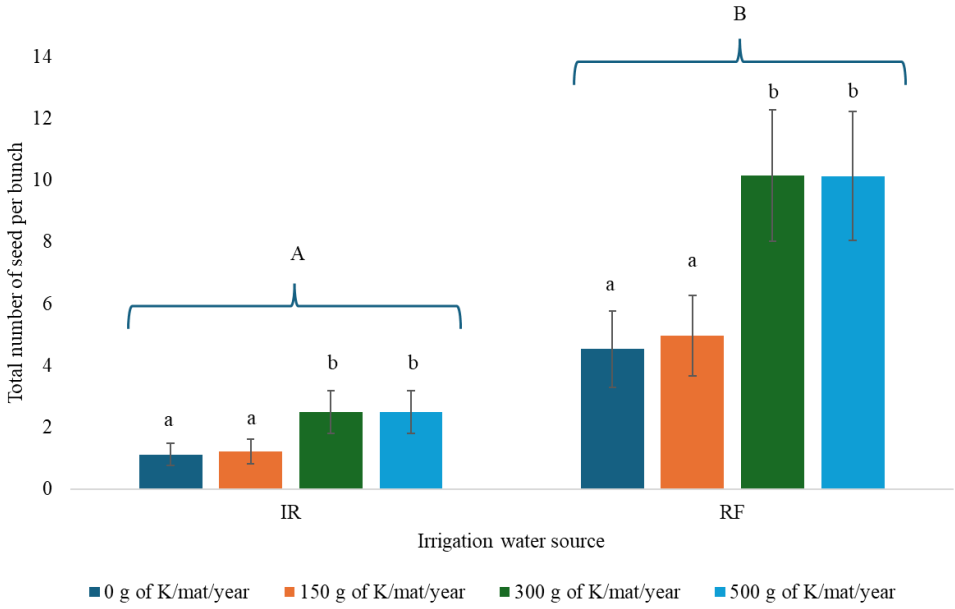


Figure 9: **The total number of seeds per bunch (TS) of Banana Huti-white under two irrigation water sources (IR: irrigation, RF: rainfed) and four K fertilisation levels (g K/mat/year) across two crop cycles (2021–2023). Means with similar letters indicate no significant difference at $P \leq 0.05$**

Table 9: **ANOVA on seeds per bunch per unit time (SPT) in Mchare banana Huti-White for two crop cycles from 2021 to 2023 as affected by irrigation and K fertilisation levels, N=320**

Fixed effect	Wald statistic	DF	Pr
block	2.19	1	0.139
cycle	3.14	1	0.076
K	16.17	3	0.001
Irrigation	27.91	1	<0.001
Cycle * K	10.403	3	0.015
Cycle * Irrigation	1.957	1	0.162
K * Irrigation	0.689	3	0.876
Cycle * K * Irrigation	2.746	3	0.432

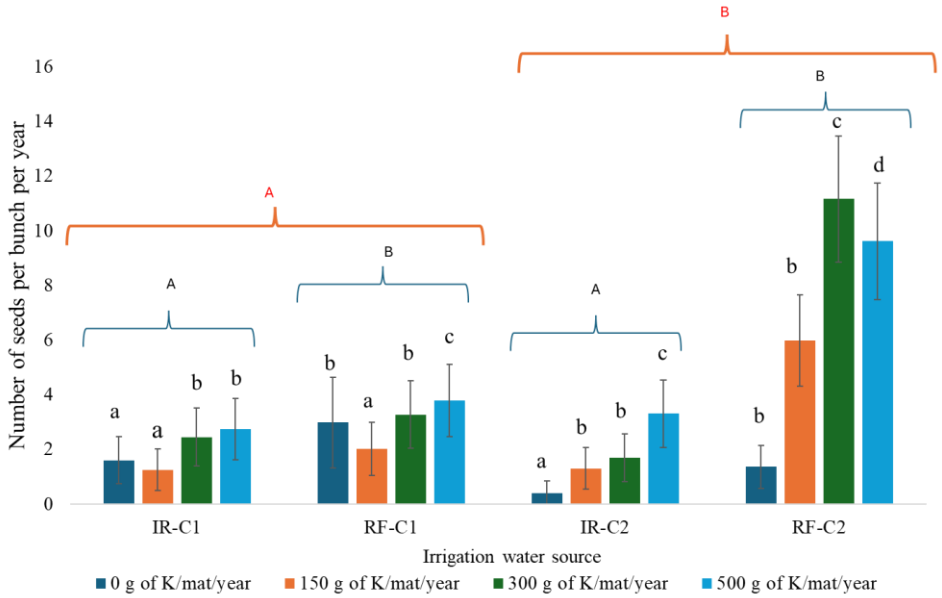


Figure 10: Number of seeds per bunch per year (SPT) extracted from banana Huti-white under two different irrigation water sources (IR: irrigation, RF: rainfed, C1: crop cycle one, C2: Crop cycle two), and four K fertilisation levels (g/mat/year) during two crop cycles from 2021 to 2023, Mean with similar letters are not significantly different at $P \leq 0.05$

The analysis of variance (ANOVA) for seed viability (SV) (Table 10), revealed the effect of block on seed viability was highly significant ($P < 0.001$), indicating that the variation between blocks contributed significantly to differences in seed viability. The cycle factor had a non-significant effect on seed viability ($P = 0.517$), suggesting that there were no notable differences in seed viability between the different cycles under study. The effect of K applications on seed viability was not statistically significant ($P = 0.141$), implying that varying K levels did not substantially affect seed viability in this study. The impact of irrigation on seed viability was highly significant ($P < 0.001$), suggesting that supplemental irrigation significantly improved seed viability compared to rainfed conditions. The interaction between potassium levels and irrigation was non-significant ($P = 0.848$), indicating that the combined effect of these two factors did not significantly influence seed viability beyond their individual effects. The results demonstrate that irrigation significantly impacts seed viability, while potassium application and its interaction with irrigation had minimal effects. This emphasizes the importance of adequate water management in optimizing seed viability. However, potassium levels alone did not appear to contribute substantially to improving seed viability under the conditions tested in this study.

Table 10: ANOVA on seed viability (SV) in Mchare banana Huti-White for two crop cycles from 2021 to 2023 as affected by irrigation and K fertilisation levels, N = 320

Term	Wald statistic	DF	Pr
block	21.81	1	<0.001
cycle	0.42	1	0.517
K	5.46	3	0.141
Irrigation	28.48	1	<0.001
K * Irrigation	0.806	3	0.848

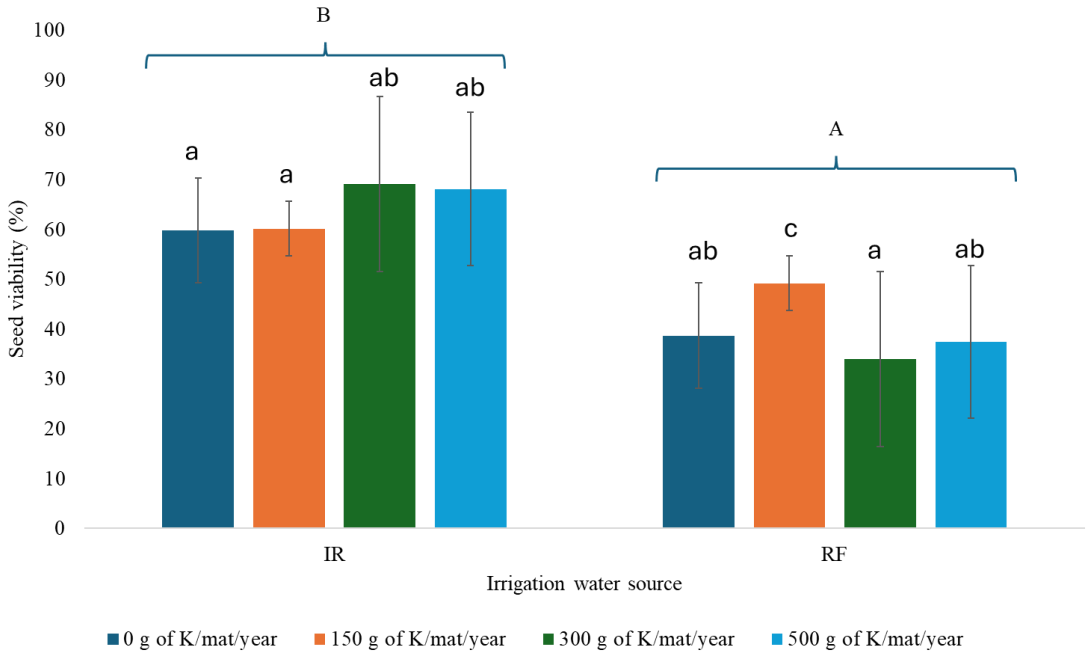


Figure 11: Banana Huti-white seed viability (SV) (proportional of the number of embryo germination to the total number of seeds per bunch) under two different irrigation water sources (IR: irrigation, RF: rainfed), and four K fertilisation levels (g/mat/year) of two crop cycles from 2021 to 2023, N=320. LSD = Least significant difference; Mean with similar letters are not significantly different at $P \leq 0.05$

The data below presented in the table 11, provides insight into the predicted mean of seeds per bunch and seed viability (%) for banana plants under irrigation and rainfed conditions with varying K treatments. The standard deviations (±) associated with these measures indicate the variability within each treatment group, offering a deeper understanding of seed production and viability consistency across different experimental conditions.

Table 11: Mean (± Standard Deviation) of the total number of seeds per Mchare banana Huti-White bunch and seed viability (%) under Irrigation and Rainfed Treatments and four fertilisation levels of K (g K/mat/year)

Treatments	Total number of seeds per bunch	Seed viability (%)
IRRIGATION	1.78±3.96	20.45±35.87
0	1.15±2.72	19.58±35.73
150	1.43±2.53	24.71±41.29
300	1.93±4.33	22.11±32.29
500	2.63±5.49	23.75±33.74
RAINFED	7.93±17.97	22.92±33.27
0	4.85±8.55	14.63±26.72
150	4.95±8.05	31.18±38.42
300	11.33±24.09	16.98±31.81
500	10.58±23.69	20.54±34.93

Under irrigation, the average number of seeds per bunch ranged from 1.15 (0 K) to 2.63 seeds (500 g K/mat/year). The high standard deviations in these treatments, especially in the higher potassium treatments (150 g, 300 g, and 500 g), suggest significant variability in seed production within each group. For instance, the 500 g K/mat/year treatment shows a standard deviation of ±5.49, which implies that, while the average number of seeds is relatively low (2.63), there is a widespread in the data with some plants producing significantly fewer or more seeds. This level of variability is consistent with other studies where potassium fertilization was shown to influence seed set but with varying degrees of effectiveness depending on the plant's specific growth conditions and nutrient uptake efficiency (Pervez *et al.,* 2004; Zahan *et al.,* 2009).

Under rainfed conditions, the average number of seeds per bunch was significantly higher, ranging from 4.85 (0 K) to 11.33 seeds (300 g K/mat/year). The high standard deviations in these treatments, particularly in the 300 g (±24.09) and 500 g (±23.69) treatments, suggest considerable variation in seed production. This variability could be due to natural factors such as plant stress, or genetic differences, which often result in more erratic seed production under rainfed conditions. This finding supports previous studies which showed that water stress could both stimulate reproductive growth and create variability in seed sets (Alqudah *et al.,* 2011; Zahan *et al.,* 2009).

The seed viability percentage under irrigation ranged from 16.98% (300g K) to 24.71% (150 g K). The high standard deviations, particularly in the 150g treatment (±41.29), suggest substantial variability in seed quality within these treatments. Previous research by Wang *et al*.

(2013) and Nieves-Cordones *et al*. (2016) has demonstrated that potassium plays an essential role in improving seed quality by enhancing nutrient transport and metabolic processes during seed development. However, excessive potassium application can disrupt other essential nutrient interactions, leading to inconsistent seed viability, as reflected in the high variability in this study.

Seed viability under rainfed conditions ranged from 14.63% (0 K) to 31.18% (150 g K/mat/year). The highest seed viability was observed in the 150g K treatment, but the high standard deviation (±38.42) indicates significant variability in seed quality. This variability is consistent with studies by Alqudah *et al*. (2011) and Wang (2007), who found that seed quality can be influenced by both nutrient availability and environmental stress. While moderate K application appears to improve seed viability, excessive K may not always result in higher seed quality, and other factors such as water availability and environmental stress may play a role in determining seed viability.

The correlation matrix for the various traits measured in this study reveals the following relationships: Bunch weight (BW) is strongly positively correlated with the number of hands per bunch (NHB) ($r = 0.95$, $p < 0.001$), number of fruits per bunch (NFB) ($r = 0.88$, $p < 0.001$), and total seed count (TS) ($r = -0.70$, $p < 0.001$) (Table 12). These results suggest that as bunch weight increases, the number of hands, fruits, and total seed count show significant changes, albeit in opposite directions for seed count. There is a negative correlation between BW and NEG ($r = -0.57$, $p < 0.01$), indicating that as BW increases, the NEG tends to decrease. The NHB shows a strong positive correlation with NFB ($r = 0.94$, $p < 0.001$) and a moderate negative correlation with TS ($r = -0.68$, $p < 0.001$), suggesting that increased NHB generally coincide with a larger number of fruits but fewer seeds produced per bunch. A similar negative correlation exists between NHB and NEG ($r = -0.55$, $p < 0.01$), indicating that more hands per bunch are associated with fewer seeds.

The average of NFB is highly positively correlated with TS ($r = -0.76$, $p < 0.001$) and negatively correlated with NEG ($r = -0.69$, $p < 0.001$), showing that increased fruit count per bunch tends to be associated with higher seed counts but fewer viable seeds. The average of TS exhibits a strong positive correlation with NEG ($r = 0.97$, $p < 0.001$), suggesting that as the total number of seeds in a bunch increases, the number of viable seeds also increases, which aligns with the notion that higher seed production generally results in better seed viability. The average of NEG (Number of Seeds per Bunch) is strongly correlated with TS, indicating that seed

production is highly consistent in terms of the total seed count and the viable seed count per bunch.

Table 12: Correlation between Yield and seeds parameters in Mchare for two crop cycles from 2021 to 2023 as affected by irrigation and potassium levels

	Average of BW	Average of NHB	Average of NFB	Average of TS	Average of NEG
Average of BW	1				
Average of NHB	0.95	1			
Average of NFB	0.88	0.94	1		
Average of TS	-0.70	-0.68	-0.76	1	
Average of NEG	-0.57	-0.55	-0.69	0.97	1

Note: BW=Bunch weight, NHB=Number of hands (fruits cluster) in a bunch, NFB=Number of fingers (Fruits) in a bunch, TS= Total number of seeds extracted and NEG= Number of embryos that germinated

4.2.2 Discussion on seed set and viability

(i) Seeds set

The results from this study underline the complex relationship between K applications and irrigation on seed sets and seed viability in bananas. While potassium application generally increased seed set, the substantial variability in both seed set and viability suggests that water availability and potassium levels play significant roles in influencing banana seed production and seed quality.

Potassium is known to support reproductive processes, such as starch production, photosynthesis, and water regulation in plants (Wang *et al.,* 2013; Nieves-Cordones *et al.,* 2016). In this study, K fertilization increased the average number of seeds per bunch, particularly under both irrigation and rainfed conditions. However, the large standard deviations indicate significant variability in seed production. This variability aligns with findings from Pervez *et al*. (2004) and Zahan *et al*. (2009), who showed that K can boost seed production in various crops, but the effects are not always uniform. This variability in seed set may be due to differential uptake and utilization of K among individual plants, suggesting that optimal K levels need to be carefully managed. Potassium is an essential macronutrient that plays a pivotal role in various physiological processes critical to plant growth, development, and reproductive success. It has been reported that K aids in starch synthesis, controls root growth, and regulates the opening and closing of stomata, all of which are crucial for efficient water use and photosynthesis (Nieves-Cordones *et al.,* 2016; Wang *et al.,* 2013; Wang, 2007). These functions of potassium are particularly important in promoting overall plant health, supporting fruit and seed development, and ensuring reproductive success.

In this study, we observed that the banana bunch yield was not significantly different between the two K treatments (300 vs. 500 g K/mat/year). However, the number of seeds produced was notably higher with the 500 g K/mat/year treatment compared to the 300 g treatment (Fig. 10). This suggests that while K does not directly affect the bunch yield at these levels, a higher concentration of K may be more effectively utilized for seed production. The increased number of seeds in the 500 g K/mat/year treatment may indicate that excess K is being directed toward reproductive processes, particularly seed development. This is consistent with the findings of previous studies, which have demonstrated that K significantly influences both fruit yield and seed set in various crops. For example, Zahan *et al*. (2009) reported that higher K application in lentils (*Lens culinaris*) resulted in increased seed production, while the lowest seed

production occurred in plants without K supplementation. Similarly, in a study on maize, Jan *et al*. (2018) found that K application significantly enhanced seed production, further emphasizing K's role in reproductive processes. Furthermore, the work of Pervez *et al*. (2004) highlighted that K application led to significant increases in seed production in several crops, underscoring its importance for reproductive success.

Additionally, Alqudah *et al*. (2011) demonstrated that water stress negatively impacted seed production and quality, but that K supplementation helped mitigate these effects, particularly in crops experiencing moisture stress. These studies support the hypothesis that K plays a key role in seed sets and that higher levels of K may be more effectively utilized for reproductive development rather than vegetative growth, as observed in our study. Potassium (K) plays a key role in helping plants cope with drought stress. It regulates stomatal opening, reducing water loss through transpiration. K also enhances root growth, improving water uptake from deeper soil layers. Additionally, it boosts osmotic balance and enzyme activation, supporting overall plant resilience under water-limited conditions.

Taken together, these studies suggest that both K and soil moisture are critical for maximizing seed production and improving seed viability. While K aids in seed set and enhances seed quality, adequate moisture availability ensures that plants can effectively utilize the nutrients for optimal reproductive success. In our study, the combined effects of K and moisture on seed production and viability suggest that careful management of both factors is essential for improving seed yield and quality in banana breeding programs. Future research could explore the precise mechanisms by which K and moisture interact to influence reproductive outcomes, potentially leading to more refined agronomic practices for seed production.

The data clearly shows that rainfed conditions resulted in higher average seed production compared to irrigation treatments, even though the irrigation treatments showed slightly higher seed viability. The large standard deviations in the rainfed treatments suggest that environmental factors, such as the timing and amount of rainfall, as well as plant stress responses, could cause considerable variability in seed production. This finding is supported by Alqudah *et al*. (2011), who demonstrated that water stress can stimulate seed production in certain crops, although the effect on seed viability is not always positive.

This study's results show that more seeds were harvested under rainfed treatment than under irrigation (Fig. 10). Cycle two seems to have more seeds than cycle one this is because in the first crop cycle, bananas may have limited nutrient reserves and resources that could hinder

maximum seed production but in the second cycle, the plant has accumulated more nutrients and energy, allowing it to allocate more resources to reproductive processes like seed set (Sun *et al.,* 2020; Uwimana *et al.,* 2020). Potassium, in particular, plays a crucial role in regulating key processes such as photosynthesis and osmoregulation, which are important for seed development. This is supported by this study as under both cycles, rainfed and 300 and 500 g of K /mat /year have the highest seed production (Fig. 11). Seed set per month shows that more seeds were obtained during low rainfall (Fig. 14).

These results indicate that the plants were producing seeds in response to the signal triggered by limited soil water availability, prompting them to prioritize seed production as a survival strategy for the next generation. This suggests that pollination is more successful during the rainy season, as the seeds that develop and are harvested during the dry season come from flowers pollinated in the rainy season, since banana bunches take three to six months to mature. This can explain that plenty of K is linked to a threshold supply in the fruit and results in higher bunch yield (and that is why it does not work with IR because the bunches are about twice the size of RF (Appendix 3). Therefore, to achieve a higher seed yield in IR compared to RF, it is necessary to significantly increase the amount of K fertilizer applied in IR. Specifically, under IR conditions, the amount of K fertilizer should be doubled to approximate the seed yield observed in RF.

The adaptation mechanisms of different angiosperms under stress have been extensively studied (Folk *et al.,* 2020; Ashapkin *et al.,* 2020). It is well-documented that plants respond to drought stress by increasing the number of seeds as a survival strategy for ensuring propagation in subsequent seasons (Norton *et al.,* 2016; Bandurska, 2022; Seleiman *et al.,* 2021; Alqudah *et al.,* 2011; McDowell *et al.,* 2008). This phenomenon is corroborated by the findings of Behboudian *et al*. (2001), who observed enhanced seed nutritive value of chickpeas under water stress conditions. These adaptations may involve increased accumulation of soluble sugars, amino acids, and proteins, contributing to seed development and viability.

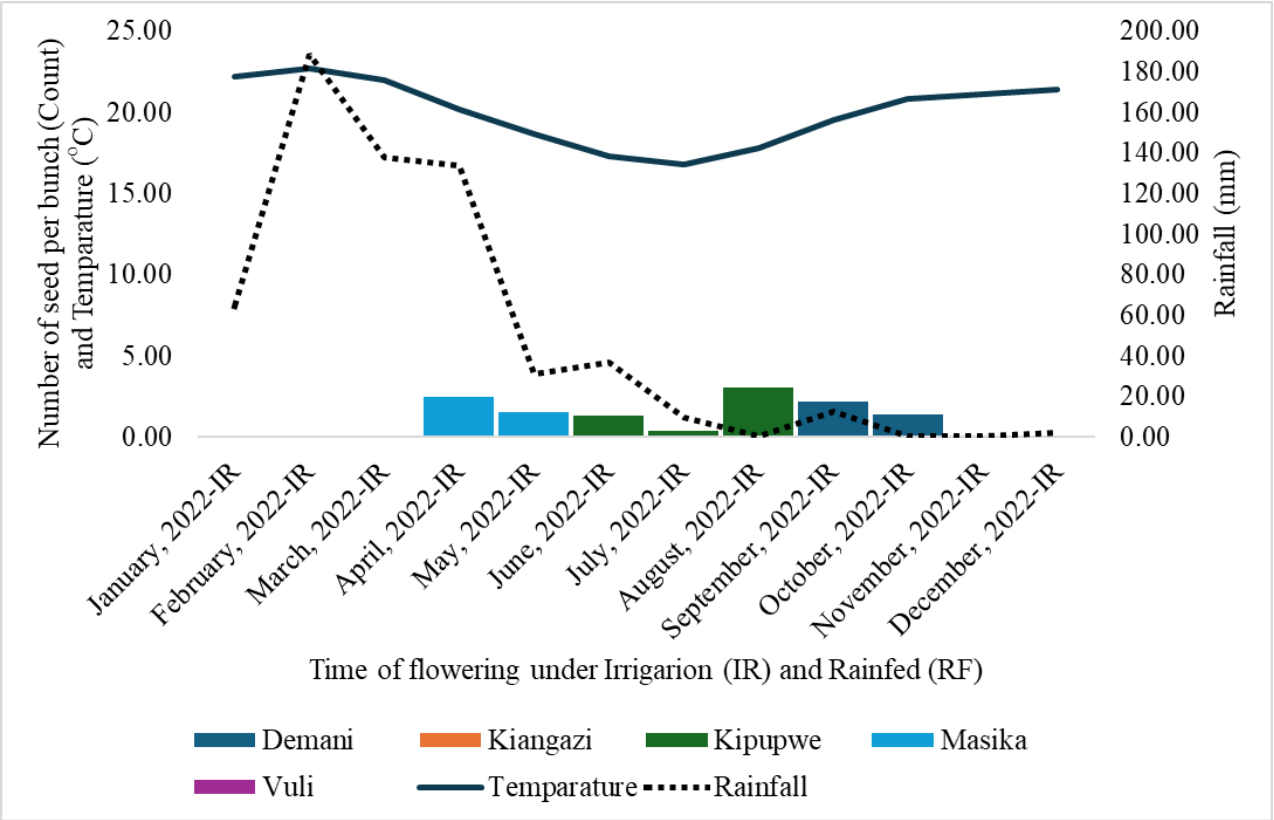


Figure 12: **Number of seeds per bunch (Count) under irrigation (IR) over twelve months 2022 indicated with bars, the colours indicate the seasons of flowering of the year, long rain season (Masika), short rain season (Vuli), dry season (Kiangazi), Cool season (Kipupwe), warm season (Demani), rainfall is shown in dotted line**

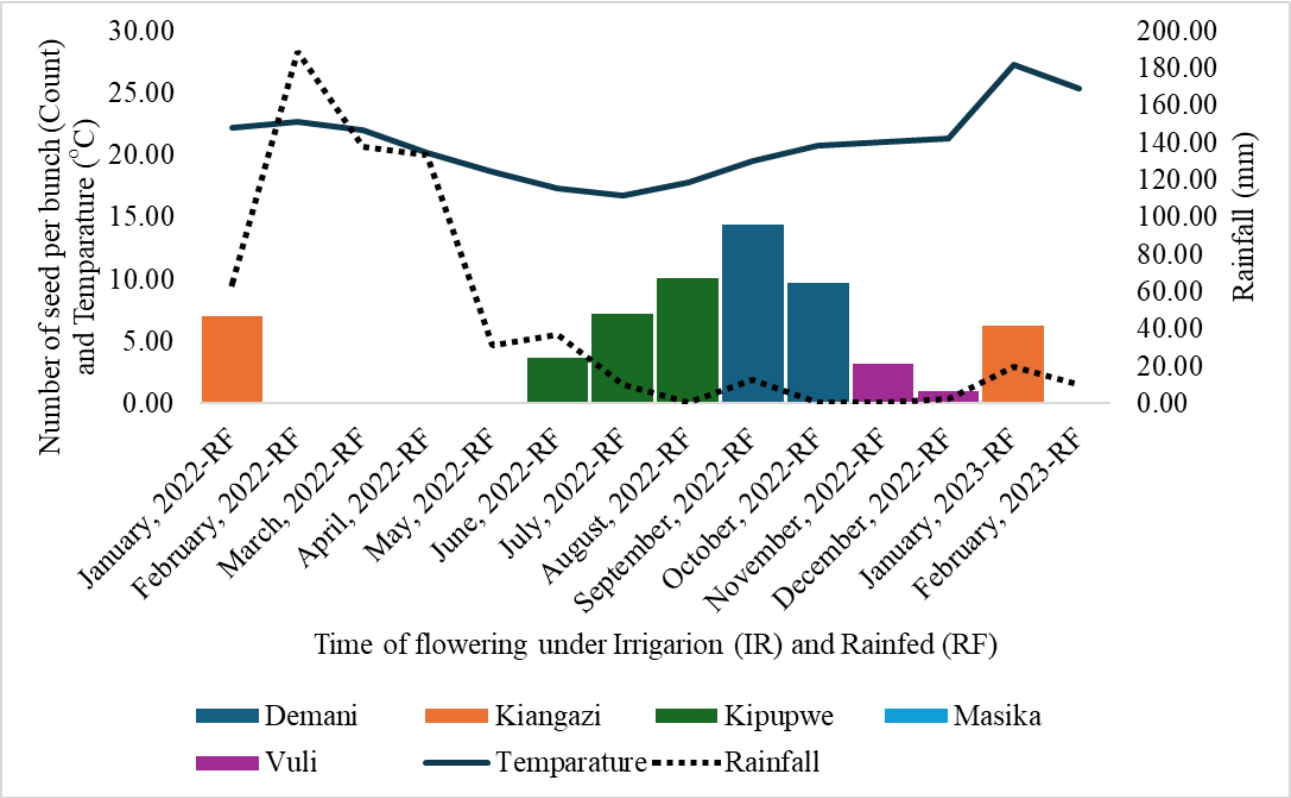


Figure 13: **Number of seeds per bunch (Count) under rainfed (RF) over twelve months 2022 and 2023 indicated with bars, the colours indicate the seasons of flowering of the year, long rain season (Masika), short rain season (Vuli), dry season (Kiangazi), Cool season (Kipupwe), warm season (Demani), rainfall is shown in dotted line**

Soil moisture, in conjunction with K levels, plays a critical role in seed production and viability. Moisture availability is essential for plants to access and utilize nutrients efficiently, including K, which can influence seed sets and overall reproductive success. Interestingly, despite supplemental irrigation resulting in fewer seeds compared to rainfed conditions, the seeds produced under irrigation were more viable. This finding underscores the importance of moisture availability not just for seed production, but also for seed quality. Inadequate water availability, as experienced in rainfed conditions, can impose stress on plants, negatively affecting the viability of the seeds they produce.

Several studies have highlighted the combined impact of moisture and K levels on seed sets and viability in different crops. For example, Pervez *et al*. (2004) demonstrated that the application of K led to significant increases in seed production, illustrating potassium's role in enhancing reproductive success. Similarly, Alqudah *et al*. (2011) emphasized that water stress significantly affects both seed production and quality, stressing the importance of adequate moisture in ensuring proper reproductive processes. These findings are consistent with our

observations that adequate soil moisture, in addition to K, is crucial for optimal banana seed development.

(ii) Seed viability

Seed viability is a critical factor in determining successful germination and plant establishment. It can be influenced by a variety of environmental and agronomic factors, such as soil moisture and nutrient levels. In this study, we evaluated seed viability in plants grown under both rainfed and irrigated conditions, with varying K levels to better understand how these factors interact to affect seed quality.

The variability observed in seed viability across both irrigation and rainfed conditions underscores the complex role of K in seed quality. Seed viability showed large standard deviations in both irrigation and rainfed treatments, indicating that factors beyond K application were likely influencing seed viability. Previous studies e.g., by Wang (2007) and Alqudah *et al*. (2011) have reported that excessive K application can disrupt the uptake of other essential nutrients, potentially reducing seed viability. On the other hand, moderate K levels have been found to positively influence seed viability. In this study, applications of 500 g K/mat/year demonstrated better seed viability, suggesting that a balanced nutrient management strategy is essential for optimizing seed quality.

Despite rainfed conditions producing a higher number of seeds, the seed viability was notably higher when plants were subjected to supplemental irrigation. Specifically, K increased seed viability by 26.12%, and irrigation improved seed viability by around 29.72%. The seed viability under irrigation was about double that under rainfed conditions, aligning with the observation that the total number of seeds produced under irrigation was approximately half that of rainfed plants (excluding the rainfed treatment with the highest K level, where the threshold K level appeared to be reached). This indicates that while higher K levels can substantially increase seed production, below a certain K threshold, water availability becomes the dominant factor influencing seed viability. Consistent water supply from irrigation led to nearly double the seed viability compared to rainfed conditions.

The present results indicated that seeds developed under irrigation conditions generally exhibited higher viability compared to those from rainfed plants. This finding is consistent with previous research, which has shown that a consistent water supply supports better physiological development in plants, leading to the production of higher-quality seeds (Rasool *et al.,* 2020; Gheysari *et al.,* 2017; Amin *et al.,* 2015). Irrigation helps maintain optimal moisture levels in

the soil, ensuring that plants are not subjected to water stress during critical growth phases, especially during flowering and seed development. In contrast, rainfed conditions are often unpredictable, with water availability fluctuating, which can lead to stress during seed formation and, consequently, reduced seed quality and lower seed viability.

In addition to supplemental irrigation, K levels played a significant role in seed viability. Seeds from plants grown with higher K levels generally showed improved viability. Potassium is a vital macronutrient that contributes to various physiological processes in plants, including enzyme activation, osmoregulation, and the regulation of stomatal openings (Wang *et al.,* 2013; Mostofa *et al.,* 2022; Rawat *et al.,* 2022; Hu *et al.,* 2018). These processes are essential for maintaining cellular functions and promoting stress tolerance during seed development (Nieves-Cordones *et al.,* 2016; Wang *et al.,* 2013; Wang, 2007). Higher K availability likely enhanced the overall plant nutrition, leading to more robust seed development.

Although both irrigation and K individually improved seed viability, their interaction did not have a significant effect on seed viability. The absence of a significant interaction suggests that, under optimal irrigation conditions, K may not further enhance seed viability to the same extent as in rainfed conditions, where water stress is a more significant limiting factor. In irrigated environments, plants were less likely to experience the physiological stress that K helps to mitigate, which may explain the lack of a pronounced interaction effect. Therefore, while both factors independently improved seed viability, their combined effect did not result in an outcome greater than when each factor was optimized separately.

Overall, both water availability and K levels were found to significantly influence seed viability. Irrigation alone improved seed viability, likely by ensuring a consistent water supply throughout the growing season. Adequate K fertilization also played a crucial role in enhancing seed quality, especially under rainfed conditions. These findings emphasize the importance of optimizing both irrigation practices and K fertilization to improve seed production. Future research could explore the mechanisms behind these effects and investigate strategies to fine-tune management practices for better seed quality under varying environmental conditions.

The high variability observed in both seed set and seed viability within each treatment, particularly under rainfed conditions, suggests that environmental stress and genetic factors may significantly contribute to the observed outcomes. Water availability, nutrient interactions, and the individual genetic responses of the plants to these factors likely influenced seed production and quality. This variability aligns with research by Zahan *et al*. (2009) and Pervez

et al. (2004), who noted that environmental stresses, such as water deficits, can lead to greater reproductive effort but also cause substantial variability in seed development and quality.

The viability of seeds is a crucial determinant of successful germination and plant establishment, and it can be influenced by various environmental and agronomic factors, including water availability and nutrient levels. In this study, we assessed seed viability in plants grown under rainfed and irrigated conditions, with varying K levels, to understand how these factors interact to affect seed quality.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The findings of this study suggest that Calcutta 4, Borneo and Huti-White, exhibit high pollen production and viability, indicating strong male fertility. This makes them suitable candidates as male parents in banana breeding programs to enhance the chances of successful pollination. This study demonstrates that warm, humid conditions and adequate soil moisture are essential for producing high pollen quantities and ensuring pollen viability. Pollen viability peaked between 0600 hrs and 1000 hrs, suggesting this as the optimal window for effective pollination. The findings underscore the critical role of supplemental irrigation in enhancing seed quality and pollen availability in banana breeding. While rainfed plants produced more seeds, those under irrigation showed greater seed viability. Additionally, potassium application—particularly at 300 and 500 g/mat/year significantly improved both seed quantity and quality under irrigated conditions.

5.2 Recommendations

This study recommends that supplemental irrigation be applied in banana experimental blocks, particularly during dry periods, to ensure consistent pollen production and its viability. Adequate soil moisture is essential for maintaining optimal pollen quality, as insufficient water can negatively impact the production of viable pollen. To manage this, breeders are encouraged to monitor soil moisture levels using moisture sensors, which will allow for the precise application of irrigation when necessary. Such a strategy will help mitigate the effects of drought and maintain high pollen viability, which is crucial for successful seed production.

Furthermore, the timing of pollination is another critical factor in maximizing seed production. This study recommends that pollination be conducted between 0600 and 1000 hours, a period during which pollen viability is at its peak. By adhering to this timing, breeders can significantly increase the chances of successful fertilization and seed set, thus enhancing the overall efficiency of the breeding program.

In terms of pollen selection, breeders are advised to consider male parent varieties such as Calcutta 4, Borneo, and Huti-White. These varieties are known for their high pollen production and viability, making them ideal candidates for use in breeding programs. This study suggests

that breeders can rely on visual observations of pollen in the field as an effective method for selecting the best-performing male parents. By doing so, breeders can ensure that only the most viable pollen is used for pollination, further improving seed production outcomes.

The application of potassium fertilizer is also recommended as a key practice to enhance seed quality and quantity. Based on the findings of this study, it is suggested that breeders apply 300 to 500 grams of potassium per meter of mat per year, particularly under conditions of supplemental irrigation. This application supports better seed development and contributes to higher-quality seeds, which is a crucial component of successful banana breeding.

Additionally, breeders should recognize the strong correlation between bunch yield and seed production traits. This study recommends that breeding strategies prioritize both fruit yield and seed set traits. Optimizing these traits will improve seed production efficiency and quality, ensuring that breeding programs not only focus on yield but also on the essential aspect of seed viability.

Finally, further studies are recommended to deepen the understanding of the factors influencing pollen viability and seed production. Future research could explore the interaction between environmental factors, irrigation practices, and nutrient management, offering insights that may refine current breeding strategies. Moreover, expanding the search for male parent varieties with superior pollen traits could provide breeders with additional options, thus enhancing the overall success of banana breeding programs.

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APPENDICES

Appendix 1: ANOVA Table on phenological events in Mchare for two crop cycles from 2021 to 2023 as affected by irrigation and potassium level

Traits	Fixed effects	SS	MS	DF	F	Pr
DFH	K	578.858	192.953	3	0.837	0.519
DFH	Irrigation	535.93	535.93	1	2.324	0.367
DFH	cycle	30.196	30.196	1	0.131	0.718
DFH	block	215.498	215.498	1	0.934	0.508
DFH	K: Irrigation	28.53	9.51	3	0.041	0.988
DFH	K: cycle	242.466	80.822	3	0.35	0.789
DFH	Irrigation: cycle	156.934	156.934	1	0.68	0.41
DFH	K: Irrigation: cycle	826.141	275.38	3	1.194	0.312
DPF	K	2090.692	696.897	3	3.241	0.126
DPF	Irrigation	683.256	683.256	1	3.177	0.324
DPF	cycle	366.247	366.247	1	1.703	0.193
DPF	block	1436.17	1436.17	1	6.678	0.232
DPF	K: Irrigation	416.591	138.864	3	0.646	0.621
DPF	K: cycle	375.436	125.145	3	0.582	0.627
DPF	Irrigation: cycle	26.906	26.906	1	0.125	0.724
DPF	K: Irrigation: cycle	373.235	124.412	3	0.579	0.63
DPH	K	1583.618	527.873	3	1.963	0.239
DPH	Irrigation	1429.991	1429.991	1	5.317	0.252
DPH	cycle	996.223	996.223	1	3.704	0.055
DPH	block	3948.706	3948.706	1	14.683	0.147
DPH	K: Irrigation	645.586	215.195	3	0.8	0.545
DPH	K: cycle	1595.975	531.992	3	1.978	0.117
DPH	Irrigation: cycle	18.705	18.705	1	0.07	0.792
DPH	K: Irrigation: cycle	717.818	239.273	3	0.89	0.447

Appendix 2: Adjusted means comparison of phenological events in Mchare for two crop cycles from 2021 to 2023 as affected by irrigation and potassium level

Traits	K	Irrigation	emmean	SE	df	lower.CL	upper.CL	group
DFH	0	IR	168.324	12.431	3.194	130.088	206.561	a
DFH	150	IR	171.642	12.502	3.259	133.584	209.7	a
DFH	300	IR	170.913	12.514	3.27	132.882	208.943	a
DFH	500	IR	173.551	12.507	3.254	135.449	211.653	a
DFH	0	RF	187.097	12.376	3.146	148.728	225.465	a
DFH	150	RF	190.443	12.374	3.147	152.081	228.805	a
DFH	300	RF	191.618	12.398	3.168	153.319	229.918	a
DFH	500	RF	194.164	12.381	3.152	155.814	232.513	a
DPF	0	IR	518.275	31.302	6.161	442.164	594.385	a
DPF	150	IR	513.168	31.328	6.18	437.048	589.287	a
DPF	300	IR	512.631	31.329	6.18	436.51	588.751	a
DPF	500	IR	509.768	31.334	6.183	433.644	585.892	a
DPF	0	RF	553.053	31.276	6.138	476.938	629.168	a
DPF	150	RF	536.885	31.273	6.138	460.778	612.992	a
DPF	300	RF	540.16	31.283	6.145	464.049	616.271	a
DPF	500	RF	538.726	31.276	6.141	462.619	614.834	a
DPH	0	IR	653.585	21.205	10.17	606.444	700.726	a
DPH	150	IR	647.985	21.246	10.248	600.801	695.169	a
DPH	300	IR	653.031	21.259	10.273	605.833	700.229	a
DPH	500	IR	643.729	21.234	10.221	596.556	690.903	a
DPH	0	RF	675.729	21.169	10.109	628.63	722.828	a
DPH	150	RF	666.485	21.162	10.102	619.399	713.572	a
DPH	300	RF	664.671	21.176	10.127	617.568	711.775	a
DPH	500	RF	666.168	21.167	10.109	619.073	713.263	a

Appendix 3: ANOVA Table on yield parameters in Mchare for two crop cycles from 2021 to 2023 as affected by irrigation and potassium level

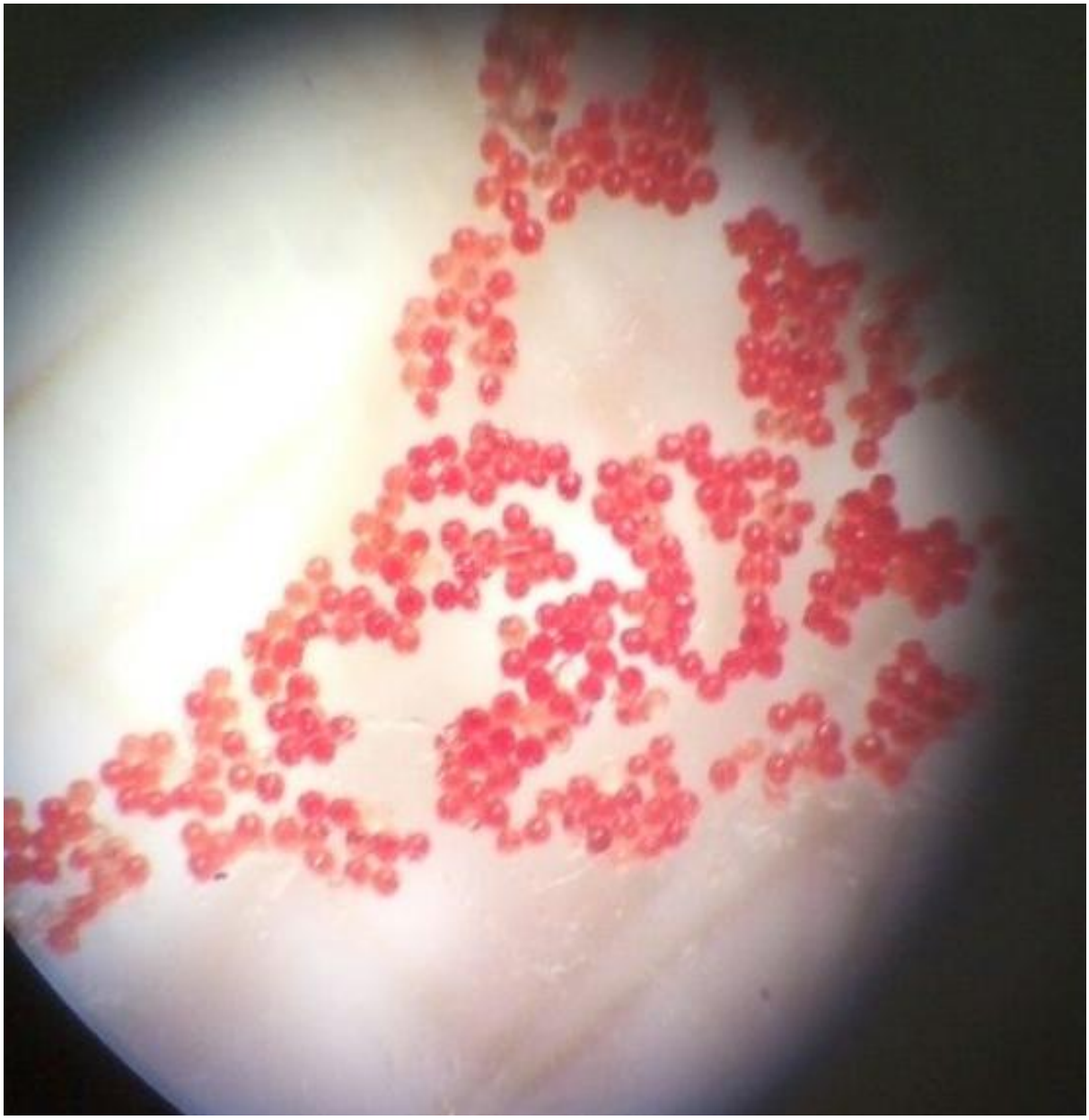
Traits	Fixed effects	SS	MS	DF	F	Pr
BW	K	1062.089	354.03	3	55.082	<0.001
BW	Irrigation	2331.098	2331.098	1	362.689	0.033
BW	cycle	0.013	0.013	1	0.002	0.965
BW	block	1.962	1.962	1	0.305	0.679
BW	K: Irrigation	972.944	324.315	3	50.459	<0.001
BW	K: cycle	23.705	7.902	3	1.229	0.299
BW	Irrigation: cycle	0.026	0.026	1	0.004	0.949
BW	K: Irrigation: cycle	4.415	1.472	3	0.229	0.876
FL	K	90.026	30.009	3	7.373	0.02
FL	Irrigation	197.858	197.858	1	48.614	0.091
FL	cycle	42.585	42.585	1	10.463	0.001
FL	block	15.401	15.401	1	3.784	0.302
FL	K: Irrigation	4.85	1.617	3	0.397	0.76
FL	K: cycle	27.062	9.021	3	2.216	0.086
FL	Irrigation: cycle	2.627	2.627	1	0.645	0.422
FL	K: Irrigation: cycle	65.691	21.897	3	5.38	0.001
NFB	K	626.712	208.904	3	1.44	0.311
NFB	Irrigation	8688.699	8688.699	1	59.899	<0.001
NFB	cycle	19.474	19.474	1	0.134	0.714
NFB	block	2558.661	2558.661	1	17.639	0.002
NFB	K: Irrigation	2994.514	998.171	3	6.881	0.018
NFB	K: cycle	395.687	131.896	3	0.909	0.437
NFB	Irrigation: cycle	1.622	1.622	1	0.011	0.916
NFB	K: Irrigation: cycle	1692.486	564.162	3	3.889	0.009
NHB	K	32.049	10.683	3	16.583	<0.001
NHB	Irrigation	184.094	184.094	1	285.764	<0.001
NHB	cycle	1.187	1.187	1	1.842	0.176
NHB	block	0.201	0.201	1	0.312	0.577
NHB	K: Irrigation	29.902	9.967	3	15.472	<0.001
NHB	K: cycle	1.299	0.433	3	0.672	0.57
NHB	Irrigation: cycle	0.177	0.177	1	0.275	0.6

Traits	Fixed effects	SS	MS	DF	F	Pr
NHB	K: Irrigation: cycle	4.937	1.646	3	2.555	0.056

Appendix 4: Adjusted means comparison of yield parameters in Mchare for two crop cycles from 2021 to 2023 as affected by irrigation and potassium level

Traits	K	Irrigation	emmean	SE	DF	lower.CL	upper.CL	group
BW	0	IR	32.838	1.108	2.654	29.036	36.639	a
BW	150	IR	39.873	1.107	2.653	36.074	43.672	b
BW	300	IR	47.732	1.119	2.653	43.891	51.572	c
BW	500	IR	51.579	1.104	2.654	47.79	55.368	c
BW	0	RF	19.807	1.111	2.653	15.994	23.62	ab
BW	150	RF	20.502	1.11	2.654	16.693	24.311	ab
BW	300	RF	20.215	1.112	2.653	16.399	24.031	ab
BW	500	RF	20.371	1.111	2.653	16.561	24.181	ab
FL	0	IR	23.932	0.813	4.542	21.778	26.086	ab
FL	150	IR	24.31	0.812	4.542	22.158	26.462	ab
FL	300	IR	26.486	0.822	4.54	24.307	28.665	a
FL	500	IR	26.653	0.809	4.542	24.508	28.798	a
FL	0	RF	18.48	0.815	4.54	16.319	20.642	b
FL	150	RF	20.091	0.815	4.542	17.932	22.25	ab
FL	300	RF	20.86	0.82	4.625	18.701	23.02	ab
FL	500	RF	21.851	0.816	4.58	19.693	24.009	ab
NFB	0	IR	91.36	2.284	5.958	85.763	96.958	abc
NFB	150	IR	95.214	2.295	6.057	89.611	100.816	ab
NFB	300	IR	96.999	2.356	6.119	91.262	102.736	ab
NFB	500	IR	98.116	2.247	5.959	92.609	103.623	a
NFB	0	RF	88.575	2.383	6.229	82.795	94.355	abc
NFB	150	RF	80.251	2.326	6.045	74.569	85.932	bc
NFB	300	RF	85.321	2.362	6.107	79.566	91.077	abc
NFB	500	RF	77.154	2.345	6.207	71.461	82.847	c
NHB	0	IR	7.5	0.134	6.293	7.176	7.824	ab
NHB	150	IR	8.3	0.132	6.277	7.98	8.62	a c
NHB	300	IR	8.625	0.139	6.187	8.286	8.964	c
NHB	500	IR	8.675	0.13	6.306	8.36	8.99	c
NHB	0	RF	6.7	0.134	6.161	6.374	7.026	b d
NHB	150	RF	6.75	0.134	6.291	6.427	7.073	b d
NHB	300	RF	7.361	0.138	6.987	7.034	7.688	ab
NHB	500	RF	6.2	0.134	6.278	5.876	6.524	d

Appendix *5*: pollen grain under light microscope after staining by TTC methods



Appendix *6*: Extracted banana seeds

Appendix 7: Germinating banana embryo in tissue culture.

RESEARCH OUTPUTS

(i) Publications

Bayo, S. J., Massawe, V., Ndakidemi, P. A., Venkataramana, P., Mlaki, A., Mduma, H., Jomanga, K., Swennen, R., & Brown, A. (2024). Pollen Amount and Viability in Mchare and Selected Wild (AA) Banana (*Musa acuminata*) Genotypes: Prospects for Breeding. *HortScience*, *59*(5), 632-638. https://doi.org/10.21273/HORTSCI17608-23

(ii) Poster presentation