CHINHOYI UNIVERSITY OF TECHNOLOGY

Genetic Conservation, Characterization and Land Suitability Modelling of Banana (*Musa Spp*.) Cultivars in Zimbabwe

By

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Declaration

I, Beaton Kumbirai, declare that the thesis entitled Genetic characterization, nutritional analysis and spatial distribution of banana (*Musa Spp.*) cultivars in Zimbabwe is my original work as well as a result of my own findings. I also declare that all the none-original sources used have been cited and well referenced. I acknowledge that this piece of work has never been applied or completed for a degree to any other organization.

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Abstract

Banana farming in Zimbabwe is a way of survival to Zimbabweans, especially in the Manicaland province of the country. Genetic and phenotypic analysis of banana varieties and cultivars paves the way for genetic conservation and improvement programs. Meaningful yields remain low owing to the high cost of propagules which are virus-free, and the absence of well characterized germplasm banks. Towards improving the yield and conserving banana genetic material in Zimbabwe, this study established a germplasm bank of banana plants characterized for genetic make-up, nutritional composition, and morpho-agronomical traits. It also modelled the suitability of banana expansion to new production areas whilst mapping out the geo-spatial distribution of bananas nationwide. Twelve banana germplasm varieties were collected nationwide, and a gene bank was established *in situ* at Chinhoyi University farm. A germplasm bank was established with 4 reps of each variety. The germplasm was then clustered into 7 distinct varieties using Random Amplified Polymorphism DNA method as well as the Inter Transcribed Spacer targeted sequencing. Growth and development characteristics of the established plants were recorded for 24 months from their planting to capture the vegetative and reproductive phases of the plants. The inflorescence of each variety gave a clear distinction between varieties. Each of the cultivars' fruits were subjected to nutritional analysis. The fruits were dried and ground into flour before chemical and physical properties were analysed. The different cultivars were then subjected to tissue culture over a period of 90 days and virus-free banana plantlets were produced. Furthermore, banana production sites were mapped out and new production areas were modelled and estimated in Maximum Entropy. In conclusion, the research offered a well-annotated gene bank with characterized, catalogued, and mapped banana cultivars that can also be mass-produced and commercialized in tissue culture at the Chinhoyi University of Technology.

List of Conference presentations

1. Oral presentation at the 5th CUT International Research Conference, 12-17th February 2023, Chinhoyi, Zimbabwe: Topic - Nutrient and flour property analysis of the banana fruits bio-banked at Chinhoyi University farm.

2. Poster presentation at the 5th CUT International Research Conference, 12-17th February 2023, Chinhoyi, Zimbabwe: Topic - Banana Cultivars in Zimbabwe: A Catalogue System

Abbreviations

A.U.C	Area Under Curve
B.Br.M.V	Banana Bract Mosaic Virus
B.B.T.V	Banana Bunchy Top Virus
B.S.V	Banana Streak Virus
C.B.F1	Centromere Binding Factor 1
C.M.V	Cucumber Mosaic Virus
D.N.A	Deoxyribonucleic acid
D.R.E.B	Dehydration Responsive Element Binding
G.I.S	Geographic Information System
G.P.S	Geographic Positioning System
I.T.S	Inter Transcribed Spacer
Maxent	Maximum Entropy
M.D.A	Malondialdehyde
M.S	Murashige and Skoog
R.O.C	Receiver Operating Characteristics
R.A.P.D	Random Amplification Polymorphic DNA
R.O.S	Reactive Oxygen Species
S.D.M	Species Distribution Model
S.O.D	Superoxide Dismutases

Table of Contents

List of Figures

List of Tables

List of Appendices

Overview of the Thesis

Chapter 1 of the Thesis presents the background of banana production in Zimbabwe. It provides the contextual framework of research done, highlighting the importance of each aspect in banana research. It stresses on the methods of germplasm conservation which include in-situ and ex situ conservation methods. Banana germplasm characterization was further analysed highlighting the taxonomy of *Musa* species. *Musa* taxonomy outlined the different banana types available as well as their uses in different parts of the world. Literature review is presented in **Chapter 2** where a review of the research area was done through literature search. The chapter culminated into a published review paper. **Chapter 3** describes the establishment of the gene bank using collected germplasm grown in Zimbabwe. The assignment of accession numbers as well as growth and setbacks due to the winter season are explained in this chapter. In the form of a research paper, **Chapter 4** highlighted the use of Random Amplification Polymorphic DNA (RAPDs), Inter transcribed spacer (ITS) markers and DNA sequencing in identifying or characterizing the collected germplasm. Morphological analysis was also inferred complimenting molecular characterization. The collected information on the cultivars was then used in creation of a catalogue to be used in identifying the bio-banked cultivars. In **Chapter 5**, the conserved and characterized germplasm was cultured *in vitro* and the performance under controlled environment for each variety was compared. As a result, the time frame for proliferation was determined for each cultivar **Chapter 6** explains the production and analysis of banana composite proximate flour. The flour was analysed for its phenotypic and physical characteristics, as well as nutritional compositions. In **Chapter 7**, banana production areas were mapped, and a Maximum Entropy suitability model was used to determine whether banana cultivation should be expanded to new areas. **Chapter 8** gives an overview of the study findings, the take home message and way forward and lastly **the appendix section** outlines all the appendices and publications generated from the research study.

Chapter 1

1.1. General Introduction

Over 10,000 years ago, humans comprehended the economic utility of plants and started their domestication. They have done this through the curation of seeds as well as vegetative propagation from season to season. This process of conservation has been an important factor in agricultural development throughout the globe. To ensure nutritional and economic security, mankind is reliant on the continuous availability of a diverse pool of plant genetic resources for agriculture (Priyanka et al., 2021). The capturing of natural and existing genetic diversity through breeding with their wild relatives is of paramount importance because wild relatives often accumulate useful variations that can be introduced into cultivated crops such that further changes can be possible. Plant genetic resource is the sum total of all allelic sources that influence a range of traits in a crop, and germplasm refers to the genetic material that is passed from one generation to the next (Wilkes et al., 1991). Genetic diversity in crops has been drawn from wild species that are either direct or distant from the crop being cultivated (Rao et al., 2004). Regardless of the availability of these wild species, the mobilization of plant genetic resources has proven to be a challenge. Although quite a number of gene-banks now exist worldwide, about 30 countries have chosen long term storage of germplasm and this is because of the limitations in maintenance systems of long-term storage (Kell et al.,2017).

Gene banks are repositories of biological materials that are collected, kept, catalogued and then made available for redistribution. Their main purpose is to preserve genetic diversity for future research and also plant genetic improvements. Ruas et al., (2018) stated that the collection, conservation, characterization and breeding of plants and their wild relatives contribute to the preservation of biological diversity, and it is an essential component in ensuring food security. Further, the 7.5 million accessions in these gene banks are primarily crops on which humans and animals rely for food and nutrition, including their diversified wild relatives and landraces. There are locally important crops and underutilized species that need to be protected. Of the 7.5 million plant accessions, approximately 15,000 accessions are banana germplasm available in a number of institutions world-wide. These accessions have been made available for use by breeders, farmers, researchers and consumers (Ho, 2010). Banana gene banks are limited and breeding programs are few due to the complexity of the banana genome. Moyer, (2022) stated

that of the conserved banana germplasm, only 1,000 varieties of bananas are under production. This therefore means that production of bananas becomes limited to a few cultivars.

1.2. Methods of germplasm conservation

In plant genetic conservation, plant collections usually become extremely large with limited characterization due to inefficient management and this is one of the main reasons why the genetic resources in gene banks remain largely unexploited. As a result, the introduction of core collection ensures efficient management and utilization of acquired accessions. Core collections, as defined by Frankel (1984), is a limited set of accessions representing the genetic diversity of a crop species and its wild relatives. They serve as an initial point for efficient utilization of the germplasm in crop breeding. The two fundamental approaches in germplasm conservation are *ex-situ* and *in-situ* conservation.as indicated in figure 1.1.

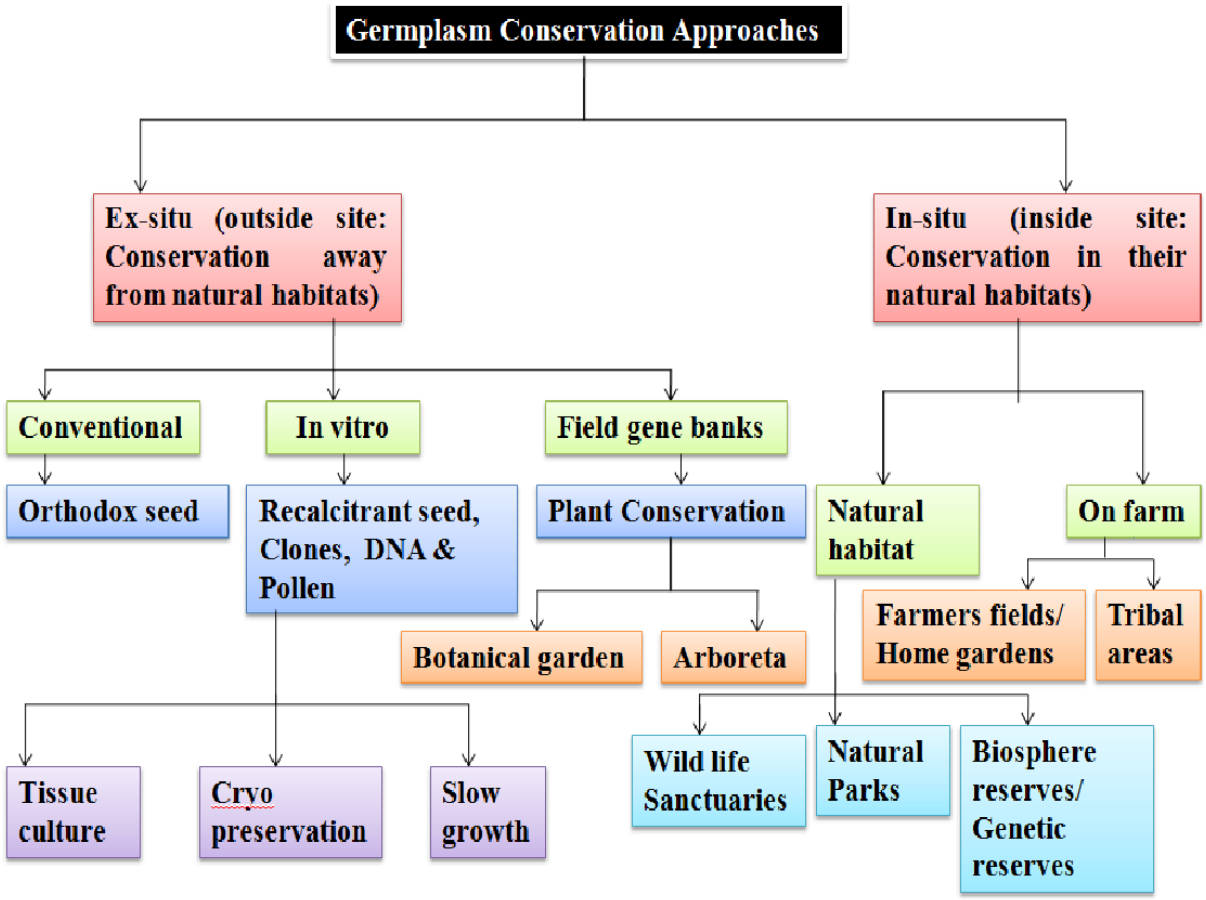

Figure 1.1. Approaches to germplasm conservation (Adapted from Priyanka et al., 2021)

1.2.1. In situ conservation

This method refers to the conservation of genetic material in natural reserves or ecosystems. It allows the species continuity without human interference.

1.2.2. Ex situ conservation

It is a cost-effective approach involving regular material viability testing. The method involves three different processes through field gene banks, *in-vitro* and conventional processes. Field gene banks involve the curation of plant material in nurseries (De, 2017). The in-vitro approach is mainly used for crops that do not produce viable seeds such as bananas, therefore plant tissue culture is employed (Ding et al., 2008). The conventional process allows the storage of germplasm in seed form (Merritt et al., 2011). For instance, Brazil has *Embrapa Mandioca Fruticultura*, an *ex-situ* centre where a germplasm collection was established in 1981. The center has over 200 accessions but despite the wide range of genetic resources, only a small number of accessions is currently being used in breeding programs due to lack of characterization and genetic identity. If *ex-situ* conservation is to completely cover the entire existing Musa diversity, including all explored and collected germplasm as well as the germplasm yet to be discovered, there is a need to correctly identify and eventually fill the possible gaps across all collections. Adequate gap identification requires that accessions be duly characterized and documented, preferably at the site of their origin, before the material is introduced into collections. Not only is the initial data vital for further studies, but without proper documentation, collections run the risk of maintaining duplicates of what is already conserved elsewhere. Correct characterization entails more than just description. Researchers must frequently compare the observed and analysed taxa with several other taxa for final assessment. Consequently, the *ex-situ* collections need to contain representative taxa across the range of diversity (MusaNet, 2016).

1.3. Banana germplasm characterization

Banana characterization defines the specific unique features of an accession that are easily identifiable and can be used to distinguish germplasm. The degree of these differences then indicates the closeness of the germplasm. In the agreed terminology of gene banks as well as germplasm management, the term characterization means the description of characters that are usually highly heritable, can easily be seen by the eye and equally expressed in all environments

(Azad et al., 2016). Understanding and cataloguing the principal characteristics of the cultivars is key to the banana improvement programme, therefore agronomic and nutritional descriptors are used in the conventional characterization of bananas (Karamura et al., 2014). Characterization of banana trees based on morphology requires clearly defined plant descriptors. With reference to IPGRI-INIBAP, (1997), characterization based on descriptors enables quick identification of phenotypes. Ortiz, (1997), explains that the most important qualitative morphological descriptors are the flower, male bud, foliage pseudo stem pigment, petiole and male flower, pseudo stem waxiness and also leaf orientation. Detailed morphological characterization is accompanied by the collection of passport data for each of the cultivars and thereafter descriptor images are taken for comparison purposes (Sunaryo et al., 2017). Molecular characterization proceeds by finding DNA sequences which reveal polymorphism between cultivars. Characterization of plant genetic diversity has been frequently assessed with the use of high-throughput next-generation sequencing methods, such as Genotyping by Sequencing (GBS) or SNP-based DNA chips (Mason et al., 2015). These have been found to be powerful methods but are not economical since they require comparability of each accession over periods of time (Christelová et al., 2017). As a result, methods such as Simple Sequence Repeat (SSR), Random Amplified Polymorphic Deoxyribonucleic acid methods (RAPD) have been employed in the identification of *Musa* germplasm. SSR and sequencing technologies have largely confirmed the morphological classification of *Musa* down to the subgroup level, and have proved to be very helpful in solving classification problems. Molecular characterization correlates to some extent with taxonomy based on morphology, but is also a complementary tool for complete characterization (MusaNet, 2016). Molecular characterization allows for the provision of reliable information for assessing, among other factors, the amount of genetic diversity, the structure of diversity in samples and populations, rates of genetic divergence among populations and the distribution of diversity in populations found in different locations (Perera et al., 2000). A molecular marker is defined as a DNA or gene sequence that is used as an identification tool (Khan, 2015). These markers have been categorized into PCR-based and hybridization-based markers and both have their pros and cons in identifying genetic variants (Čížková et al., 2015). Identification of these genetic variants through characterization therefore allows for the distinction of Musa taxa across the globe.

1.4. *Musa* Taxonomy

Bananas belong to the family *Musaceae* and are cultivated throughout the humid tropics and sub-tropics. The family *Musa* is composed of the genera *Ensete*, *Musa* and *Musella* and all the edibles are from the Musa genus. The genus comprises of approximately 65 species basing on the morphology as well as chromosome number, the *Musa* genus is then sub-divided into 4 groups, which are Eumusa with (2x=22), Rhodochlamys (2x=22), Australimusa (2x=20) and Callimusa (2x=20) (S. The 2x refers to the chromosome number at diploid state in the nucleus. The Emusa is the one which is mainly focused on with species, *M. acuminata* (A-genome $2n = 2x = 22$; *n* represents the haploid chromosome number), *M. balbisiana* (B-genome, $2n = 2x = 22$), *M schizocarpa* (S- genome, $2n = 2x = 22$) and the *M textillis* (T-genome, $2n = 2x = 20$) (Davey et al., 2013). The majority of edible cultivated bananas originated from intraspecific or interspecific hybridization between wild diploid *M. acuminata* (A-genome) and *M. balbisiana* (B-genome) species. Combinations of these A- and B-genomes have resulted in various genotypes of cultivated edible bananas, including diploid (AA, BB and AB), triploid (AAA, AAB and ABB) and tetraploid (AAAB, AABB, ABBB) variants (Simmonds et al., 1955). The triploid genotype variants constitute the predominant cultivated varieties that are planted worldwide.

1.5. Banana types under production

The adoption of banana cultivars corresponds to need and preference by the consumer. Globally, different types of cultivars are grown for a variety of uses. In Asian countries, the Cavendish banana (AAA genome) accounts for 59% of total production making it the most produced variety. The remaining dessert bananas constitute 16 % in production whilst the remaining 25% constitute the production of cooking bananas of the AAB genome. The Cavendish banana is also a major type in the Caribbean as well as Latin America followed by cooking bananas. It accounts for 66% of the global Cavendish export and 62% of produced bananas in that region are consumed locally. In West and Central Africa, the cooking banana (AAB genome) is the most produced banana.it accounts for 69% of total production and is mainly consumed locally. The dessert bananas constitute 24% in production. East and Southern Africa mainly focus on dessert bananas. Together with cooking bananas, they constitute 76% of total production.

Banana production in Zimbabwe is constituted by both the commercial and the landrace varieties. Landraces are the local varieties that largely result from adaptation and continuous selection by farmers and are usually more diverse within field populations than modern

cultivars (Azad et al., 2016). They have a relatively high level of genetic variation and are highly stable in the face of adverse conditions. However, they are hardly virus-cleaned and as such their yield levels are lower than their yield potential. Examples of such varieties are the *Nzarayapera,* and the Gold finger (Azad et al., 2016). Banana production in Zimbabwe is ranked 57 amongst 132 banana producing countries in the world. Banana farming in Zimbabwe produces more profit compared to maize and tomatoes as far as the dollar to ton ratio is concerned and therefore can be a major contributor to the economy (Chitamba, 2016). Commercially, the Dwarf Cavendish and the Williams are the most produced cultivars (Mustaffa et al., 2012). Williams is a cultivar which belongs to the Giant Cavendish group. Since they are both of the Cavendish nature, their difference is viewed in terms of plant height and features of their fruits. Dwarf Cavendish is a shorter plant compared to Williams, which grows to a height of about 3.7 m, producing approximately 300, 15- 20 cm long sweet and identical fingers per bunch. Amongst other characteristics, the fruits have a bottle neck apex (Kepler et al., 2011). The Dwarf Cavendish is known to have a height of approximately 2- 3 m whilst producing an average of 100, 15-20 cm long fingers. The fruits are characterized by a slightly pointed apex (Damasco et al., 1998). The banana fruit is highly nutritious, it acts as a good source of carbohydrates and energy, is rich in vitamins A, B1 and B2 and minerals such as iron and calcium (Chitamba, 2016). One medium- sized finger (126 grams) is known to provide 110 calories, 422 milligrams of potassium, 30 grams of carbohydrates, 3 grams of dietary fibre amongst other nutrients (Azad et al., 2016). It is its high yield, high sugar content and high nutritional values that makes Williams one of the most commercially produced cultivars in Zimbabwe and the world at large.

1.6. Nutritional composition of banana fruits

Disregarding their global market status, bananas and plantains are referred to as the poor man's apple. This is because they have been a major source of calories of many ethnic tribes of Africa and the Pacific Islands and all the developing countries of the world (Singh et al., 2011). In most African countries such as Nigeria, bananas are part of the staple food and therefore it is essential to know their nutritional and biochemical composition. Bananas and plantains are very rich in nutrients, such as starch, vitamins, sugar, potassium, sodium, calcium, and magnesium as indicated in table 1. Plantains are nutritionally low protein food material but have very high carbohydrates, vitamins and mineral content (Azad et al., 2016).

Table 1. 1. Nutritional composition of bananas and plantains per 100 g serving

Constituents	**Units**	**Banana (*Musa Spp.*)**	**Plantains (*Musa Spp.*)**
Energy	Kcal	89	122
Water	g	74	65
Protein	g	1.1	1.3
Total Lipid	g	0.3	0.4
Carbohydrate	g	21.8	32
Fibre	g	2	2.0-3.4
Sodium	mg	1	4
Potassium	mg	385	500
Calcium	mg	8	3
Magnesium	mg	30	35
Iron	mg	0.4	0.6
Phosphorus	mg	22	30
Vitamin C	mg	11.7	20
β- carotene	µg	68	390-1035
Thiamin (Vitamin B1)	µg	40	80
Riboflavin (Vitamin B2)	µg	70	40
Niacin	µg	610	600
Pantothenic acid (Vitamin B5)	µg	280	–
Pyridoxine (Vitamin B6)	µg	470	–
Folic acid	µg	23	–

(Modified Table adapted from Aurore et al., 2009

Vitamins (riboflavin, folate, and vitamin C), carotenoids (-carotene, lutein, and zeaxanthin), and minerals (P, K, Ca, Mg, Na, Fe, Mn, Zn, Cu and B) compositions vary widely among banana cultivars. Inadequate intake of some of the nutrients such as iron and Vitamin A is known to cause nutritional disorders in East Africa and Asia (Ashokkumar et al., 2018).

1.7. Banana uses

Due to the differences in their colors, flavors, textures, sizes, and forms, bananas and plantains have various functions. While a large number of culinary variants are utilized in recipes that call for cooking bananas and plantains, many cultivars are eaten fresh as dessert fruits (Singh et al., 1998). It is usual in South India to serve meals on banana leaves, which are also used when steaming food, since they prevent food ingredients from burning (Lal et al., 2017). Asian cuisine can also be prepared with the banana trunk. Asian cuisines more frequently use the plant's big, flexible, and waterproof leaves as disposable, eco-friendly "plates."

The plant is also used in industries other than food, such as textile. The plant's fragile leaves and shoots are plucked and used to make fibre for manufacturing yarn because of its fibrous nature (Fan, 2023). This Japanese method, which produces tablecloths and kimonos, is carried out solely by hand, unlike the machine used in India to separate banana fibres. Together with the plant's bark, banana fibre is used to make paper. Also, it has been demonstrated that heavy metals from nuclear and fertilizer industries may be filtered out of river water using banana peel powder (Arunakumara et al, 2013).

1.7. Banana production expansion and limitations

Globally, challenges in production of bananas are as a result of pests and diseases. In other countries, especially those that are landlocked, transportation is a major hindrance in the production and marketing of the fruit (Azad et al., 2016). According to Azad et al., (2016), banana production is mainly hindered by pests and diseases, high cost of production, lack of high yielding cultivars and lack of suitable planting soils amongst other factors. Constraints of expanding production in Zimbabwe are centred on climatic conditions. Expanding banana production to new regions requires that the climatic conditions be similar or be tweaked in the littlest way possible since the crop is highly susceptible to climatic changes. Major banana plantations in Zimbabwe are located in the Manicaland province where they occupy approximately 45000 ha of land, with an annual yield of 189 499 tonnes (Muhoyi et al., 2021). The harvests accrue an average of USD$ 450 / ton (knoema.com/atlas/Zimbabwe/topics/Agriculture/Crops-Production-Quantity-tonnes/Bananas-production). The average yield potential of Cavendish banana ranges from 40-

50 tonnes per hectare therefore expansion of unit area results in higher yield. Potential interventions to production expansion are therefore the extrapolation of the production to new environments with similar climatic conditions, or where few changes can be made to suit the production requirements of bananas. Mimicking or finding similar environments in other regions in Zimbabwe as well as identifying suitable accessions for the targeted regions has remained a major challenge due to a number of reasons.

1.8. Statement of the problem

The main problem being addressed by this research is the unavailability of proper documentation of the currently produced banana cultivars, such as origin, genetic information and nutritional composition, such that germplasm conservation and improvements can be employed. The fruit requires an identification system for breeding purposes and assessments, which would then pose as an easy way of knowing characteristics, strengths and weaknesses of a particular cultivar before production. Apart from knowing the genetic characteristics of bananas, its geo-spatial distribution also needs to be assessed in the country.

1.9. Justification

Creating repositories or gene banks not only preserves samples of the banana germplasm of all the cultivars currently being produced, but it potentially creates a huge reservoir of knowledge about plant performance (including stress tolerance, nutritional quality and overall yield) in a wider range of climates, soils, and management regimes than could be tested on the same environment. This information, if made available to breeders and biological engineers, has great potential to then feed back into further improvement programs.

Defining accessions based on their genetics reveal their closeness and origin, while morpho-agronomic traits provide informative conclusions on cultivars just by looking at them, which is very important especially to communal farmers. Since time immemorial, genetic variation has always been recognized and tracked based entirely on the visual assessment of phenotypic variation. Since the 80's assessment is now assayed directly at DNA level. Genetic characterization identifies differences in the genetic sequences of individuals recording polymorphisms whilst monitoring their absence or presence in an organized way using a genome coordinated system (Halewood et al., 2018). Genetic characterization allows the level

of variation within a gene pool to be ascertained and accurately quantified and this in turn gives an informative presentation as far as the quality, distribution and use amongst other factors are concerned. Extensive genetic characterization linked to phenotypic traits allows gene banks to be searched for lines containing desired genetic elements and/or trait characteristics, and the production of a new and better crop that improves the sustainability, diversity and resilience of crops which is an important matter in food security. Food security lies within crop diversity and greater diversity of genetic resources provides the raw genetic material required to breed for a more nutritious and fast-growing banana fruit.

1.10. Objectives

The aim of the study was to establish a genetic pool of Zimbabwean grown bananas with lines suitable for breeding. To achieve this successfully, it was important that germplasm be collected from across the country and planted on an *ex-situ* gene bank and the plants characterized genetically and morphologically before, during and after fruiting.

The specific objectives were:

1. To establish an *ex-situ* banana gene bank
2. To characterize the banana germplasm both genetically and morphologically
3. To determine the fruit nutritional composition among the banana accessions
4. To evaluate the performance of conserved cultivars *in-vitro*.
5. To determine the geo-spatial distribution of banana production and extrapolate it to new areas for production expansion

1.11. Hypothesis

1. Ho: A banana gene bank can not be established at Hunyani Farm
2. Ho: The genetic and morphological characteristics of bananas in Zimbabwe are the same
3. Ho: The nutritional composition of banana accessions in Zimbabwe is the same
4. Ho: Banana cultivars perform the same way *in-vitro*
5. Ho: There are no new areas that bananas can be grown in Zimbabwe

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Chapter 2

2.1. Banana production in Zimbabwe: An analysis from a biotechnological perspective

2.2. Abstract

Globally, banana (*Musa Spp.)* is ranked fourth in terms of gross value production following rice, wheat and maize and its production in Zimbabwe is ranked 57 amongst 132 producing countries. Banana farming in Zimbabwe has been found to produce more profit as compared to maize and tomatoes as far as the dollar to ton ratio is concerned and therefore can be a major contributor to the economy of Zimbabwe. The review serves to troubleshoot and address the unavailability of proper documentation of the currently produced banana cultivars' origin, genetic information and nutritional analysis such that germplasm conservation and improvements can be employed.

The fruit requires an identification system for breeding purposes and assessments, which would then pose as an easy way of knowing characteristics, strengths and weaknesses of a particular cultivar before production. Apart from knowing the genetic characteristics of bananas, its geo-spatial distribution requires assessment. Banana production in Zimbabwe is concentrated in the warm and humid regions 1 and 2 of the six Natural Regions of Zimbabwe but similar climatic conditions are found in areas dotted across Zimbabwe. Using species distribution models, cultivars can be mapped to new environments mimicking the original production conditions.

Keywords: *Musa Spp*. germplasm, genetic characterization, gene pool, morphological characterization, species distribution models

2.3. Introduction

2.3.1. Banana production in Zimbabwe

Zimbabwe is an agriculture-based economy (Maiyaki, 2010). The country has over 39 million hectares of land with 33.3 million reserved only for agriculture. According to FAO statistics, agriculture contributes only 17 % to Zimbabwe's gross domestic product (GDP), an increase from 2017's 10.46 %. Despite the low GDP percentage, agricultural activities provide employment and income for 60-70 % of the Zimbabwean population. Agriculture supplies approximately 60 % of the raw materials required by the agro-industrial sector contributing up

to 40 % of the total export earnings (FAO Zimbabwe, 2018). Mutenga, (2019) , supported these statistics highlighting that banana production amongst tobacco, cotton, sugar, horticulture and tea, collectively contributes 40 % by value of national export to the country's GDP.

Generally, bananas grow very well in areas with an optimal mean monthly temperature of 27 ℃ and require a mean annual temperature of 12-13 ℃ (Tushemereirwe 2001). The plant generally has higher water requirements of approximately 25 mm per week for minimal optimal growth. The annual rainfall required by bananas is in the range of 1500-2500 mm but with very good management or irrigation schemes, they can grow well in areas with a mean annual rainfall of 1200 mm and below. These conditions are the major reason why banana production is high in the Eastern Highlands. Banana production in Zimbabwe has proved great potential in becoming a major income generating crop as highlighted by Chaipa (2015), Murendo et al. (2018) , Draft (2018) , and Mutenga (2019) . Banana production in Zimbabwe has proved to be more viable compared to commonly grown field and horticultural crops (Matimaire, 2018). Findings from research surveys indicated that 1 hectare of tomato, for example, yields 15,000 kg, and with the average price of US $0.50 cents per kilogram, US$7500/ ha can be generated (ZFU, 2019). Maize, a staple field crop, has an average yield of 5 tons per hectare and a price of $300 per ton, thus capable of generatingUS1500/ ha (ZFU, 2017). Banana on the other hand, the 45 ton/ ha average yield has a market value of $550 per ton (Matimaire, 2018), which converts to USD 24 750 (ZFU, 2017). Being a perennial crop, banana, like all the other perennials, offers food security making it a favorable crop for developing countries. Production in Zimbabwe is being conducted on both large and small scales. Internationally, production in Zimbabwe is ranked 21 in Africa and 57 in the world among 135 producing countries. Surveys of banana production showed that production in Zimbabwe is mainly done by small holder farmers (Chaipa, 2015, Murendo et al., 2018) under contract system (Murendo et al., 2018). Large scale banana producers in Zimbabwe have large estates that include Matanuska, Rosywood Mahemu, Roscorn Estate, Tagumi Estate and Rift Valley in the Eastern District of the country (Chidavaenzi, 2014) . From 2007 to 2011 banana production average output hovered around 1 million metric tons per year, but a sharp decline, attributed to high nematode infestation, was noticed in the year 2012 (Mutenga, 2019). An increase in cropped land area between 2016 and 2017 failed to correspond to output and yield per hectare fell from an average of 50 metric tons per hectare to 0.6 metric tons per hectare (Mutenga, 2019).

The paper highlights *Musa Spp*. production in Zimbabwe per sector, areas and scale of production, as well as germplasm improvement efforts thus far. To the best of the researcher's knowledge, there is no comprehensive research done in Zimbabwe on the genetic,

morphological or nutritional analysis of the locally produced cultivars as well as setting up gene pools for banana germplasm conservation therefore this paper serves as an initial step towards achieving these goals.

2.3.2. Constraints of Banana Production in Zimbabwe

The economic meltdown in Zimbabwe resulted in the reduction and complete halt of most of the operations in the smallholder irrigation schemes (Union, 2019). This has been due to high cost of maintenance against poor output markets, low liquidity amongst smallholder farmers (Union, 2019). Banana yield levels realized by smallholder farmers in Zimbabwe are generally low and do not bring high revenues due to suboptimal timing of harvest. Farmers are either not technically equipped or have insufficient resources to produce high yield at the right time. They generally lack important production skills, including pest management practices, improved plant nutrition, irrigation and soil health practices (Chitamba et al., 2016, Abdoulaye et al., 2014). Chaipa (2015), highlighted that smallholder farmers who operate under contract farming are greatly affected by pricing and market opportunities. The Food and Agriculture Organization of the United Nations (FAO) initially implemented a contract farming project in Zimbabwe in the 2010/11 agricultural season (FAO, 2013). The Sentinel Survey, (Murendo et al., 2018), revealed that some banana growers under contracts are provided with loans payable after 6 months at an interest rate of 12 %. With such problems as market price fluctuation, poor road networks, load shedding and old irrigation equipment amongst other factors, banana growers fail to sustain continuous profitable production and as a result, fail to repay the loans. Contracted growers are required to also provide collateral in form of land, money or group membership status and failure to provide also results in farmers being unable to access credit for farming (Murendo et al., 2018). Zim-AIDED (2017), however highlighted the help provided by Zim-AIDED to Honde Valley farmers towards good production practices and higher yield.

Plant parasitic nematodes hinder banana production causing yield loss of up to a minimum of 30 % and cumulative losses due to reduction in bunch weight may reach as far as 75 % if effective control measures are not implemented (Murendo et al., 2018). Tissue culture technology should be effectively used to produce clean pest free planting material. The unpredictability of natural disasters may contribute to difficulties in banana production. The Cyclone Idai of 2019, hit hard on banana production in the Eastern District of Zimbabwe (Zinyuke, 2020a). The produce, planting material, fertile soils, market and transportation

networks were destroyed by the national disaster and, as the farmers were still recovering from the cyclone, the Covid-19 pandemic surfaced destroying the hopes of many farmers (Zinyuke, 2020b).

2.3.3. Banana Repositories

According to Ruas et al., (2018), the collection, conservation, characterization and breeding of plants and their wild relatives contribute to the preservation of biological diversity, and are essential components in ensuring food security. Their role is to preserve genetic diversity for future research and plant breeding (Africa, 2018). PGRFA (2007) indicated that the most outstanding constraints in the use of plant genetic resources is the lack of publicly available evaluation data for most banana accessions and the capacity to manage the data. Researchers and breeders require such information for the selection of germplasm and further studies as highlighted by (Musa Net, 2006). INIBAP, (2006) highlighted that there are as many as 1,000 different banana cultivars and possibly up to 70 wild species in the world. Currently 56 institutes worldwide are conserving over 15,000 accessions that are made available for use by breeders, farmers, researchers and consumers. Major 'players' in the banana industry such as Latin America, Asia and the Caribbean Islands have managed to characterize their banana plants, a factor which has contributed to massive and successful production as indicated by Mattos et al. (2010), Mukunthakumar et al. (2013), Sunaryo et al. (2017) and Mattos et al. (2010b). In Africa, countries such as Nigeria, Uganda and Kenya amongst other producing countries have characterized their lines (Qaim, 1999) and a few have managed to introduce GM bananas for better yield (Reuben et al., 2016). Zimbabwe does not have a banana gene bank but rather it has production areas and tissue culture facilities (TRB, 2020, Dube, 2018). The Chiredzi Research station, an arm of the Department of Research and Specialist Service (DRSS), harbors banana germplasm that can be included in gene banks. The coffee research station in Chipinge also has an established *ex situ* site of *Musa Spp* that can serve as a gene bank. Apart from these, most estates and plantation which include the Honde valley and Chipinge plantations, the Nyanyadzi, Middle Sabi, Mupangwe irrigation schemes, Njikizana and the Baguti plantation to mention but a few, are production sites as well as vital sources of germplasm for the establishment of banana gene banks. The study was therefore aimed at assessing the characterization of bananas in Zimbabwe .

2.4. Methodology

2.4.1. Database Search

Meta-analysis allows quantitative analysis of experimental results reported by other authors and the estimations of effective size. The literature for this review was obtained through literature search on peer-reviewed publications and also greys literature. The process is highlighted by fig 2.1. For searches that could not yield results from the internet, information was gathered through phone calls to different institutions and banana plantation sites. Peer-reviewed articles from databases such as PubMed, NCBI, Directory of Open Access Journals (DOAJ), Web of Science, Science Direct, SciELO, BMC, Research gate and Academia were used. For a wider scope of information, grey literature searches were obtained using search engines such as Google Scholar and FAO. The literature that was used dated prior to 2020 and since research of bananas is quite extensive, constructs were used both as individual words and in their combinations and these were, [world] +[Africa] + [Zimbabwe]+[*Musa spp*. genetic characterization]+ [*Musa spp*. Improvement]+[*Musa spp*. Tissue culture]+[virus indexing]+ *Musa spp*. morphological and agronomic characterization]+[nutritional and chemical composition of *Musa spp.*]+ [*Musa spp.* breeding] + [species modelling] + [GIS in species modelling]. The resultant publications were of no exact time limits as relevance was of the greatest priority.

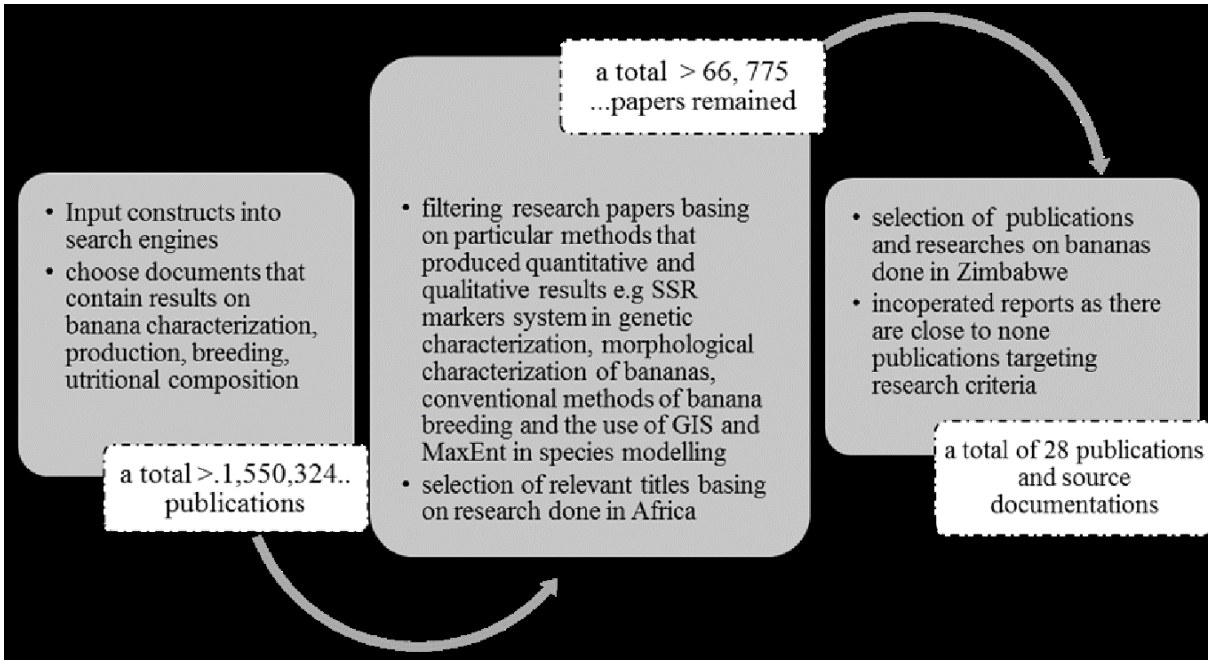

Figure 2.1. The schematic flow diagram of the literature database search process (Source: Author's own)

2.5. Results and Discussion

2.5.1. Banana Identification

Just like how citizens or non-citizens undergo registration and identification card collection in a nation, plant species need identification and registration. Identifying how a plant can qualify to be called native or foreign/alien in a particular region is a highly debatable topic amongst botanists and word play has more often than not come into play during such circumstances (Pysek, 1994). Time factor has, over the years, been considered to be of paramount importance in an attempt to explain the stage at which a plant species can be classified as a native species, but to some extent it has been found to be of no scientific basis (Ansong et al., 2014). According to Pysek (1994), plants occurring in regions where they are not native have been termed aliens, exotics, introduced, translocated, neophytes, naturalized, invaders amongst other names. Webb (1985) suggested eight criteria which may be employed in defining the status of plants within a country which are fossil evidence, historical evidence, habitat and geographical distribution, frequency of known naturalization, genetic diversity, and reproductive pattern and possible means of introduction. In reference to Webb's criteria, Pysek (1994) went on to discover that only fossil and historical evidence can, without being scanty, prove the actual status of a plant as supported by Belnap et al., (2012) and Smith, (1986). Determination of the native-ness of a plant species can therefore be guided by three factors which are: i) species that arrived before

the neolithic period should be considered native even if introduced by man ii) species that became extinct during the last glacial period and were re-introduced by man cannot be native and iii) the species that arrived in the area in recent years by means independent of human activities should be regarded as native. A majority of researchers concur that these are a population of plants that have been cultivated for many generations in a certain region, being shaped by the biotic and abiotic stresses, crop management, seed handling and also eating preferences (Karamura et al., 2016; Introduction, (2009), Bioscience for Farming in Africa, (2016), Fischbeck, 2013). Most of the bananas being grown by local farmers in Zimbabwe are landraces that, according to Breseghello & Coelho (2013), constitute a dynamic genetic entity that are continuously changing and, in some cases, showing quick changes when they are subjected to different climatic regions. Using Pysek's, (1994) methods of giving status to species, the majority of bananas being produced in Zimbabwe are of the 'alien' status and have naturalized over the years. It is important therefore to document and distinguish these species as to which are of the native or alien status. This is a vital step towards cataloguing, characterizing and target improving the banana collection. The Rosywood Mahemu Estate for example, introduced Du Roi's, sourced from South African Laboratories. These are agronomically superior cultivars which are under production in Zimbabwe even though they are not of Zimbabwean origin. Plantations in the country produce cultivars that have naturalized over the years and have adapted to the Zimbabwean climate. Field research in the Eastern Highlands identified a massive site (GPS coordinate -20.190558, 32.626010) of what can be referred to as "invasive" banana species. Among them is a variety which constitutes a huge plant that gives short, starchy golden fingers, locally called *Nzarayapera.* Such banana sites constitute a reservoir of unexplored germ lines, which can be used for further *Musa spp* improvement.

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2.5.2. Phenotypic Characterization

Genus *Musa* was firstly named by Carl Linnaeus in 1753, using the Linnaean binomial system and up until the 1940's different types of bananas and plantains were given Linnaeus' binomial names. The nomenclature was later abandoned for an alternate genome-based system (Cheesman, 1947). Classification of *Musa* species based on the morphological traits was firstly published in 1887 based on the flesh of the fruit, inflorescence and the fruit size (Sagot, 1887). It was only five years later that Baker (1893), firstly designated the *Musa* sections. The process was carried out for over a decade until *Musa* was designated into five (5) sections namely *Emusa, Rhodochlamys, Callimusa, Australimusa* and the *Ingentimusa*. This process was pioneered by (Cheesman, 1947) basing on the morphological features and the chromosome number. The introduction of genomics in the 21st century led to the discovery that suggested that the five sections were not monophyletic, hence the groups were re-grouped into two classes, but there are still unidentified banana species to date. Bananas have three ploidy levels in different combinations of the A (M. *acuminata*) and B (B. *balbisiana*) genomes (Jesus et al., 2009). America & Montcel (2008), described bananas as herbaceous plants which can confer the aspect of a tree. The plant has a pseudo-stem which is formed by the concentric assembly of leaf sheaths, crowned by a rosette of large oblong to elliptic leaves. From a study by Allen et al, (1988), the leaves of a banana plant are produced successively until the inflorescence is cast. Wide morphological variability has been observed in the plant's inflorescence type, size, fruit orientation, apex shape, pseudo-stem and fruit color (Vuylsteke et al., 1995). Cultivars differ in phenotype due to genetics and the environmental factors which is why the same Chinese Cavendish from the Honde Valley can be phenotypically different when grown in the Middle Sabi region of Zimbabwe. Characterization of banana plants based on morphology requires that particular plant descriptors be used. According to IPGRI-INIBAP, (1997), the use of plant descriptors in phenotypic characterization enables quick discrimination between phenotypes. The scoring methods of qualitative and quantitative data can also be presented differently in accordance to the type of system being used. Morphological descriptors should be easily scored and must have a constant phenotypic expression in all environments that is low environmental influence and high heritability, therefore, strong and important descriptors are those that are not easily affected by the environment (Ortiz, 1997). Descriptors that possess low genotype by environment interaction are more important for agronomic evaluation and for classification. Ortiz, (1997), went on to explain that the most important qualitative

morphological descriptors are the male bud, hermaphrodite flower, pseudo stem pigment, foliage, petiole and male flower, pseudo stem waxiness and leaf orientation. The important quantitative morphological descriptors are the pseudo stem girth, height, fruit number and size. The researcher also highlighted that the quantitative descriptors selected generally have high heritability (>0.8), high repeatability (>2.0) and low coefficient of variation (9-15%) with the exception of height, therefore, can be regarded as morphological traits of importance in banana plants. Detailed morphological characterization is accompanied by the collection of passport data for each of the cultivars and thereafter descriptor images are taken for comparison purposes (Sunaryo et al., 2017). According to Ruas et al., (2018), it is deemed wise to consult with local agricultural experts on the varieties that are present within a particular location and to have photographic evidence of each, because vernacular names may differ with location therefore duplication may occur. Difficulties in the identification of genotypes that are closely related using morphological descriptors has increased with the development of new varieties. Since these descriptors are influenced by the environment, some can only be evaluated during the late stages of development, demanding time and physical space for evaluation (Jesus et al., 2009). Literature search, however, did not yield any record of morphological characterization of banana plants in Zimbabwe unlike other African countries like Kenya and Uganda that have managed to characterize their landraces and commercially produced bananas (Commission of the European Communities., 1971, Vuylsteke et al., 1995, Njuguna et al., 2008, Karamura et al., 2016). However, the Chiredzi Research Station, an arm of the Department of Research and Specialist Services (DRSS), is said to be carrying out the banana characterization process as well as micro-propagating the plantlets.

2.5.3. Genetic Identification/Characterization

Genetic characterization allows the identification of duplicates, estimate the extent of accessions of particular genetic diversity and also determine the phylogenetic relationships thereby allowing for mapping out origins of the cultivars (Park et al., 2009). The morphological, agronomical and physicochemical characterization of the banana fruit allied with estimates of genetic variability assessed using molecular markers is of paramount importance in the selection of progenitors in order to explore heterotic characteristics and breed bananas (Mattos et al., 2010). Molecular markers have been used extensively for germplasm characterization. For example, assessment of genetic diversity within the Musa genotypes has been done using random amplified polymorphic DNA markers (RAPD's) with over a thousand

research publications, (Gubbuk et al., 2004, Mukunthakumar et al., 2013, Jesus et al., 2009, Azad et al., 2016), using restriction fragmentation length polymorphism markers (RFLPs), (Beata & Andrzej, 2011), microsatellites or simple sequence repeats (SSR), (Oriero et al., 2006, Karamura et al., 2016, Mattos et al., 2010), fragment length polymorphism markers (AFLPs), (El-khishin *et al*., 2009), and diversity array technology markers (DArTs), (Martin et al., 2017, Beata & Andrzej, 2011, Paridah et al., 2016) each method having its own pros and cons. Due to modern DNA sequencing technology and bioinformatics tools, the sequencing and assembly of genomes for economically important crops like bananas are becoming common hence understanding the genetic make-up allows for the detection of regions that could represent polymorphism associated with agronomic traits. Heslop-Harrison (2007), explained that the knowledge of genomics and understanding of the crop allows for domestication, involving interactions of plant breeders and genomic scientists to then design the characteristics required from a banana cultivar and consider how to produce the ideal cultivar.

2.5.4. Nutritional Composition

Academia journals alone showed over 50,000 journals worldwide that explained the nutritional composition of bananas and plantains. According to literature, bananas may vary in their genetic characteristics but rarely do they give a huge difference in their nutritional composition (Pareek, 2015, Awedem et al., 2015, Ashokkumar et al., 2018). Ashokkumar et al., (2018) showed that carotenoid rich cultivars were identified by a number of researchers, however, limited knowledge is available on the micronutrient composition differences between banana and plantains.

Bananas are vegetative parthenocarpic and develop their fruit from the inferior ovary of the female flower. It is during the development of the fruit that the pulp to peel ratio increases from 1:1 to 4:1 depending on the variety and maturity at harvest. It is at the storage ripening stage that the starch decreases from about 22% to 1% and at the same time soluble sugars increase from 1 to 20% (Forster et al., 2003). The hydrolysis of starch at this stage results in the increase in pulp to peel ratio. The fruit is rich in nutrients, starch, sugar, vitamin A and C, potassium, sodium, calcium and magnesium (Ashokkumar et al., 2018). Studies in Africa and South America revealed the relationship between yellow to orange flesh coloration and higher carotenoid content (Fungo & Pillay, 2011).

2.5.5. Banana Germplasm Improvement

Plant breeding is a science driven process of developing new plant varieties. There is a general consensus amongst researchers and breeders that the process involves the creation of multi-generation genetically diverse populations on which selection is practiced to create adapted plants with new combinations of specific desired traits (NAPB, 2019). According to Breseghello & Coelho, (2013), the challenge of plant breeding resides in improving all of the desired traits of interest simultaneously. Improving the desired traits simultaneously is mainly hindered by the genetic correlations between traits which is caused by genes with pleiotropic effects, physical linkage between genes in the chromosomes and the population genetic structure amongst other factors.. Breseghello & Coelho, (2013), further explained that the selection for a single trait changes the correlated traits, sometimes in the desired direction whilst in the other times it goes in an unfavourable direction. For this reason, selection therefore can lead to unanticipated changes which are normally within the range that is normally observed in the crop and thus assumed to pose no risk to consumers or the environment.

Over the years, numerous research programs have introduced several methods for the improvement of bananas and these include, conventional breeding, marker assisted selection, genetic engineering, induced mutation breeding, protoplast fusion and selecting some clonal variants. Amah et al., (2018) highlighted that the marker-assisted selection technique of breeding is becoming more popular in the breeder's community as it offers the possibility of significantly reducing the amount of time required for banana improvement. However, despite its potential to enhance and hasten banana improvement, the application of molecular markers remains largely unexplored because of the intricate genetics, high genetic similarity and high polyploidy nature of bananas as well as the difficulty in developing segregating populations attributable to either male or female sterility (Amah et al., 2018). Amah et al. (2018) explained that productivity, disease resistance and yield have been priorities of banana breeding with an emphasis on simply inherited agronomic traits. The nutritional qualities however have been secondary due to limited resources, knowledge on genetics of these traits and also the overwhelming dire impacts associated with major diseases of bananas. However, the recent advances in banana genomics and increased awareness of the nutritional importance of bio-fortification surfaced by The Banana Genome Hub, developed by the French Agricultural

Research Centre for International Development (CIRAD), and the Biodiversity International proves that the interest in this area has risen (Rateng, 2018).

Banana breeding is complicated by parthenocarpy, low fertility, low seed viability, polyploidy and associated irregular meiotic behavior, long generation times, diverse genome configuration and a narrow genetic base (Amah et al., 2018). The breeding cycle of bananas from crossing to release of a new variety takes up to 15 years as described by Pillay & Tenkouano (2011), but despite the long and complex breeding cycle, hybrids have successfully been developed containing diverse agronomic and disease resistance traits. According to an article from Kenya (Rateng', 2018), Scientists in Nairobi have demonstrated that through genomic prediction models, that the time frame for banana breeding can be reduced to less than 15 years. Literature in the public domain did not yield much as far as biotechnological applications to bananas are concerned which highlights the need for a board in Zimbabwe that specifically targets banana crop. Virus indexing, tissue culture and characterization of the banana plants are the future of the banana industry and therefore institutions such as the Chiredzi and Grasslands Research Stations may need to focus on these techniques to help improve banana production. Besides targeting bio-fortification, molecular markers and other biotechnological methods can be applied to locally grown bananas to improve drought resistance. As in the case of maize varieties that were bred to suit the different agro-ecological zones of Zimbabwe, bananas can also be bred for a similar objective. Breeding can therefore be used as an expansion tool of banana production in such semi-arid parts of Zimbabwe.

2.5.6. Distribution Patterns of *Musa Spp*. In Zimbabwe

In Zimbabwe, extensive banana production is practised in the Manicaland province. The province produced 81% (396 975 tons) of the total annual output during the 2017/ 18 cropping season, with the balance of 19 % (8 800 tons) coming from the rest of the country (Tsiko, 2018). Region 1 is the most favored banana producing area due to high rainfall. Bananas grow well in the tropical climate, which is found mainly in the Manicaland province of the region 1 of Zimbabwe. Findings from recent surveys (Matimaire, 2018a) , have shown that vast hectares of land in the Eastern Highlands have a potential of producing over 32 550 tons of bananas per annum, but only 1600 hectares are currently under banana production and Chipinge alone has 1130 hectarage available for production but only 100 hectares are being utilized. Region 1 harbors plantations in the low-lying areas such as Rusitu Valley, Honde Valley, Burma Valley,

Chipinge, Chibuwe and Mutema (Chitamba, 2016). More than 4,000 people in Honde Valley alone depend on bananas for more than a third of their income (Lacey, 2018).

Apart from the Eastern Highlands, other parts of the country produce bananas on a smaller scale. Production in other regions of the country is not as large as that of region 1 although with irrigation schemes the plantations have successfully yielded quality fruits for example Charara Farm in Kariba produces over 100 tons per year on an 80 hectare farm (The Herald, 2020). The Middle Sabi's farm 32 has managed to produce bananas on over 160 hectares of land, positively transforming lives of A2 farmers (The Herald, 2020). The Herald, (2020), reported that it was through the partnering of the Farm with a private contractor, the MacCarter Bananas, that great success in production was noted. Statistical results of the reviewed studies showed that banana yield is highly dependent on climate and rainfalls (Sabiiti et al., 2016). This however does not have to be the end of the story as cultivar can be mapped to new areas where they have a higher probability of thriving. Cultivar mapping is a technique that uses reviewed cultivar characteristics and performance within a locality and extrapolates the production of respective cultivars to new locations with similar climatic conditions.

2.5.7. The use of species distribution models in identifying banana geo-spatial distribution

The spatial and the temporal distribution of plant and animal species is affected by the quality and quantity of habitats (Fars, 2019). Beane et al., (2013), revealed that SDMs are useful in both generating maps that ensure identification of suitable habitats and also determining suitable variables which are primary drivers for a species occurrence on the landscape. The species distribution models (SDMs), according to Elith et al., (2010), estimate the relationship between species records at the site and the environmental or spatial characteristics of those sites. They predict the habitat potential and spatial distribution of a species according to the occurrence data and different environmental variables (Fars, 2019). SMDs with low accuracy modelling techniques produce poor predictive performance since the most important aspect of SDMs is the choice of the appropriate algorithm or model because one of the basic questions about SDMs is whether the type and quality of data can produce the information needed for the intended application. Matching the SDMs to the requirements of applications is very critical to avoid poor inference and management outcomes; therefore, it is necessary to specify a framework within a structured decision-making context for the use of SDMs. The use of these models at local scale has provided vital information about habitat desirability and help with resource management.

Different probabilistic and statistical models are currently being used to determine plant species' spatial distribution. Amongst the common SDMs are the statistical models that include generalized linear models (GLMs), generalized additive models (GAMs) and the probabilistic models such as Maximum Entropy (MaxEnt) and the frequency ratio (FR). The result from predictive modelling methods can be used to identify the factors affecting the distribution of species, to introduce the appropriate species and also to determine habitat suitability for plant establishment on a local scale (Sahragard, 2016).

2.5.7.1. *MaxEnt* in Species modelling

MaxEnt is a machine learning technique that can be used to predict geographical distribution of any spatial phenomena, plants and animals included (Endries, 2001). According to Merow et al., (2006), Maximum Entropy (MaxEnt) is one of the most popular software packages for species distribution and environmental niche modelling. Its popularity is brought about by two reasons which are its highly predictive accuracy and the fact that it is very easy to use. MaxEnt has been identified to be a strongly competitive or superior modelling method particularly for the species with limited distribution (Beane et al., 2013). The software takes a list of species' present locations as its input (which is called the presence-only (PO) data as well as a set of environmental predictions (which include rainfall and soil types amongst others) across a user-defined landscape. Since its availability in 2004, it has been utilized extensively for modelling species distribution.

However, because it uses the present only data, the absence of data or the species at a particular location may affect the output. When for an example, a banana species is absent from an environmentally suitable area maybe because of past disturbances, the signal of absence is found in the distribution of presence records and therefore the location is marked as without the species and the pattern in the presence-only will suggest the area is unsuitable affecting the modelling pattern. Countering the biases that can be brought about when using MaxEnt is the use of presence-absent modelling method since the models are less susceptible to problems of sample bias (Elith et al., 2010).MaxEnt contracts presence against back ground locations where the presence or absence of a species is unmeasured. The background samples (sometimes referred to as pseudo-absence) are desired because according to Phillips & Dudı, (2008), MaxEnt allows one to predict the probability of presence within a particular location. By

default, the MaxEnt software uses a prior which assumes that a species is equally likely to be in anywhere on the targeted landscape that is on the Zimbabwean map, every pixel has the probability of being selected as background (Merow et al., 2006). MaxEnt then contrasts the environmental conditions (through the use of different climatic layers) at background locations (all the Zimbabwe area) with those at observed presence locations (those with banana production) and then shows the new areas in which banana production can be done.

2.5.7.2. ArcGIS in MaxEnt modelling

Brown (2014), highlighted that almost all species distribution model methods require extensive data preparation in GIS prior to the building of the model. GIS effectively provides an ideal tool for the assessment of landscapes, development and application of habitat models as well as modelling the potential distribution of species and habitats (Endries, 2001). Woods (2014) explained that ArcGIS performs the roles which include creating snap grid for the study area, creating environmental layers, aligns layers to snap grid, creating CSV of occurrence data (locations of interest), converting environmental data to ASCII, converting the results from ASCII to Grid and also symbolizing the results on a map. Generally, the geographic information system (GIS) manages, analyses and displays geographic information using three main views which are the Geodatabase, the Geo-visualization and the Geo-processing view in ArcGIS (ESRI, 2001). The Geo database contains the generic data models that are necessary for the creation of the mapping system whilst the geo-visualization shows features and their relationships to the earth's surface. Using these features the modelling of the local *Musa* plantations becomes easier and more visual. According to the ESRI, (2001), it is the geo-processing view that is of fundamental importance in the mapping of new areas for banana production in ArcGIS. The geo-processing view is a set of information transformation tools that derive new geographic datasets from the existing ones. In this manner, new areas can be modeled using different and useful climatic layers (SDM, 2011). Modelling or mapping out of new banana production sites using ArcGIS require the input of specific data apart from layers so as to narrow down regions of importance.

The mapping procedure therefore extends the banana production area, increasing production yield. This process can be aided through banana breeding programs thereby producing tailor-made cultivars that suit particular climatic conditions.

2.6. Conclusion

Literature research has revealed that little has been achieved on banana characterization in Zimbabwe and there is a large gap in comparison to other crops and therefore engaging and implementing research can help extend production as well as provide a resilient crop across the country. Towards satisfactory analysis of the *Musa spp*. production in Zimbabwe, a germplasm collection needs to be created in order for accessions to be closely monitored to consider possible gene-environment influence on the phenotype. As such, multi-locational gene banks have to be established across the geographical regions of Zimbabwe. By doing so, accurate multifactorial assessments of genetic lines are made for the betterment of banana production in the country.

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Chapter 3

3.1. *Musa* Spp. Gene bank establishment

3.2. Abstract

The extent and the distribution of genetic diversity in any plant species is dependent on ecological and geographical factors as well as evolution and breeding systems. The study was conducted with the aim of collecting banana species, under commercial production as well as landraces, establish an *ex-situ* gene bank and characterize the collected accessions using morphology and molecular methods. A total of forty-eight plants were collected from production areas across the country and were planted at the Chinhoyi University of Technology owned Hunyani Farm each with 4 replicates. To each plant, an accession number was assigned. The germplasm was evaluated based on the fruit, bract, male flower as well as the inflorescence for two fruiting seasons. The germplasm was subjected to target sequencing, which identified five genetic groupings. Seven cultivars were identified using morphological characteristics and morphological features. Three of the five genetic groupings were from the culinary family, while the other two were from the dessert family. The collection's characterization allowed one cultivar to be distinguished from another. Characterization of two cultivars which are locally known as *Nzarayapera*, a local landrace, demonstrated that they had two distinct genomes. One was genetically identified as an apple banana, an AB hybrid whilst the other, a *Nzarayapera*, a B genome banana. Consequently, more cultivars found in the country need to be characterized in order to ensure proper classification and conservation.

Keywords: banana**,** genetic conservation, germplasm,

3.3. Introduction

Gene banking operations and facilities are likely to vary substantially from country to country, in terms of both the size and capacity of the bank and the types and amount of equipment needed. These factors are dependent upon the quantities of germplasm to be placed in the gene bank, which will in turn depend upon the objectives of the gene bank, the range of species and breeds to be conserved, and the financial resources available for the conservation programme. Plant germplasm conservation and maintenance in *ex situ* gene banks is mainly done through active or base collections. Active collections of germplasm require storage methods that can retain the viability of each plant for a short period of time (Makhathini et al., 2002). This is usually carried out in green houses or in the field. Bananas stored in field gene banks have a lower risk of losing genetic integrity (due to genetic drift) if the mother plants are maintained for many years and are readily available for study and use. However, bananas maintained in field gene banks are considerably more exposed to physical risks (climate, diseases, pests) and costs are higher for storage (labour, inputs and space) hence in-vitro gene banks are more preferred (MusaNet, 2016).

Characterization and evaluation of germplasm are parts of plant genetic conservation activities and the layout of the gene bank takes such activities into consideration. This therefore means that a field gene bank is normally set up as a field experiment or trial using an appropriate experimental design. There are a number of factors that may mislead a field trial and this causes bias in experimental results which include moisture, temperature, soil texture, fertility and many others factors. Complete elimination of these factors may not be possible therefore, require minimization. Minimization includes soil heterogeneity, replication of genotypes and randomization of germplasm. While establishment of field gene banks is laborious and requires close monitoring, it is important in conservation of genetic diversity.

Germplasm collection has been applied towards the preservation of genetic diversity. It is an essential component in ensuring food security and indigenisation. The objective of field germplasm collection is to obtain maximum diversity from minimum sample size and number. Both random and non-random sampling can be employed while collecting the samples. Non-random sampling allows the selection of germplasm with clear morphological characters leaving those associated with disease resistance and other physiological characteristics. In nature, the modern banana is parthenocarpic hence collection can only be done through germplasm sucker collection. Target germplasm suckers are always expected to be 10 cm in

diameter at the base or more with minimal damage on the corm**.** The objective of this study was to collect banana germplasm across Zimbabwe, annotate and bio-bank it in an *ex-situ* gene bank.

3.4. Materials and methods

3.4.1. Germplasm collection and establishment

Forty-eight banana germplasm plants were collected from different areas dotted across the country and these include Chipinge, Honde Valley, Nyanyadzi, Chimanimani, Chiredzi, Zaka, Chinhoyi and Harare. Germplasm was collected in the form of young sword suckers from local farmers and the GPS of the collection site was also taken. At each plantation, 4 replicates of each variety of the healthy-looking sword suckers were taken.

At the Hunyani Farm, a 1000 m^2 piece of land was identified for germplasm establishment and the pH of the soil was confirmed using the litmus paper test as indicated in Figure 3.1. The area was cleared, burnt and ploughed before, 60 x 60 x 60 cm holes were dug with a spacing of 3 x 3 m as indicated by Tushemereirwe, (2001). The holes were left exposed to the sun in order to get rid of soil nematodes that sterilize naturally and then they were filled with a mixture of organic manure and soil (in the ratio 1:1) up to the 40 cm mark.

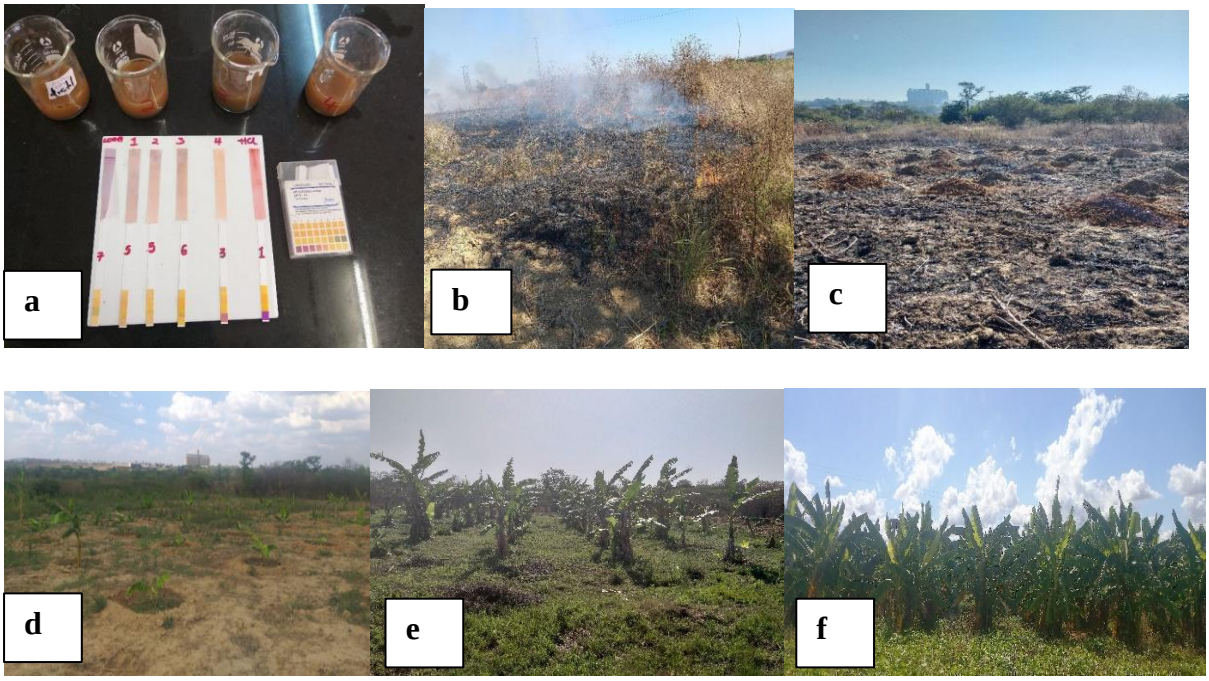

Figure 3. 1. Gene-bank establishment at the Hunyani Farm in time series: (a)soil testing, (b) land clearing, (c) hole digging for planting, (d) established germplasm, (e) winter period, (f) gene bank at fruiting stage

Using a complete randomized block design, the plants were then planted in each hole where more soil was then added to cover the base of each plant. To each plant, an accession number was assigned according to where the plant was obtained as well as its position in the gene-bank as shown in Figure 3.2. Growth and development characteristics of the established plants were recorded for 24 months from their planting to capture the vegetative and reproductive phases of the plants.

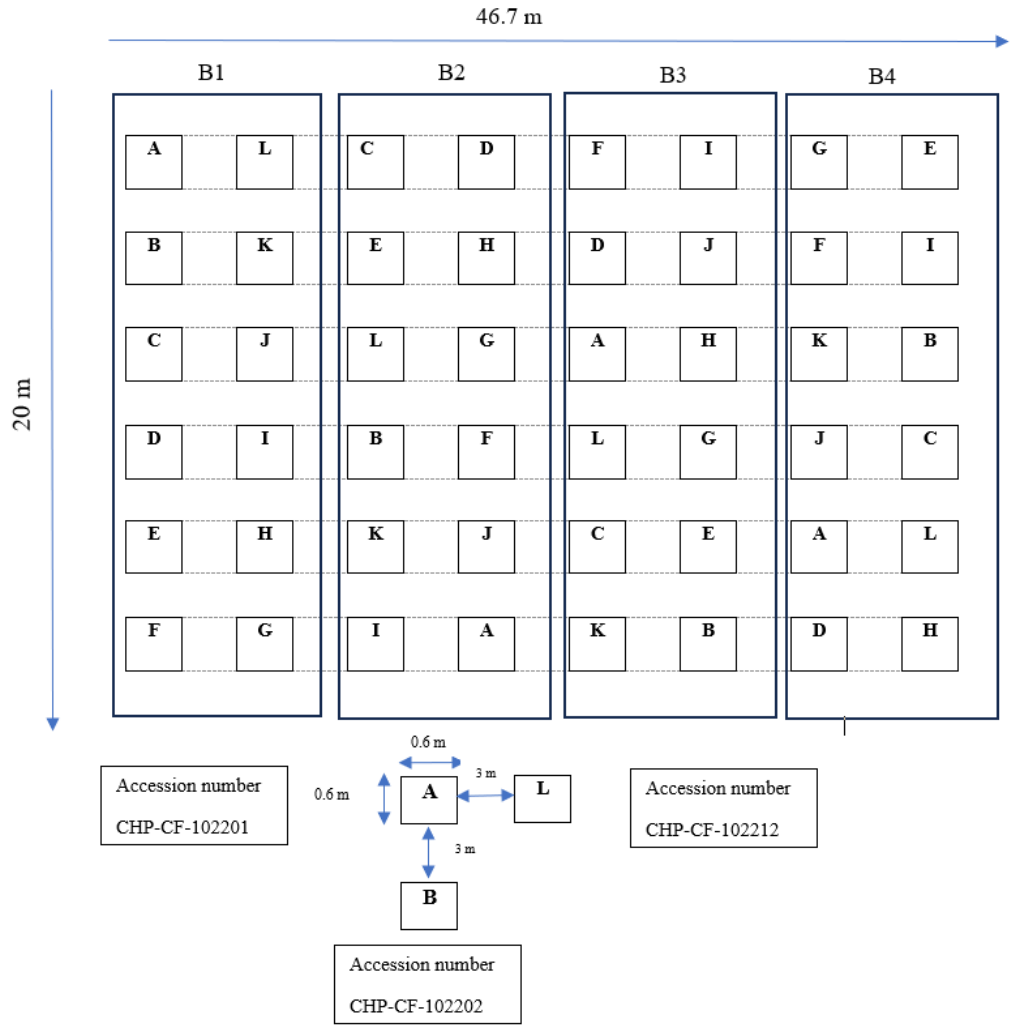

Figure 3. 2. Layout of the gene bank at the Hunyani Farm. The letters represent the different varieties collected. (GPS coordinates (-17.350621, 30.239247).

3.4.4. Irrigation

Each station's basin was filled with water after every 3 days until the germplasm was well established then watering was done twice a week.

3.4.5. Gene bank maintenance

Plants were de-suckered and young plants (three months old) were used in tissue culture. Three plants per banana station were maintained. Leaves were pruned after the winter period. Fertilizer was not used but rather organic manure application was maintained. Harvesting was done in batches due to different maturity stages.

3.5. Results and discussion

3.5.1. Germplasm establishment and accession number assignment

The gene bank was established in October 2020 with four replicates for each collected variety. To the bananas collected from Chiredzi, accession numbers CH-CF102201-05 were assigned. CH being the source of the material, CF being the current plant site, the Hunyani Farm, 10-22 being the 22nd of October, the planting date and 01-05 the position of the plant in the gene bank. The same was done for Nyanyadzi (NY), Chipinge (CHP), Chinhoyi (CHN), Honde Valley (HV), Zaka (ZK) accessions.

3.5.2. Irrigation

The hydric state of banana plants is considered one of the main factors responsible for the growth and development as well as fruit production. The plant has a high-water requirement due to its fast development and large foliar area, but it has some tolerance to drought apparently by closing stomata and maintaining strong root pressure (Turner et al., 2007). Biological studies have shown that the daily transpiration varies from 5.6 mm under direct sun and 1.9 mm in cloudy conditions (Turner et al, 2007). The required amount therefore increases as temperatures increase. Irrigation was done through filling each plant station basin with water. During the summer/ hot period, the plants were watered every 3-4 days and 7-8 days in the cold/winter period.

3.5.3. Temperature and its effect on banana growth

The May to July winter period of 2022 had an effect on the plants with other cultivars being affected more than others. The plantation was a year old. Temperature range was a minimum of 10 ℃ to a maximum of 24 ℃. Recorded rainfall was less than 14 mm hence it was a cold and dry period. The degree of damage done on the plants by the cold temperatures were scored in Table 3.1. The dwarf Cavendish was mostly affected by the cold season as shown in Figure 3.3. The pseudo-stem was completely destroyed which shows that cold temperatures in Chinhoyi had a significant effect on its growth as compared to its collection source. This may also be attributed to water shortage as the irrigation system did not properly function during the winter season. Difference in the effect of temperature was attributed to the plant's origin as well as morphology and genetics The AB hybrid, *Nzarayapera* was least affected by the cold as most of its leaves remained intact. The hybrid was sourced from Chipinge which has lower temperatures compared to Chinhoyi. The rate of plant growth and fruit maturity are determined by the temperature. Studies have shown that the optimum temperature for foliar growth ranges from 26-28 ℃. High temperatures of about 37 ℃ or more result in burnt leaves and stunted growth. Low temperatures of 10 ℃ stop the growth of any developing folia (Pegg et al, 2019). The development of the banana flower is affected by temperature. Post-harvest low temperatures can also damage the fruit through uneven pulp softening and shelf-life reduction. Ramirez et al., (2011) highlighted that below 9 ℃, latex coagulation occurs in the pericarp and the ripening capacity of the banana is lost.

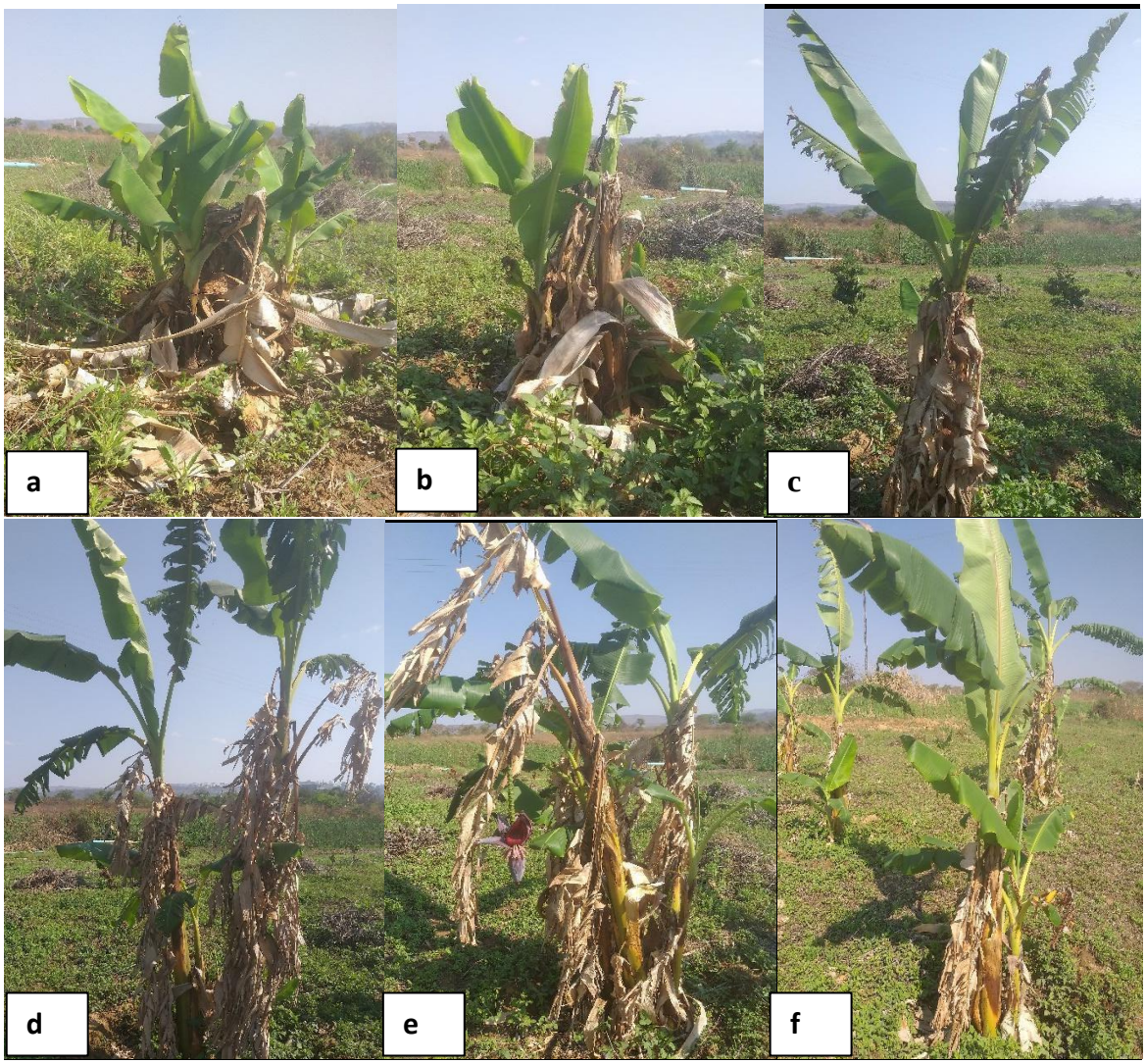

Figure 3. 3. Banana plants affected by the cold weather: (a) and (b) shows the dwarf Cavendish, (c) shows the Nzarayapera, (d) shows the giant Cavendish, (e) shows the apple banana and (f) shows Grand Naine.

The apple banana variety, an AB hybrid, had already cast its inflorescence before the winter period but due to the low temperatures, the fruits did not develop and the rachis dried together with the bract. This is because low temperature reduces the photosynthesis through photo-inhibition which reduces the development of banana plants (Joshi et al., 2023). In addition to photo-inhibition, low temperatures induce production of reactive oxygen species (ROS) such as singer oxygen ($^1O_2$), superoxide ($O_2\cdot^-$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($\cdot OH$). These species are critically toxic to plants as they lead to malfunctioning of tissues and ultimately death (Kumar & Yadav., 2009). Turner et al (2007), mentioned that when low temperatures occur prior to flowering, the emergence of the inflorescence has to overcome the pressure from the leaves. Low temperatures are one of the major environmental stresses in plants which influences growth, development, yield quality, post-harvest -shelf life as well as

the geographic distribution. Studies have shown that Musa exhibit high genetic variability for cold tolerance with the AAA group being more sensitive than the plantain (Yang et al, 2012). Reports have been made that temperatures below 12 ℃ inflict irreversible damage to the Cavendish group but the B genome cultivars can tolerate up to 0 ℃ (Lü et al., 2000). Even though *Musa spp*. appear to lack a cold acclimation mechanism, however plantain constitutively overexpress the *Arabidopsis* transcription factor Dehydration Responsive Element Binding Factor (DREB) or C-repeating binding factor (CBF1) thereby becoming highly tolerant to the cold temperatures by increasing *Superoxide Dismutases* (SOD) activities, decreasing *Malondialdehyde* (MDA) content as well as the fluid leaking rate. The mechanisms that plantains use for low or cold temperature resistance are associated with increased redox potential characterized by adapted Reactive Oxygen Species (ROS) scavenging capability, reduced ROS production, decreased lipid peroxidation, and cell wall stabilization (Sivirihauma et al, 2014). The expression of genes under cold stress depends on the species, the temperature and the length of exposure to low temperature. A family of transcription factors CBF 1-3 or DREB 1B, 1C and 1A which belong to the APA2 family of DNA binding protein, bind to the cold and dehydration responsive DNA regulatory element (DRE). Over-expression of the DREB1B/CBF1 therefore results in the expression of downstream *cld*-inducible genes increasing freezing tolerance.

Table 3. 1.Banana germplasm response to Chinhoyi cold temperatures from *May to July over a period of 24 months*

Accession number	Local name	Genome	Assigned score
CH-CF-102201	Williams	AAA	3
HV-CF-102206	Giant Cavendish	AAA	3
CH-CF-102211	Dwarf Cavendish	AAA	4
CHN-CF-102216	Fig Banana	ABB	3
CHP-CF-102221	*Nzarayapera*	BB	2
QNY-CF-102226	Grand Naine	AAA	3
CHP-102231	*Nzarayapera*	AB hybrid	2

Scoring scale key: 1- no leaves affected; 2- yellow leaves; 3-brown leaves falling off; brown leaves, destroyed stem

3.5.4. Harvesting

Fruit harvesting started in April 2022, 24 months after gene bank establishment and Figure 3.4 shows some of the harvested bananas. The Fig, apple banana and *Nzarayapera* (all containing the B genome), were the first to ripen whilst the dwarf Cavendish ripened last. The Fig banana bunches produced not more than 20 fingers whilst the rest produced up to 250 fingers. Harvested bunches were ferried from the gene bank to the Chinhoyi University of Technology in a truck. During transportation, bunches were cushioned to allow minimum bruising. Bunches were stored in a dark room in the Biotechnology laboratory until they ripened. The

determination of the ripening stage is usually observed by the bunch-filling degree, fruit angularity, and pulp to peel ratio as well as the size of the fruit.

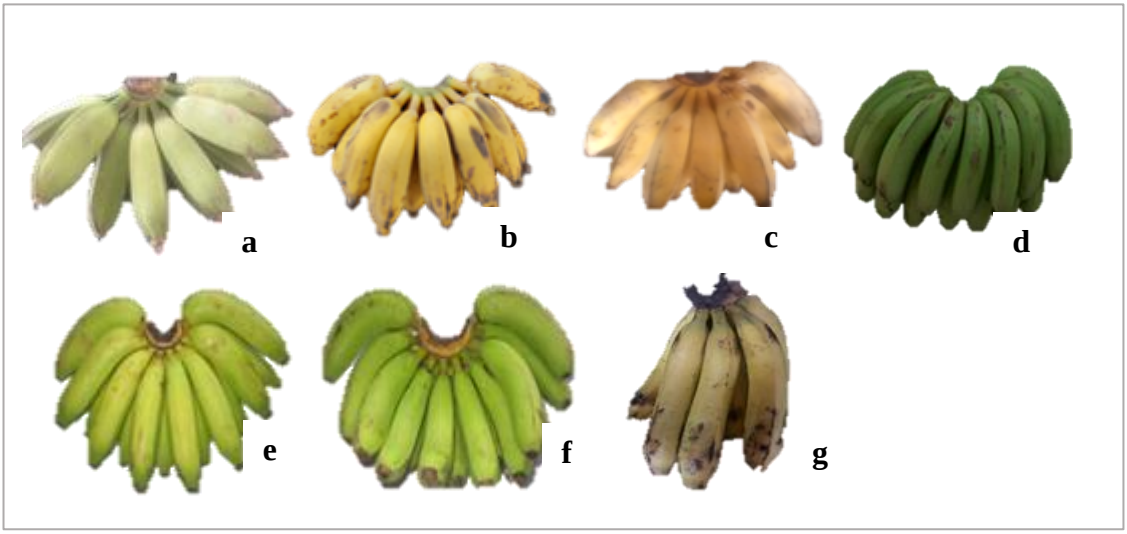

Figure 3. 4. Harvested bananas at different ripening stages: (a) fig banana. (b) Apple banana, (c) Nzarayapera (d) Grand naine (e) dwarf Cavendish (f) Williams (g) Giant Cavendish.

3.6. Conclusion and Recommendation

Banana germplasm was collected from different parts of the country and a *Musa ex-situ* gene bank was successfully established. This paved the way for the conservation of banana genetic material in Zimbabwe. Efforts in conservation of genetic material require an integrated approach of *in situ* and *ex situ* methods such that diversity is understood at species, genus as well as ecosystem level. For detailed information and data, the collections can be created in different regions of the country. In this manner, accurate and informed assessments of genetic lines become enhanced due to environmental variation. This then means that the gene pool can be sampled extensively under the widest range of environmental conditions possible allowing for the development of new breeds tailored specifically to those particular environmental conditions.

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CHAPTER 4

4.1. Genetic and morphological characterization of the established gene bank

4.2. Abstract

Banana production is increasingly under threat due to harsh weather conditions as a result of climate change and different diseases. As such there is a need for the preservation and the characterization of the banana cultivar population for the purposes of crop improvement. The identification of collected banana germplasm in Zimbabwe was conducted based on Random Amplified Polymorphic DNA (RAPD), the Inter-transcribed spacer region as well as morphology. The study was conducted with the aim of distinguishing one cultivar from another towards genetic conservation as well as banana improvement. ITS 1 and ITS 4 region targeting primers were used to amplify the DNA from twelve cultivars as well as sequence. Blast results identified five *Musa* groups which are *Musa balbisiana (BB), Musa ABB, Musa AB hybrid, Musa acuminata (AAA) and Musa acuminata subsp. Malaccensis (AA).* Phylogenetic analysis was done on the sequences under study and a Maximum Likelihood tree was generated to determine relationships between the sequences. RAPD evaluation produced a total of 80 amplification products and 62.5% of the DNA fragments were polymorphic whilst 37.5% were monomorphic. Primer fragments ranged from 250 bp to 1000 bp and the results were subject to cluster analysis which was performed using the unweighted pair group mean average. Further identification was done using the inflorescence, bract, male bud and fruit characteristics of each cultivar complementing the molecular evaluation. Genetic and morphological identification of locally grown bananas was therefore successful. An important step towards identifying pure lines suitable for breeding

Keywords: Banana, RAPDs, Target sequencing, genetic diversity, Inter transcribed spacer

4.3. Introduction

Bananas, as described by Campos et al., (2017), are herbaceous plants that can confer the aspect of a tree. The plant has a pseudo-stem which is formed by the concentric assembly of leaf sheaths, crowned by a rosette of large oblong to elliptic leaves. The leaves of a banana plant are produced successively until the inflorescence is cast (Aeberli et al., 2023). Being a perennial crop, banana, like all the other perennial crops, offers food security to most developing countries in the world (Ghag et al, 2017). Globally, banana (*Musa Spp)* is ranked fourth in terms of gross value production following rice, wheat, and maize (Chitamba et al., 2016). Its production in Zimbabwe is ranked 57 amongst 132 producing countries. Banana production in Zimbabwe is constituted by both the commercial varieties and the landrace varieties. Genetic characterization in Zimbabwe has mostly targeted crops such as maize, (Semagn et al., 2012), sunflowers, (Masvodza et al., 2015), sorghum (Cuevas et al, 2014), sugar-cane (Mugehu et al., 2020), tobacco (Mwadzingeni et al., 2013) and wheat (Mutari et al., 2022) amongst other crops. Documented characterization in bananas has been carried out on plant parasitic nematodes from Rusitu Valley cultivars but to the researchers' knowledge, no information has been made available on banana genetics in Zimbabwe (Chitamba, 2016).

Regardless of the social, cultural and economic significance of bananas, little is known about their organization and evolution. The *Musaceae* family, which belongs to the order *Zingiberales,* consists of three *genera Musa*, *Ensete* and *Musella*. The traditional morpho-taxonomic classification of bananas is based on a set of morphological descriptors and basic chromosome number. This conventional taxonomy has been often questioned and the phylogenetic relationships within the family *Musaceae* remains subject to debate. Different types of molecular markers have been used until now to investigate the diversity of bananas at

various taxonomic levels but the taxonomy and phylogenetic relationships still remain unresolved.

Molecular markers have been used extensively for banana characterization which include random amplified polymorphic DNA markers (RAPD's) with over a thousand research publications, (Mukunthakumar et al., 2013), restriction fragmentation length polymorphism markers (RFLPs), (Devarumath et al., 2002), simple sequence repeats (SSR), ((Karamura et al., 2016), fragment length polymorphism markers (AFLPs), (El-khishin et al., 2009), and diversity array technology markers (DArTs), ((Beata & Andrzej, 2011) each method having its own pros and cons. Genetic analysis using ribosomal DNA (rDNA) has often been used to analyze genetic variability and relationships amongst banana cultivars. The rDNA is the coding region of the RNA component of the ribosome which constitutes the 18S sub-unit, the 5.8S and the large subunit which is the 26S. According to Baldwin et al., (1995), an internal transcribed spacer (ITS) separates these units. The use of the ITS region in investigating phylogenetic studies has become widespread and this is mainly because the region is highly variable due to frequently occurring nucleotide polymorphisms or insertions/deletions in the sequence and is phylogenetic inference at the specific and generic levels (Feliner and Rosello, 2007) . It also has a bi-parental inheritance compared to the uniparental inheritance of chloroplast and mitochondrial markers and the intra-genomic sequence uniformity caused by active homogenization of repeat units, also known as concerted evolution. Owing to these and other varied properties, the ITS region became one of the most widely used sequences for phylogenetic inferences. ITS 1 and ITS 4 primers have been used in the identification of Truffles (Bertini et al, 1999), (Martin & Rygiewicz, 2005), *Trichoderma* , (Iqbal et al, 2022) amongst others.

The objective of this study was to characterize and assess the genetic relationship amongst collected accessions grown in Zimbabwe targeting the ITS regions of the accession sequences

as well as morphological characterization based on selected descriptors of the accessions with a working hypothesis that states that there is no variability in the collected accessions.

4.4. Materials and Methods

4.4.1. Morphological Characterisation

Morphological Characterization of the *Musa* cultivars was done focusing on the inflorescence, bract, male flower and the fruit according to 'Descriptors for Banana (*Musa* spp.)' IPGRI-INIBAP/CIRAD (1996). Qualitative criteria were used and morphological character traits at fruiting stage were matched against the IPB-INIBAP Banana catalogue as well as the Musa Germplasm Information System (MGIS) database.

4.4.2. Molecular Characterization

4.4.2.1. DNA extraction

Three months old leaves of banana plantlets were collected from the Chinhoyi University Farm gene bank and DNA was isolated using the modified CTAB method from Siddique et al., (2014). The CTAB buffer was pre-heated at 60 ℃ in a water bath before being added to 1 g of fresh leaf sample in a preheated mortar. The sample was ground and incubated at 60 ℃ for 30 min with occasional swirling. Sample was then cooled for 5 min and centrifuged for 1 min at 6000 g. Using equal volumes, chloroform-isoamyl alcohol (Skylabs, South Africa) extraction (in the ratio 24:1, v/v) was performed once and centrifuged for 5 min at 6000 g. The aqueous phase was transferred to a new tube and chloroform-isoamyl extraction was repeated until the upper phase was clear. To the aqueous phase, Proteinase K (New England *BioLabs*, U.K) was added and the mixture was incubated at 37 ℃ for 30 min. Sample was placed on an ice-bath

and ice cold iso-propanol alcohol (Glassworld, South Africa) was slowly added down the side of the tube until white stringy DNA precipitated. The DNA containing phase was transferred to a new tube and centrifuged at 10 000 g for 5 min until a pellet was recovered. The liquid phase was decanted and pellets were washed in 70 % ethanol and dried. The pellet was dissolved in the TE buffer and DNA was ready for amplification. The genomic DNA concentration was analysed using the BioDrop µLite 1380 machine. The µLite 0.5 mm path length was used with a dilution factor of 1.

4.4.2.2. DNA Amplification

Amplification of the genomic DNA was carried out using ITS 1 and ITS 4 primer sequences (synthesised at Inqaba Biotech, South Africa). ITS 1 5' (TCCGTAGGTGAACCTGCGG) and 3' (GGCGTCCAAGTGGATGCCT) and ITS 4 3' (TCCTCCGCTTATTGATATGC) and 5' (CGTATAGTTATTCGCCTCCT).

PCR was carried out in 25 µL reaction volumes containing One Taq DNA polymerase 2X Master Mix with standard buffer (12.5 µL), 10 µM, forward primer (0.5 µL), 10 µM reverse primer (0.5 µL) and nuclease free water (9.5 µL) (New England *BioLabs*, UK). Initial denaturation and the activation of enzyme step of 10 minutes at 95℃ was followed by a 35cycle amplification at the following conditions: 30 seconds at 95 ℃, 40 seconds at 60 ℃, 40 seconds at 72 ℃. A final 5-minute extension at 72 ℃ completed the protocol and was held at 4 ℃. The PCR products were separated on agarose gel (1 %) in 0.5 X TBE buffer and stained with ethidium bromide for band visualization (Oxford Laboratory Reagents, India).

RAPD amplification reactions were carried out using 10 random primers. The 25 µL volume reactions were carried out using the following conditions: initial denaturation for 1 min at 95 ℃ was followed by 45 cycles at the following conditions; 1 min at 94 ℃, 1 min at 35 ℃ and 2 min at 72 ℃. A final 7 min extension at 72 ℃ completed the protocol. The amplified

fragments were separated The PCR products were separated on agarose gel (1 %) in 0.5 X TBE buffer and stained with ethidium bromide for band visualization

4.4.2.3. RAPD evaluation

The bands that were distinct were used for the genetic divergence analysis. Using Multivariate Statistical Package v.3.22 and Nei Li index, a distance matrix and a dendrogram was constructed using Unweighted-Pair Group Means Average (UPGMA) cluster analysis.

4.4.3. Sequencing

The ITS 1 and ITS 4 amplicons were purified and then sent for Sanger sequencing at Inqaba Biotec, South Africa. The ITS 1 and the ITS 4 regions of the DNA were sequenced.

4.4.3.1. Sequence alignment

The 24 obtained sequences were received as Chromatogram files hence they were trimmed in Chromas v2.6.6 before being exported as fasta files.

4.4.3.2. Phylogenetic analysis of Musa sequences

Phylogenetic analyses were conducted using MEGA version 11 (Tamura K, Stecher G, and Kumar S 2021). Additional ITS sequences were downloaded from NCBI for comparison with the sample sequences. Clustal W was used for sequence alignment and evolutionary analysis of the *Musa* species was analysed using the Maximum Likelihood method and Tamura-Nei model. Bootstrap consensus tree for the Maximum Likelihood method was obtained from 1000 replicates. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with superior log likelihood value. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 11

(Tamura K, Stecher G, and Kumar S 2021). The phylogenetic trees were rooted using an outgroup species *Ensete ventricosum*.

4.4.3.3. Sequence Blasting

BLAST program was inferred to identify similar sequences. The NCBI website https://blast.ncbi.nlm.nih.gov/Blast.cgi was accessed to compare obtained protein sequences to sequence databases as well as calculate the statistical significance. On the web page, blastn was inferred. BLASTN search program searches for nucleotide databases using nucleotide queries. FASTA sequence files were uploaded and a descriptive title was added to each uploaded sequence. The nucleotide collection nr/nt database was used. The Megablast BLAST algorithm was used to optimize for highly similar sequences. The sequences were then blasted and the results were displayed in another window.

4.5. Results

4.5.1. Morphological Characterization

Figure 4. 1.The inflorescence of banana cultivars where (a) shows Williams, (b) sweet fig, (c) Nzarayapera, (d) Grand Naine, (e) apple banana, (f) dwarf Cavendish, (g) giant Cavendish

Morphological characterization of the cultivars was done focusing on the inflorescence as shown in Figure 4.1. The bract, male flower and the fruit were also analysed as indicated in Table 4.1. The descriptors used provided a distinct variation in the cultivars.

Table 4. 1.Banana plant descriptors used for morphological identification

1- Fig, 2- Apple banana, 3- Nzarayapera, 4- Williams, 5- Giant Cavendish, 6- Dwarf Cavendish, 7- Grand Naine.

descriptor	**Musa ABB**	**Musa AB hybrid**	***Musa balbisiana***	***Musa acuminata***	***Musa acuminata malensis***

Bract					
Bract base shape	1-medium	2-medium	3- medium	4-medium 5-medium 6-medium	7-medium
Apex shape	intermediate	intermediate	intermediate	4-Slightly pointed 5-Slightly pointed 6-Slightly pointed	intermediate
Bract shape	intermediate	lanceolate	intermediate	4-Intermediate 5-intermediate 6-intermediate	intermediate
Bract lifting	Lifting 2/more	Lifting 2/more	Lifting 2/more	4-Lifting 2 /more 5-Lifting 2/more 6-Lifting 2/ more	Lifting 2/ more

Bract external colour	Purple-brown	Red-purple	Orange red	4-Pink-purple 5-Red purple 6-Deep purple	Purple
Behaviour before falling	revolute	revolute	revolute	4-Revolute 5-revolute 6-revolute	Revolute
waxiness	Very waxy	Very waxy	moderate	4-Very waxy 5-moderate 6-few wax	Very few wax
Male flower					
Dominant colour	Pink-purple	yellow	yellow	4-Yellow 5-yellow 6-yellow	Yellow
Free Tepal colour	Tinted pink	Tinted yellow	Tinted with yellow	4-Tinted yellow 5-Tinted yellow 6-tinted yellow	Tinted yellow
Free tepal appearance	corrugated	corrugated	corrugated	4-Corrugated 5-corrugated	Simple folding under the apex

				6-corrugated	
Free tepal apex shape	triangular	triangular	triangular	4-Oval 5-triangular 6-oval	Triangular
Inflorescence					
Male bud type	normal	normal	normal	4-Normal 5-normal 6-normal	Normal
Male bud shape	intermediate	intermediate	intermediate	4-Intermediate 5-intermediate 6-intermediate	intermediate
Bunch position	Vertical hang	slightly angled	Vertical hang	4-Vertical hang 5-Vertical hang 6-vertical hang	Vertical hang
Bunch shape	cylindrical	cylindrical	cylindrical	4-Cylindrical 5-cylindrical 6-cylindrical	cylindrical

Bunch appearance	Lax	compact	compact	4-Lax 5-Lax 6-Lax	Lax
Rachis position	Falling vertically	Falling vertically	curvy	4-Angular 5-angular 6-falling vertical	Curvy
Peduncle colour	Light green	green	Light green	4-Light green 5-green 6-green	Light green
Fruit					
shape	Straight in distal part	straight	straight	4-Slightly curvy 5-curved 6-curved	Curved
apex	Blunt tipped	Bottle-neck	pointed	4-Blunt tipped 5-Bottle-necked 6-blunt tipped	Blunt tipped

Pulp colour at maturity	White	yellow	yellow	4-Cream 5-cream 6-cream	Yellow
Days from planting to flowering	366	250	396	4-360 5-400 6-423	380
Days from flowering to harvest	135	100	145	4-120 5-118 6-135	137

4.5.2. DNA extraction, PCR amplification and DNA sequencing

The amplification and sequencing and identification of the *Musa* samples was successfully done with evidence from the Blast results. Twelve DNA samples were purified and sequenced using an ABI 3500XL Genetic Analyzer Sanger sequencing machine.

4.5.3. RAPD analysis

The genotypes were subjected to RAPD primer analysis. The results are highlighted in Figure 4.2 and 4.3. A total of 80 amplification products were generated and 62.5% of the DNA fragments were polymorphic whilst 37.5% were monomorphic.

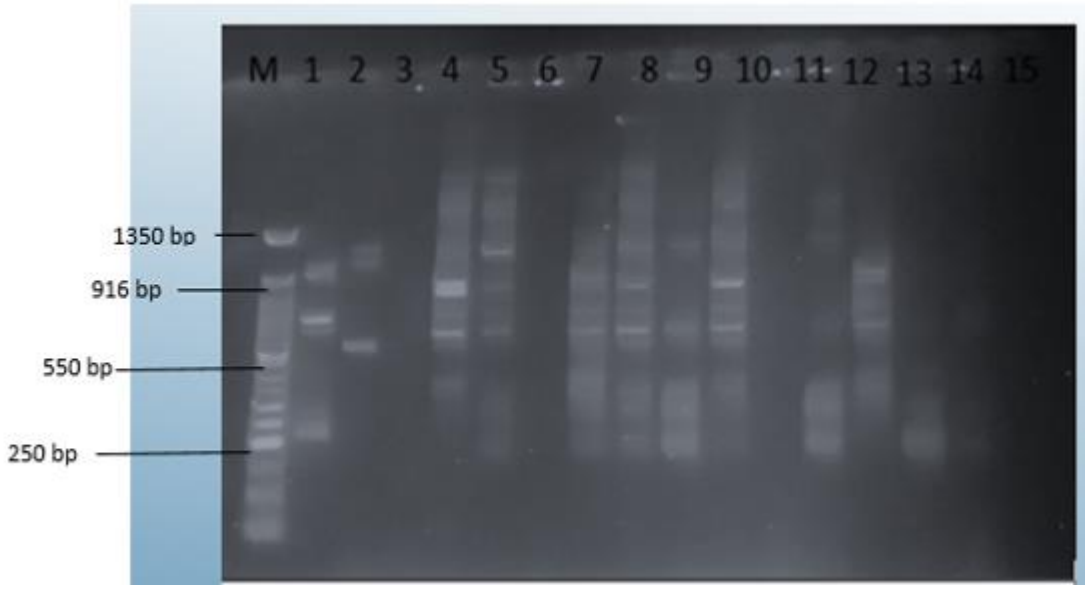

Figure 4. 2. OPAY 12 RAPD primer run

M being a 50 bp NEB DNA ladder, well 1-15 being the Musa samples

Figure 4. 3. UBC 788 RAPD primer run

M being a 50 bp NEB DNA ladder, well 1-15 being Musa samples

Primer fragments ranged from 250 bp to 1000 bp depending on the primer. A matrix of genetic similarities and genetic distances was created based on Nei Li's index. Genetic differences and high genetic similarities were observed

4.5.4. Phylogenetic and Blast Analysis

The phylogenetic tree for ITS 1 sequences resulted in four clades as shown in Figure 4.4. Clade 1 consisted of 7 sequences, 4 of them being downloaded NCBI sequences. Whilst blast inference identified the 3 sample sequences as Musa ABB Sabeh Biru cultivars. All sequences in this clade contains a B genome. The second clade consisted of 2 NCBI downloaded sequences of the *Musa acuminata* species. Clade 3 consisted of 8 sequences. 7 out of 8 of these sequences were blast identified as *Musa acuminata subsp malaccensis* with the 8th being *Musa acuminata var. nakai* isolate, an NCBI downloaded sequence. *Musa acuminata subsp malaccensis* sequence samples 12, 03, 09 and 07 showed more relationship distance from being *Musa acuminata var. nakai* isolate as compared to sample sequences 02, 08 and 10. Sample sequences 01 and 04 identified as Musa acuminata AAA group cultivar *Grito* from the blast search and therefore made up the fourth clade. *Ensente ventricosum*, which belong to the genera *Ensente* was the outgroup sequence.

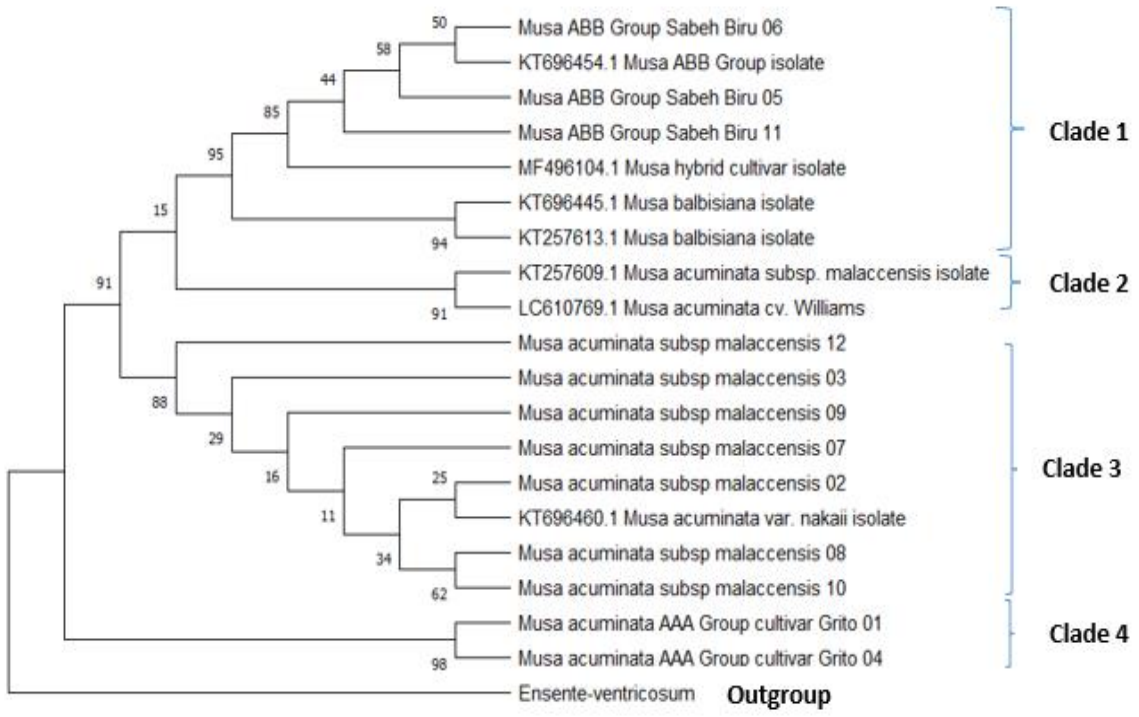

Figure 4. 4. Evolutionary analysis of ITS1 sequences by Maximum Likelihood method.

The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model [Tamura and Nei, 1993]. The tree with the highest log likelihood (-10841.64) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with superior log likelihood value. This analysis involved 20 nucleotide sequences. There was a total of 6810 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 [Tamura et al., 2021]

The phylogenetic tree for ITS 4 sequences resulted in four clades as shown in Figure 4.5. Clade 1 consisted of 9 sequences, 6 of them being blast inference *Musa acuminata subsp malancessis* species and the other three being blast inferred *Musa balbisiana* sequences. Clade 2 consisted of all NCBI downloaded sequences. The third clade comprise of only 1 sequence, a blast identified sample sequence, *Musa acuminata subsp malaccensis*. Clade 4 consisted of 2 blast inferred Musa hybrid cultivars.

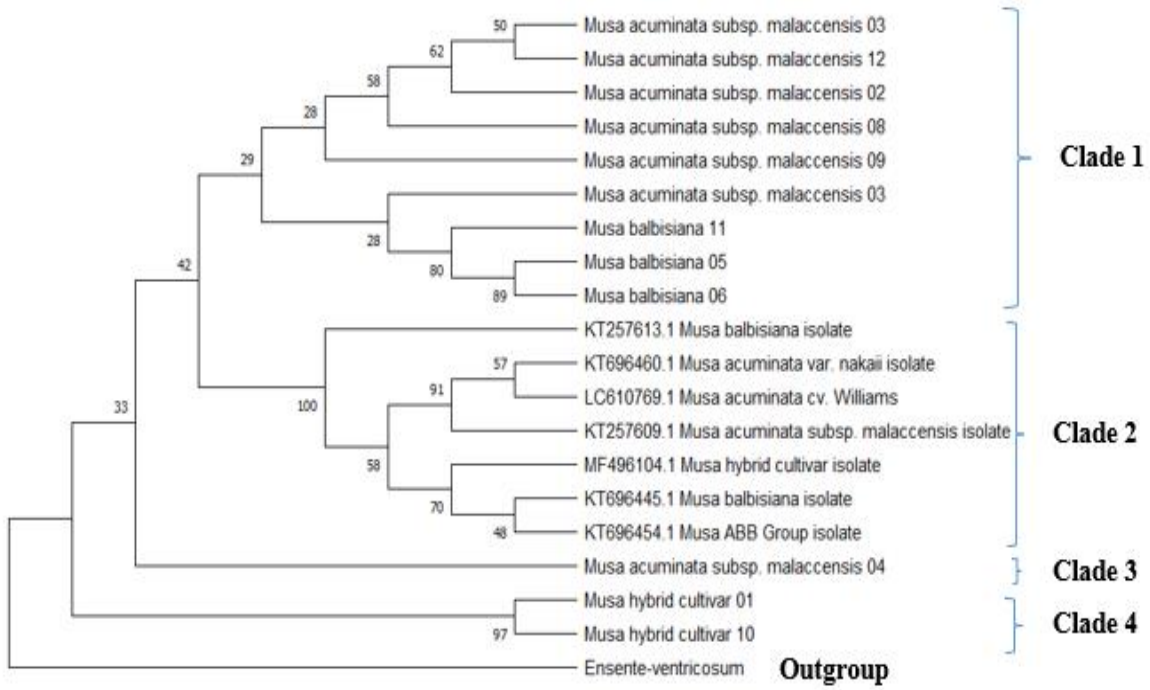

Figure 4. 5.Evolutionary analysis of ITS4 sequences by Maximum Likelihood method

The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model [Tamura and Nei, 1993]. The tree with the highest log likelihood (-14146.13) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with superior log likelihood value. This analysis involved 20 nucleotide sequences. There were a total of 6843 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 [Tamura et al., 2021].

4.5.5. Efficiency of ITS1 and ITS 4 primers for genetic identification

The average GC contents for ITS 1 and ITS 4 was shown to be 63.06% and 61.60% respectively https://jamiemcgowan.ie/bioinf/gc.html. ITS4 showed a range of 84.39%-99.68% blast identification rate for the 12 *Musa* samples whilst ITS 1 showed an almost similar range of 84.32-99.84 %. The length of the ITS 1 varied from 622 to 630 whilst those of ITS 4 ranged from 630 to 958 bp. Higher ambiguity percentage in identification using ITS 1 was noted in sample sequences 01, 04, (which were identified as *Musa acuminata*) as well as in samples 01, 04, 05 and 10 (*Musa* hybrid cultivar, *Musa acuminata* subsp. *Malaccensis,Musa balbisiana and Musa* AB hybrid respectively) in ITS 4 sequences. Sequences were identified to cultivar level hence morphological identification was employed to name the local bananas.

4.5.6. Dendrogram Analysis

The dendrogram (Figure 4.6) showed three groups that have the almost same genetic distances. *Musa acuminata malaccensis* 03, 09 and 12; *Musa Balbisiana* and *Musa* hybrid cultivar group align at the same Euclidean mark. *Musa acuminata* 02 and 07 demonstrate the longest distances.

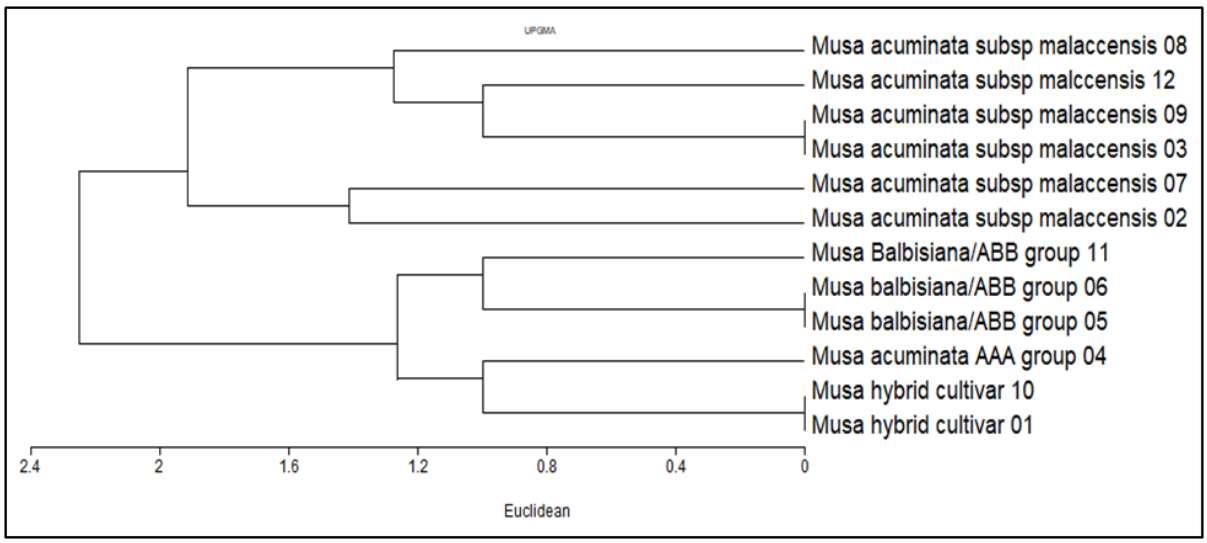

Figure 4. 6.Genetic distances showing relatedness of Musa cultivars

4.6.5. Discussion

4.6.5.1. Morphological analysis of identified cultivars

Characterization of the germplasm using morphological traits resulted in genomic groups being identified with international as well as local names. *Musa* ABB identified cultivar as a Fig banana type also known as the quince, *Sapa*, *Tanga* or *Couruda* banana depending on the country. It is a hybrid of *Musa acuminata* and *balbisiana* and originates from the Philippines. The tree is a medium sized plant that produces 20-35 plum straight, sweet fruits with cracks on their peels.

The *Musa* AB identified cultivar was identified as the apple banana which is locally called '*Nzarayapera*' which translates to 'no-more hunger'. This is because the fruit is starchy and filling. Literature states that the variety originated from Ecuador and is referred to as the

Manzano banana characterized by a sweet apple aroma hence its English name (Minneopa Orchards, 2022).

Musa Balbisiana cultivar was identified also as *Nzarayapera*.it is a starchy plantain that has tiny seeds at its centre. The cultivar takes time to flower unlike the *Musa AB* hybrid.

Musa acuminata identified cultivars were the Williams, Giant Cavendish, Dwarf Cavendish and the Grand Naine. These cultivars are mostly grown for commercial purposes unlike the first three. The bract external colour, male flower, and fruit apex give differentiation of one Cavendish from the other. Bracts, male bud shape and mature fruit colour allows the differentiation between ploidies and banana subgroups (Vilhena et al., 2019),

4.6.5.2. Genetic evaluation analysis

Molecular identification has a higher ability of differentiating one cultivar from the other. The use of ITS in genetic diversity and phylogenetic studies showed high levels of interspecific divergence and have been used frequently. The study utilised maximum likelihood models to infer phylogeny based on the ITS1 and ITS4 regions. The sequences all clustered into 4 clades for both the ITS1 based tree (*Fig 1*) and the ITS4 based tree (*Fig 2*). In the present study, ITS1-5.8S –ITS2 region was found to have higher discriminatory ability in comparison with the ITS 4 region as it managed to accurately separate different genome types that possessed a close genetic resemblance as supported from researches by Bartoš et al, (2005) and Hřibová et al., (2011). It can also be shown that the genetic resemblance is also shown in the phenotype of the banana isolates.

Based on the BLAST results, the Fig banana of the ABB genome showed 99.6 % similarity to the Sabeh Biruh cultivar. The apple banana is identified with two genomes using both ITS1 and ITS 4 sequences. It showed 88.6 % AB hybrid genome whilst a 99 % *Musa acuminata subsp. malaccensis* identical. *Musa balbisiana* commonly known as *Nzarayapera* showed a 99 % similarity. Williams, giant Cavendish, dwarf Cavendish of the AAA genome showed a higher similarity to the *Musa acuminata* Grito cultivar. Cultivar Grand naine showed a 99% identity to *Musa acuminata sub malaccensis*.

The distance- based identification system revealed that the *Musa balbisiana (Nzarayapera)* and the hybrid cultivars have similar Euclidean distance whilst *Musa acuminata* grand naine demonstrated the longest distance.

The results of the study shows that the phylogeny of the *Musa* family is controversial but the discriminatory power of the ITS sequences gives conclusive evidence of distinct cultivars. However, there is need for further identification and possibly barcoding of the cultivars.

4.7. Conclusion

This study showed that molecular characterization of banana cultivars in Zimbabwe is essential. The study revealed that there is indeed variation amongst the banana cultivars being grown in Zimbabwe. Genetic relatedness of the cultivars was unravelled and unique and rare cultivars such as the Fig banana were identified. The *Nzarayapera* was correctly identified according to its genetics tackling one major challenge of cultivar synonyms that is faced in banana farming. . Morphological analysis gave an insight into the important stages of each of the cultivars which is useful to farmers as well as breeders The study also showed that the ITS sequencing coupled with morphological traits are a useful tool in characterization of bananas.

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Chapter 5

5.1. *In-vitro* propagation: Growth characterization of conserved *Musa* germplasm from Zimbabwe

5.2. Abstract

The use of tissue culture in the propagation of bananas and plantains has proved to be of paramount importance due to faster propagation as well as virus elimination. In this study, three months old suckers belonging to the seven identified cultivars (Grand Naine, Giant Cavendish, Dwarf Cavendish, Williams, Fig banana, Apple banana and '*Nzarayapera*') were investigated for their growth performance *in vitro*. Suckers were washed and excised such that the meristematic tissue was cultured. The tissue was cultured on Murashige and Skoog media supplemented with plant growth regulators. A variation in the proliferation and growth of the cultures was observed amongst the genomic groups as well as among cultivars of the same group. The genomic group AAA produced shoots at the fastest rate whilst the BB genome, *Nzarayapera* cultivar, had the slowest proliferation. This therefore showed that the A triploid bananas have the ability to multiply at a faster rate as compared to their counterparts and therefore can be mass produced more efficiently.

Key words: banana, meristematic culture, micro propagation

5.3. Introduction

The banana fruit is parthenocarpic therefore it is propagated by vegetative methods through suckers. This method is relatively slow for large scale production and it also allows the transfer of virus and diseases from one generation to the other hence plant tissue culture offers an alternative rapid and disease-free route, the production of banana plantlets *in-vitro*. Micro-propagation through shoot tip culture has been done by a large number of researchers using different media and varieties of banana. Research has shown that plants can exhibit great variation under in vitro conditions with regards to shoot establishment, proliferation, and regeneration of roots (Babu, 2019). This is due to different factors such as the culture environment, genotype, hormone ratios, explant type and the culture media composition (Dagnew et al., 2012).

Tissue culture has been defined as the propagation of group cells, single cells as well as a specific part of a plant under controlled aseptic conditions. Tissue culture has over the years laid a foundation of uniform, high quality, true to type disease free planting material at a large scale (Suman, 2017). The steps that are involved in banana tissue culture include the selection of a superior mother plant, culture initiation, multiplication, rooting, hardening and dispatch. Tissue culture propagation does not exclude virus infection unless the mother plant as well as the sub-cultured plantlets are tested and maintained virus free. The process of testing and maintaining the plantlets virus-free is therefore an essential step in the production of tissue cultured material.

Virus indexing plants for the presence or absence of transmissible virus is important in the mass production of bananas. The status of the donor plant as well as the resultant plantlets health-wise is an important factor in the success of tissue culture (Selvarajan et al, 2009). The major viruses that naturally infect bananas include Banana Bunchy Top Virus (BBTV), Banana Streak Virus (BSV), Banana Bract Mosaic Virus (BBrMV) and Cucumber Mosaic Virus (CMV). These viruses propagate easily through clones therefore sensitive and very accurate detection is critical in ensuring virus free planting material (Viswanath, 2021). Research has shown that standard serological and molecular based tests have a highly positive impact in tissue culture production

Genetic uniformity with regards to homogeneity in planting material is important in banana farming. Genetic variability is mainly induced by somaclonal variation which has an effect on the yield. If they go unnoticed, these variants can therefore be mass-produced hence PCR-based analysis needs to be carried out to test for genetic fidelity (Bhalang et al., 2018).

In this study, tissue culture was used as a tool to assess how the bio-banked germplasm proliferated under a controlled environment taking note of whether there is a difference in their performance.

5.4. Materials and Methods

5.4.1. Plant Material

Seven banana varieties from *Musa* genomic groups obtained from the Chinhoyi University *ex-situ* gene bank were used in this study. The banana varieties which are namely, the Dwarf Cavendish, Giant Cavendish, Williams, fig banana, Grand Naine, Apple banana, and the

Nzarayapera were used as experimental material each being replicated thrice. Explants were obtained from at most three months old healthy-looking sword suckers. The study was conducted in the Biotechnology Laboratory at Chinhoyi University of Technology, Zimbabwe.

5.4.2. Explant preparation and surface sterilization

Sword suckers were excised by a knife and washed thoroughly under running water for 20 minutes. The explants were excised to a length of 2 cm and immersed in 70 % ethanol for 2 minutes. The explants were then immersed in 5 % sodium hypochlorite for 10 min and then rinsed thrice with sterile distilled water. Excess water was allowed to drip onto a sterile multi-wipe before the final excision and removal of young leaves around the meristem.

5.4.3. Media preparation and culturing

The culture media used was a modified Murashige and Skoog basal medium (MS) containing 25 g/l sucrose, 0.70 g/l activated charcoal, and 12 g/l plant agar. The medium was autoclaved at 1.2 KPa and 121^0C for 15 minutes after pH adjustment to 5.8. Prior to sterilization, plant growth regulators were added. The autoclaved media was allowed to cool at room temperature before meristem culture.

The surface-sterilized explants were placed on the MS medium which contained 6-benzylaminopurine (BAP) 1 mg/l, Indole-3-butyric acid (IBA) 1.5 mg/l, and naphthalene acetic acid (NAA) 2 mg/l for shoot initiation. The cultures were placed in a growth chamber in a complete randomized design with three replicates and were incubated for 8 weeks aseptically at 25 ℃ under a cool white fluorescent light and the rate of leaf proliferation was observed.

5.4.4. Initiation and sub-culturing

Observations were made after every two days noting the browning rate of cultures as well as the contamination /survival percentage. The length of the proliferating shoots was recorded at weekly intervals. Sub-culturing of cultures was also done at one-week intervals. The percentage browning rate of the culture was calculated as follows:

Percentage browning (%) = B_1/A_T *100…… (1) where B_1 is the number of browning tissue and A_T is the total number of cultured tissues.

5.4.5. Plantlet acclimatization and hardening

After a period of 8 weeks, surviving plantlets with 3 to 4 leaves and developed roots were taken out of the bottles and washed thoroughly with running water, removing the agar. In a completely randomized design, three plant replicates from each genomic group were potted in autoclaved soil and kept inside the lab for a week before exposure to the outdoors.

5.5. Data Analysis

Statistical analysis was done using SPSS v 28.1.1.0 where data was subjected to one way ANOVA and means were separated using Fisher's Least Significant Difference (LSD).

5.6. Results and discussion

5.6.1. Shoot development

The plants-initiated growth at different rates. Shoots developed from all the surviving inoculated meristems within 8 weeks. As indicated in Table 5.1, there was no significant difference in the height of the meristematic tissue of all genomic groups at 95% confidence interval in week 1. From week 1 to week 2, the meristematic tissues showed an increase in shoot length. *Musa balbisiana* and *Musa* ABB genomic groups showed a significant increase whilst the increase in *Musa* AB hybrid and *Musa* AAA were not significant at $p>0.05$. As a result, the coefficient of variation between *Musa* AB hybrid and *Musa* AAA was the highest (recorded at 15.99 %). From week 2 to week 8, the shoots heights increased significantly and their difference in growth rate was also significant at $p>0.05$ for each of the genomic groups. *Musa* AAA recorded the highest shoot length at 3.9 cm whilst *Musa* BB recorded the least growth.

Table 5. 1. Length of shoots for the Musa genomic groups (cm)

Week	AAA	ABB	AB Hybrid	BB	LSD	CV	P value
1	0.1±8.01E-18[a]	0±0.00[a]	0±0.00[a]	0±0.00[a]	0.00	0.00	P<0.05
2	0.7±0.049[c]	0.5±0.024[b]	0.7±0.049[c]	0.3±0.036[a]	0.163	15.988	P<0.05
3	2.1±0.036[d]	1.2±0.00b[b]	1.5±0.014[c]	0.5±0.024[a]	0.090	3.636	P<0.05
4	2.5±0.014[d]	1.9±0.054[b]	1.7±0.000[c]	0.7±0.036[a]	0.133	4.180	P<0.05
5	2.9±0.014[d]	2.4±0.024[b]	2.2±0.014[c]	1.9±0.014[a]	0.067	1.50	P<0.05
6	3.1±0.054[d]	2.7±0.014[b]	2.4±0.014[c]	2.1±0.000[a]	0.116	2.382	P<0.05
7	3.4±0.000[d]	2.8±2.56E-16[b]	2.5±0.027[c]	2.3±0.014[a]	0.061	1.167	P<0.05
8	3.9±0.027[d]	3.4±0.027[b]	2.9±0.000[c]	2.5±0.014[a]	0.082	1.366	P<0.05

LSD= least square difference, CV= Coefficient of variation. Values with different letters indicate significant differences at (p>0.05)

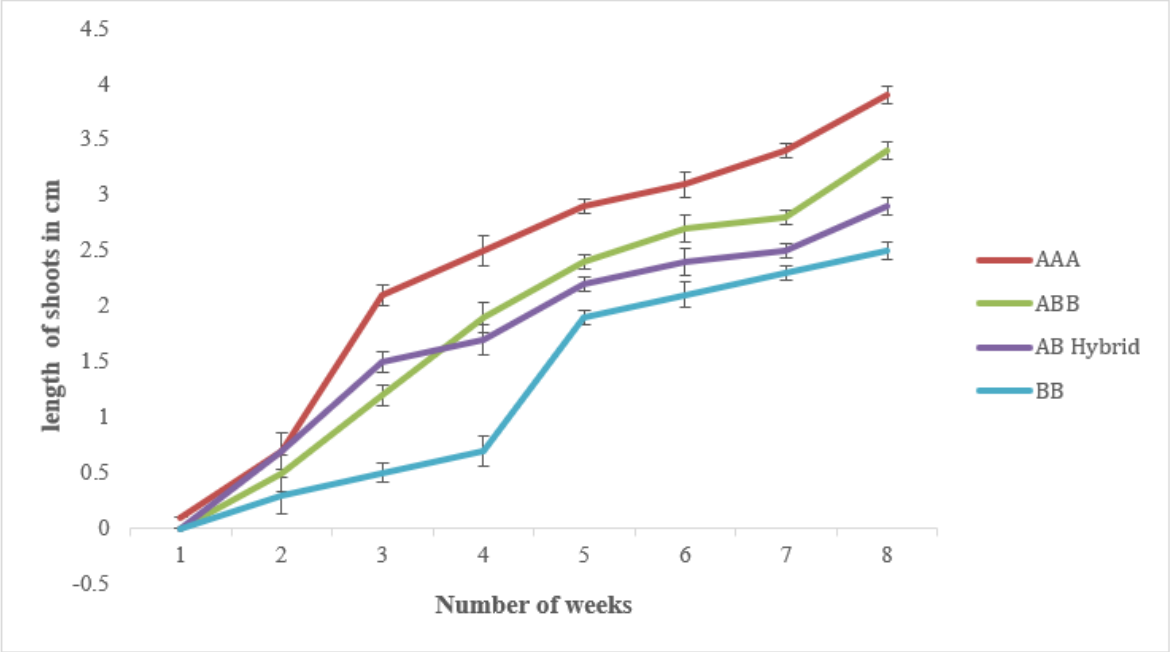

Figure 5. 1. Shoot development of Musa genomic groups

Musa ABB and BB genome were late to proliferate due to browning of the meristematic tissues. The browning effect of the B genome has shown to have an effect on the shoot proliferation. As indicated in Table 5.2, the B genome containing cultivars were all brown by the third culturing week

Table 5. 2. Percentage browning of banana meristematic tissue over a period of 8 weeks

Week	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
AAA	0	0	16.67	16.67	25	25	25	25
ABB	16.67	16.67	25	25	25	25	25	25
AB Hybrid	0	8.33	25	25	25	25	25	25
BB	25	25	25	25	25	25	25	25
Percentage browning (%)	41.67	50	91.67	91.67	100	100	100	100

Studies have shown that the B genome banana enhances extensive oxidation of phenolic compounds as well as tissue death (Hirimburegama et al., 1997). Babu, (2019), noted that when B genome explants were treated with 50 mg/l citric acid and ascorbic acid for a period of 30 minutes, no browning was observed. The inhibition of the browning was attributed to ascorbic acid's ability to scavenge oxygen radicals that are produced during plant cell injury. Similarly, according to Irshad et al. (2018), supplementation of 15 mg/L ascorbic acid to basal media minimizes the phenolic secretion. Similar effects of ascorbic acid in tissue culture medium were reported by Ko et al., (2009) during the micro propagation of Cavendish banana. Ascorbic acid has been shown to inhibit the browning of cultured tissues and improve morphogenesis in Cavendish banana (Ko et al., 2009)

5.6.2. Tissue browning

Tissue browning was observed in where the cultured meristem changed colour from creamy white to brown due to phenolic content as shown in Figure 5.2 As a result, the cultured meristematic tip was transferred to fresh media after a week.

It was observed that cultivars with a B genome showed more browning tissue as compared to the A genome. Contamination was also observed in the media and the media was changed as soon as contamination was noticed. The contamination as well as the browning of tissue explant is considered as a major challenge in tissue culture. The discolouration or browning of the explant tissues during the first culture is the main challenge because it is genome related. Contamination has over the years been controlled through proper explant handling and different surface sterilisation techniques.

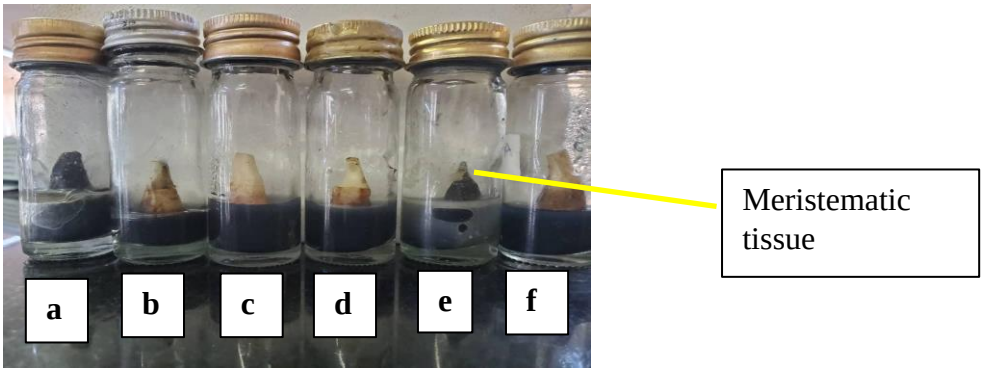

Figure 5. 2.Browning meristematic cultures of cultivars from different genomic groups from (a) Musa BB, (b) Musa AB hybrid, (c) Musa AAA, (d) Musa AAA, (e) Musa ABB and (f) Musa AB hybrid.

Explant browning observed in cut surfaces has shown different intensity. As indicated in Table 5.3, the intensity of browning differs from one cultivar to another (Bhalang et al., 2018).

Table 5. 3.Colour scale showing the browning intensity of each of the genomic groups

Week	AAA	ABB	AB Hybrid	BB
1	0	1	0	1
2	0	2	0	2
3	1	2	3	2
4	1	2	3	3
5	1	3	3	3
6	2	3	3	4
7	2	3	3	4
8	2	3	3	4

Scoring key:0-cream, 1-creamy brown, 2-brown, 3-deep brown, 4-deep brown/black

The BB genome has been found to produce a deeper and more pronounced black colour as compared to other genomic groups. This is in agreement with the reports that *Musa balbisiana* contributes more to browning and death of tissues (Kariyana et al., 2013). Browning in plants is attributed to oxidation of polyphenols which also affect the rate of shoot development (Ko et al, 2009). This fact may be correlated to the poor rate of shoot proliferation observed in the BB and ABB cultivars. The prevalence of browning varies among species, cultivars, the physiological state of the plant/tissue, and size of explants and age of explants (Ahmad et al., 2013; Ozyigit, 2008). Oxidative browning is a common problem in plant tissue culture; resulting in reduced growth (Krishna et al., 2008), lower rates of regeneration and can ultimately lead to cell and tissue death.

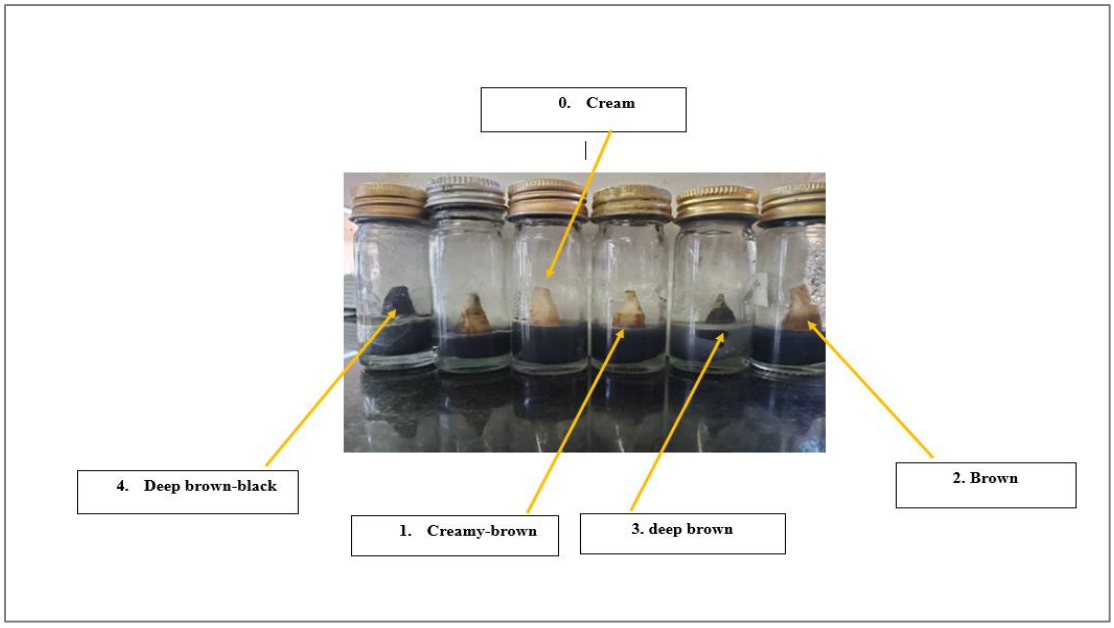

Figure 5. 3.Different browning effect on banana genomic groups

5.6.3. Plant acclimatization

Plantlets that had fully developed a root system were transferred into small polythene bags before being transferred into pots as shown in Figure 5.4. In polythene bags, the plantlets were covered with transparent plastics in the lab. The plantlets were then moved out of the lab after a week and 100% of the acclimatized plantlets survived.

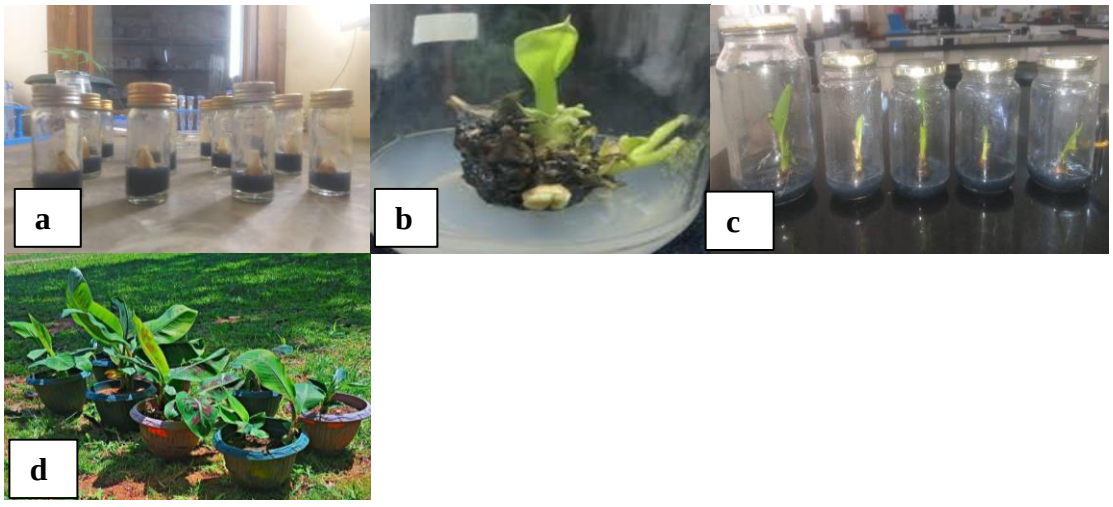

Figure 5. 4. In-vitro propagation of bio banked Musa cultivars from (a) initiation phase (b) multiplication phase (c) rooting phase and (d) hardening

5.7. Conclusion and Recommendation

The banana genomic groups showed a significant difference in their growth rate one from another under controlled environment. The AAA genome bananas are highly recommended for in vitro propagation due to their growth rate as evident from the study. The B genome in *Musa* contributes to changes in growth rate as well as the browning rate of the genomes. This therefore means that the BB, AB hybrid and ABB bananas slow proliferation is attributed to their B genome. The browning problem encountered therefore would require investigations in auxin and cytokine hormone ratios for each genomic group as well as the addition of phenolic reduction chemicals such as ascorbic acid that help reduce the browning effect.

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Chapter 6

6.1. Nutrient and flour properties of the bio-banked banana

6.2. Abstract

This study was aimed at evaluating the proximate compositions and flour properties of established banana germplasm at the C.U.T owned Hunyani Farm. Samples were taken from the following *Musa* genome groups namely *Musa acuminata*, *Musa balbisiana*, *Musa ABB* and *Musa AB hybrid*. The composite flour was analysed for two physical properties which were water and oil holding capacity. Organoleptic evaluations were carried out on aroma, colour and the texture of the flour whilst moisture, ash and crude fibre content were quantitatively assessed. Total soluble solids of dessert banana pulp were found to be lower ($P<0.05$) (2.5±0.51 g/kg) than those of culinary bananas (3.6± 0.39 g/kg). Dessert banana flours showed higher general acceptance compared to culinary banana flour. *Musa ABB, AB hybrid* and *balbisiana* cultivars have lower moisture content (68.32 %, 68.90 % and 65.80 % respectively) as compared to *Musa acuminata* (73.76 %). A higher ash content was also noted in *Musa AB* hybrid and *balbisiana* which both recorded 1.25 % and 1.10 % respectively. Crude fibre percentage content was highest in *Musa AB* hybrid (1.60 %) following *Musa balbisiana* (1.54 %) *and Musa ABB* (1.50 %). Results suggested that culinary bananas *Musa* BB and *Musa* ABB genomic groups have a great potential in becoming a good source of banana flour.

Keywords: unripe banana flour, nutritional value, proximate analysis, physical properties

6.3. Introduction

Bananas are ranked fourth most important food crops worldwide after rice, wheat and maize and , approximately a total of 300 cultivars are in existence in production countries (Tinzaara et al., 2017). Regardless of their abundance, only a few are being produced on a large scale and this is largely because of the lack of consumption appeal for the market due to non-superior preferences like palatability, taste, physical and nutritional characteristics. Evaluating nutritional composition of the least favoured cultivars together with those in large scale production contribute to value addition. Bananas are staple food to some of the developing countries hence it is of paramount importance to evaluate their nutrition. The use of green

bananas in production of flour has since been employed in the country to mitigate post-harvest losses as well as generate revenue (Farmers Review Africa, 2022).

Kaur et al (2017) described banana flour as a gluten-free starchy food that contributes greatly to the gluten intolerance problems. These include celiac diseases, an immune-mediated enteropathy which is characterized by diarrhoea, anaemia, abdominal pain amongst others as a result of gluten intake (Rubio-Tapia et al, 2013). Green bananas are used in flour production due to low sugars and high starch composition. The presence of type 2 resistant starch provides benefits for the gut as it possesses prebiotic properties (Orvando-Martinez et al., 2009). Companies such as Nezox Brands and Greenit Diversified Group in the Honde Valley, Manicaland have since engaged in the production of banana flour (Farmers Review Africa, 2022) and snacks hence this study was aimed at analysing the proximate composition of locally grown bananas. Researchers concur that the proximate analysis of samples provide valuable information on nutritional composition and assess the quality of the sample therefore moisture, ash and crude fibre (Obiageli et al, 2016).

According to Campbell et al, (2012), flour is a product whose quality is a variable and therefore is subject to erraticism. Flour quality is measured by its water absorption capacity, particle size and distribution, starch rheology and retrogradation, colour amongst other factors. Physical measures may be easier to analyse for quality control in small and medium-sized enterprises with limited funds and staff. The determination of chemical flour components such as sugar, starch, and dietary fibres is more difficult and time intensive. As a result, it is worthwhile to investigate the physical qualities of banana flour, and to design ways for distinguishing banana flour based on its physical data. Hence, the primary objective of this study was to characterize specific physical properties of banana flour and utilize the data to establish a distinction amongst the genomic groups' banana flours.

6.4. Materials and Methods

Green mature bananas (in the range of 70-90 days period of development) were harvested from the Chinhoyi University Farm. The experimental treatments were banana fruit cultivars from the genomic groups namely *Musa balbisiana, Musa ABB, Musa acuminata and Musa AB hybrid.* Soluble solids were determined from each genomic group where 25 g of pulp was suspended in 90 ml of distilled water, blended for 2 min before being measured using a refractometer for total soluble solids (TSS).

6.4.1. Flour preparation

Harvested fruits were washed with tap water, hand peeled and sliced using a kitchen knife. The slices were dipped in 0.3% w/v citric acid for preservation before being oven dried at 50 ℃ for 72 hours (Ovando-Martinez et al., 2009) as indicated in Figure 6.1.

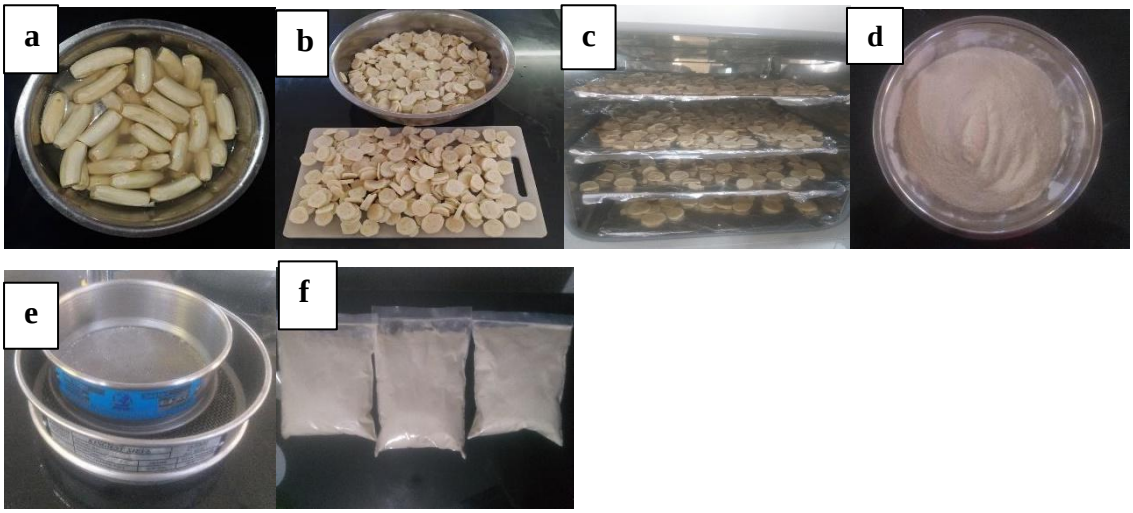

Figure 6. 1. Flour production process from a-f where a) peeled green bananas, b) cut bananas dipped in citric acid, c) shows banana slices drying process, d shows ground banana flour, e shows sieves used to refine ground flour and f shows packaged flour.

Using a pestle and mortar, the dried product was ground to powder then sieved through a 0.5 mm sieve. The flour was kept at room temperature in airtight containers for further analysis.

6.4.2. Chemical composition

The qualitative proximate analysis of different banana cultivars included moisture content, ash content and crude fibre. They were determined according to AOAC methods (AOAC 2012).

6.4.2.1. Moisture Content

A mass of 2 g each of the banana flour samples was weighed and heated in an oven at 105 ℃ for 6 hours until a constant weight was reached. The Moisture content of the samples was calculated using the formula

Moisture content (%) = $[(W_1-W_2)/W_1] *100$…… (1) Where W_1 was the initial weight of the sample and W_2, the final weight of the sample after drying.

6.4.2.2. Ash content

Ash content was also determined. 2 g of the sample was heated in a furnace at 550 ℃ for 6 hours until a constant colour change was observed. The ashes were then weighed and the percentage ash content was calculated using the formula:

Ash content (%) = $[(W_1-W_2)/W_1]$ *100 …… (2) Where W_1 was the initial weight of the sample and W_2, the weight of the sample after ashing.

6.4.2.3. Crude fibre

Determination of crude fibre was done following the Wende method (James, 1995). A mass of 2 g of the ground sample was weighed and soaked in 200 ml of sulphuric acid (H_2SO_4) at 1.25 % before being heated for 30 min. The mixture was filtered using a pH indicator paper before being washed with hot water. This was done until the residue was no longer acidic. The residue was then re-soaked in 200 ml of 1.25 % sodium hydroxide (NaOH). It was then boiled for 30 min, filtered and the paper was oven dried and weighed. The filter paper was placed in a crucible and burnt to ash before being cooled in the presence of a silica gel pack to absorb any moisture. The ash was weighed again. The crude fibre weighed was calculated using the formula:

Weight of fibre = residue weight – ash weight…….. (3)

% crude fibre = [fibre weight/ sample weight] * 100…… (4)

6.4.3. Flour hydration properties

Flour hydration properties were determined through assessing the water holding capacity as well as the oil holding capacity. The hydration properties were determined by methods with reference to Campuzano et al, (2018) and Rodriguez et al, (2008).

6.4.3.1. Water holding capacity

To evaluate and determine the water holding capacity, three different temperatures were used. 1 g of flour was mixed with 25 ml of distilled water. The mixture was then incubated at 40 ℃, for an hour before being centrifuged at 3000 g for 20 min. The supernatant was then decanted and the residue was left to drain for 10 min at 45 ℃. The residual substance was then weighed and the water holding capacity of the flour was calculated following methods by Laraurri et.,al (1996). The process was repeated at 60 ℃ and 80 ℃.

6.4.3.2. Oil holding capacity

To evaluate the oil holding capacity, 1 g of flour was mixed with 25 ml of olive oil. The mixture was then incubated in a water bath at 40 ℃, for an hour before being centrifuged at 3000 g for 20 min. The supernatant was then decanted and the residue was allowed to drain for 10 min at 45 ^{0} C. The residual substance was then weighed and the oil holding capacity of the flour was calculated following methods by Laraurri et al., (1996). The process was repeated at 60 ℃ and 80 ℃.

6.4.3.3. Organoleptic evaluation

The flour was analysed for colour, texture and aroma by a panel of 10 evaluators using the five-point hedonic scale. Data analysis was done in SPSS v 28.0.1.1.

6.4.4. Data Analysis

Statistical analysis was done using SPSS v 28.0.1.1 where data was subjected to one way ANOVA and means were separated using Fisher's Least Significant Difference (LSD).

6.5. Results and discussion

6.5.1. Flour preparation and total soluble solid content

The average lengths and diameters of the fruits used were taken as well as the weights as represented in Table 6.1. On average, the dessert bananas gave a yield of 154, 23 g per kg of green bananas whilst the culinary bananas produced on average 221.35 g per kg of green

plantains. The average total soluble solids (TSS) of dessert bananas were lower than those of culinary bananas and this may be attributed to the difference in starch and sugar compositions.

Table 6. 1.Physical parameters measured in preparation of green banana flour

Parameter (Genomic group)	***Musa Acuminata***			***Musa ABB***	***Musa Balbisiana***	***Musa AB hybrid***
Cultivars	W*	G*	D*	Fig	*Nzarayapera*	Apple
Weight (g)	100.4	126.4	142.1	161.4	69.7	65.3
Length (mm)	124.7	145.3	140.6	135.8	96.8	96.4
Width (mm)	35.6	37.7	33.8	43.2	34.8	32.6
TSS (º Brix)	1.7±0.93	2.9±0.44	3.1±0.19	3.6±0.34	3.5 ±0.67	3.8 ±0.18

W*=Williams, G= Giant Cavendish, D= Dwarf Cavendish

6.5.2. Chemical composition proximate analysis

The proximate analysis of green banana flour from the five genomic groups were summarised in Table 6.2 below.

Table 6. 2.Percentage proximate composition of green banana flour

Proximate Composite	***Musa* ABB**	***Musa Acuminata***	***Musa balbisiana***	***Musa* AB hybrid**	**LSD**	**P value**	**C.V**
Moisture Content	10.28 ± 0.009^{a}	10.69 ± 0.005^{c}	10.36 ± 0.005^{b}	10.32 ± 0.005^{a}	0.026	P<0.05	0.13
Ash Content	1.34 ± 0.009^{b}	2.36 ± 0.000^{c}	1.23 ± 0.007^{a}	1.25 ± 0.003^{a}	0.0244	P<0.05	0.84
Crude Fibre	1.62 ± 0.005^{d}	0.68 ± 0.007^{a}	1.54 ± 0.000^{c}	1.51 ± 0.003^{b}	0.019	P<0.05	0.75

LSD= least square difference, CV= Coefficient of variation. Values are expressed as mean ± standard deviation. Values with different letters indicate significant differences (p>0.05)

6.5.2.1. Moisture content

The moisture content of the banana flour ranged from 10.28% to 10.69% (Table 6.2). *Musa* AB hybrid and *Musa* ABB moisture contents were not significantly different (P>0.05). *Musa acuminata* genomic group had the highest moisture content. The least moisture content was found in the ABB genomic group. Moisture content is inversely related to the amount of dry matter. It contributes greatly to fruit deterioration, browning dehydration and oxygen absorption. (Pomeranz, & Meloan, 1994). Moisture content defines the shelf life of processed goods, the reactivity of chemical compounds or the bulk material's binding characteristics. As a result, the capacity to precisely identify and control moisture content levels throughout processing procedures is important towards the success of numerous commercial and scientific operations (Arceusz et al, 2013). Suas (2009) stated that the moisture content of green banana flour should not exceed 14%. This is because moisture content above 14% affects the quality of flour and shortens the shelf-life flour. The lower the moisture content, the longer the shelf life of the flour.

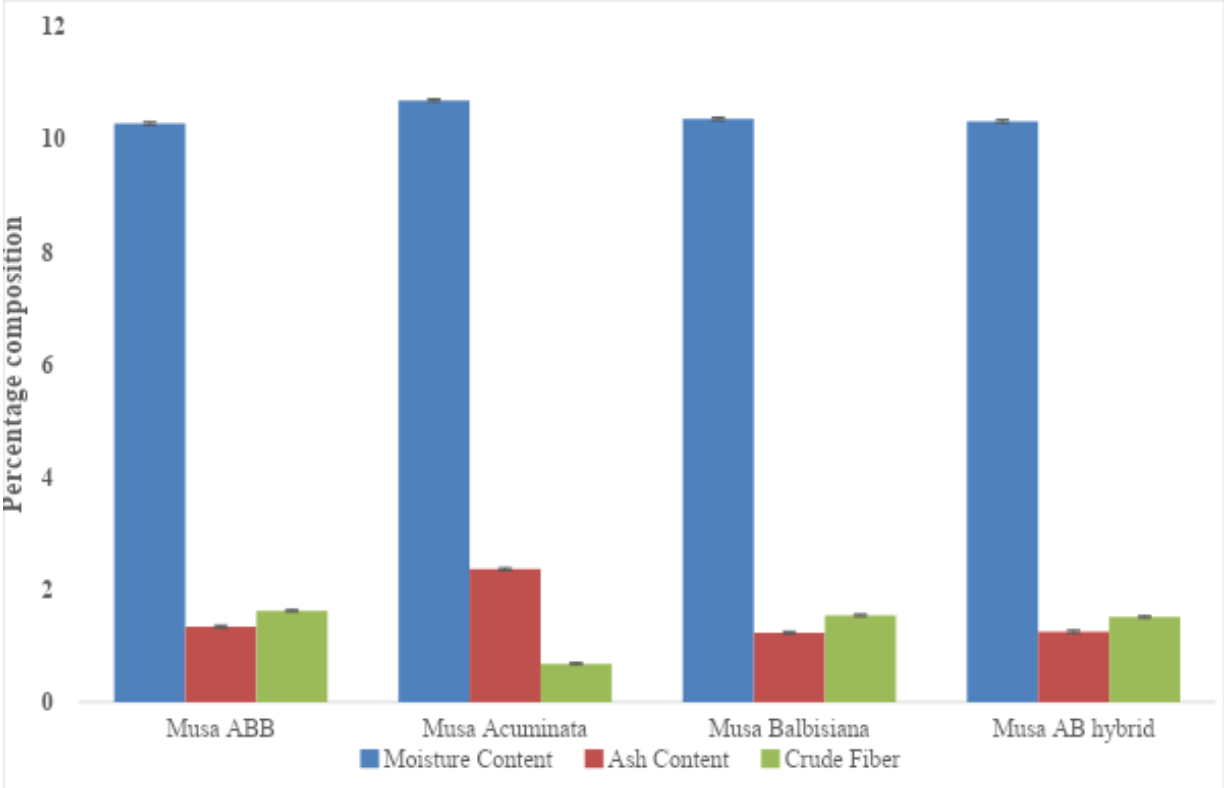

Figure 6. 2.Percentage moisture, ash and crude fibre content from green banana flour

6.5.2.2. Ash content

The ash content of the banana cultivars were significantly different (P<0.05) (Figure 6.2). The ash content ranged from 1.23% to 2.38%, with *Musa balbisiana* and *Musa* AB hybrid exhibiting the lowest ash content. *Musa acuminata* showed the highest ash content amongst the banana genomic groups. There was no significant difference in moisture content of *Musa balbisiana* and *Musa* AB hybrid. Ash content is regarded as the inorganic residue that remains after the removal of organic matter and water through heating. It then provides a measure of total mineral amount within the flour of each genomic group. This can be attributed to differential absorption capacity of banana genomic groups.

6.5.2.3. Crude fibre content

The crude fibre content ranged from 0.68% to 1.62%. The crude fibre content of the genomic groups differed (P<0.05). *Musa* ABB genomic group had the highest crude fibre content (1.60%) followed by *Musa balbisiana* and *Musa* AB hybrid with a percentage composition of 1.54% and 1.50% respectively. Higher crude fibre content is directly proportional to high dietary fibre content (Hasmadi et al., 2018). With reference to Williams et al, (2019), consumed fibre poses a physiological effect on the gastro-intestinal function where it helps alleviate intracolonic pressure in diverticular diseases. Dietary fibre has a chemical effect on the absorption and reabsorption of bile acid and the adsorption of dietary fats and cholesterol.

6.5.2.4. Correlation of proximate composite

Crude fibre and ash content have a significant and strong negative correlation (r= -0.98). Research on wheat flour has reported that ash and crude fibre content have no correlation as reported by Schopf et al., (2021) and National Research Council, (1989). Ash content and moisture content of the banana genomic groups showed a strong positive correlation (r=0.97) at (P>0.05). The moisture and crude fibre content of the banana genomic groups showed a strong negative correlation (r= -0.99). Studies have shown that carbohydrate and crude fibre content in banana flour can influence its moisture content. As the drying temperature increases, the moisture content of banana flour decreases, while the carbohydrate content remains consistent or even increases. This is observed in the studies conducted by Syed Abu Bakar et al (2018). Syed Abu Bakar et al (2018) also show that the crude fibre content decreases as the drying temperature increases. These findings suggest that higher levels of carbohydrate and

lower levels of crude fibre contribute to lower moisture content in banana flour and as a result a negative correlation is attained.

6.5.3. Flour hydration

6.5.3.1. Water holding capacity

The water holding capacity (WHC) of the dessert bananas were generally higher as compared to the culinary group. As the incubation temperature increased, the WHC increased significantly. Measurements at 40 ℃ and 60 ℃ showed that the starch was not affected and hence the WHC was low. Measurements at 80 ℃ resulted in an increased capacity and this is attributed to gelatinization and protein denaturation. These processes allow the availability of more hydroxyl groups and the release of amylose into the water thereby increasing the WHC (Agama-Acevedo et al, 2016). The Cavendish group had an average of 2.5 water/g whilst the culinary group had an average of 2.3 water/g. There was no statistical difference between these two groups at $p>0.05$. According to Waliszewski et al, (2012), the water holding capacity is related to the physical state of starch, dietary fibre and protein content in the flour. It is the release of amylose in starch which has the capacity to effectively bind the water molecules yielding a higher water holding capacity.

6.5.3.2. Oil holding capacity

The oil holding capacity of green banana flour (OHC) of the *Musa* groups had an average of 2.1 g of oil/g and did not change regardless of an increase in temperature. There was no statistical difference in the oil holding capacity of the *Musa* groups at $p>0.05$ at 40 ℃, 60 ℃ and 80 ℃. The oil holding capacity is the ability of food material to absorb oil. The OHC in banana flour signifies the hydrophobic nature of the starch. Gomes et al, (2021), stated that the stabilization of food systems using high fat content can easily be achieved through emulsification especially in bakery products. Emulsification can be improved through the formation of protein-starch formulations and gluten-gluten interactions thereby improving the aeration in confectionaries (Aziah et al., 2009).

6.5.4. Organoleptic analysis

The Table 6.3 shows the sensory metrics that were analysed using a five-point hedonic scale. The average overall acceptance for flour colour was high in *Musa acuminata* genome group. At P>0.05, the colour of the banana flour was significant across all genomic groups.

Table 6. 3.Hedonic test evaluation on organoleptic analysis

	***Musa* ABB**	***Musa Acuminata***	***Musa balbisiana***	***Musa* AB hybrid**	**LSD**	**P value**	**C.V**
Aroma	6.65±0.003^{c}	6.44±0.003^{a}	6.52±0.003^{b}	6.52±0.005^{b}	0.013	P<0.05	0.109
Texture	6.48±0.003^{a}	7.19±0.005^{c}	6.48±0.005^{a}	6.5±0.005^{b}	0.012	P<0.05	0.137
Colour	8.17±0.007^{c}	8.29±0.003^{d}	7.5±0.003^{b}	7.18±0.005^{a}	0.019	P<0.05	0.129

LSD= least square difference, CV= Coefficient of variation. Values are expressed as mean ± standard deviation. Values with different letters indicate significant differences (p>0.05)

The texture of the *Musa* ABB and *Musa balbisiana* genome groups did not show a significant difference at p>0.05. flour. As shown in figure 6.3, the acceptance of aroma for *Musa* ABB was distinctively high showing a significant difference from the other groups. *Musa acuminata* possessed higher acceptability levels in terms of texture and colour.

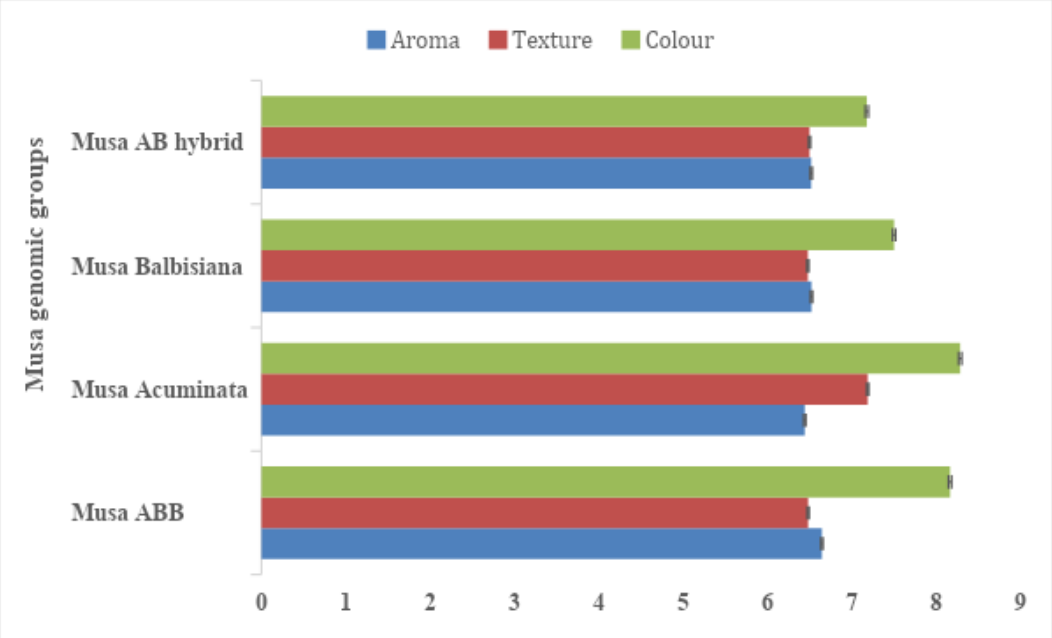

Figure 6. 3.Comparative analysis of green banana flour sensory metrics

A higher acceptance in the colour of *Musa acuminata* genomic group flour may have been attributed to the difference in colours as the Cavendish showed a lighter yellowish colour whilst

its counterparts showed a darker colour as shown in Figure 6.4. Colour is an important parameter in consumer acceptance of food products. The darker colour obtained in other genomic groups may be attributed to the drying process of the flour. Bananas usually change colour due to oxidation if they are not treated before flour production., Ohizua et al, (2016) stated that banana flours generally have whiter colour, therefore drying process becomes the main source for the difference in colour of the flour. Because a majority of people perceive flour to be white, they therefore tend to lean towards that which they are accustomed to (Luo et al., 2019). According to Ayofemi et al., (2014), one of the most essential qualities that attributes to food acceptability is texture. Texture manifests as a combination of physical qualities felt by touch (which include kinaesthetic as well as mouthfeel), sight and also sound.

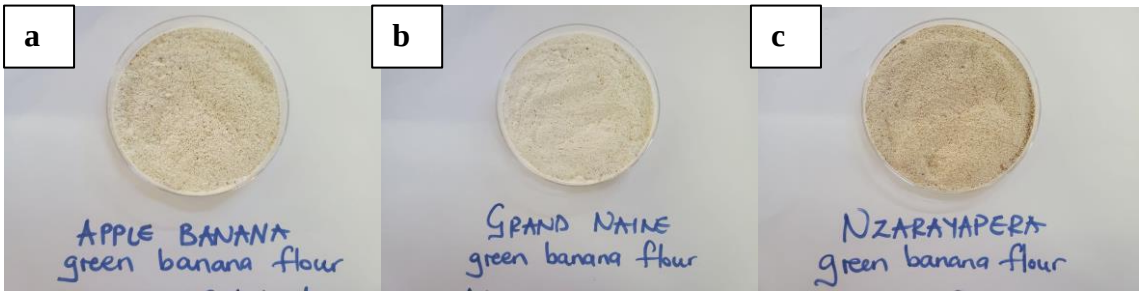

Figure 6. 4. Proximate flour composites obtained from harvested green banana where (a) shows apple banana flour, (b) shows Grand Naine banana flour and (c) shows Nzarayapera flour.

6.6. Conclusion and Recommendation

Based on the physical qualities of the flours, the Fig banana, apple banana and *Nzarayapera*, which are culinary bananas, pose as great sources of banana flour due to their fibre, moisture content, starch quantity as compared to the Cavendish group at green mature stage. These cultivars are not being commercially produced at a large scale therefore this research can pave the way for large scale production. More food products have the potential to be designed and be adjusted to meet customer sensory approval and preference. Further analysis on the banana flour's rheological properties and determination of starch compositions needs to be assessed for downstream processes of banana flour-based products.

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Chapter 7

7.1. Suitability modelling of banana production in Zimbabwe using Maxent

7.2. Abstract

Large scale banana production in Zimbabwe is confined to agro-ecological zone 1, although other regions have managed to produce to a limited extent. This is mainly because of the favourable climatic conditions for banana growth found in this region. Across Zimbabwe, there are agro-ecological zones that possess similar or can be modified to mimic climatic conditions in agro-ecological zone one. In this study, new potential production areas were prospected using Maximum Entropy (Maxent). The data output was mapped in ArcGIS. An average of training and test Area Under Curve of 0.920 with a standard deviation of 0.03 was achieved after 10 replication trials. The Area Under Curve was represented by showing sensitivity on the y-axis and 1-specificity on the x-axis for all potential thresholds. Based on the bioclimatic data, the outputted model showed Area Under Curve values greater than 0.9 indicating a good prediction ability. Distinction of suitable areas from unsuitable areas was established using an automatic generated threshold. Low suitable areas ranged from 0-0.46, medium suitable areas ranged from 0.46-0.77 and high suitable areas ranged from 0.77-1. Potential production areas lied in the medium range of the suitability model due to a number of factors. An approximate total of 4757.112 km^2 was found to be suitable for banana expansion in Zimbabwe and this equates to over USD $ 1,813,715.9844 billion dollars annual returns when fully utilised. The main environmental factor affecting banana distribution in Zimbabwe was found to be precipitation therefore irrigation schemes could be of great help towards banana production expansion.

Keywords: banana production expansion, GIS, species distribution models

7.3. Introduction

Amongst Zimbabwe's agricultural regions, agro-ecological region 1 is the most favoured banana producing area with a hectarage of approximately 3,255 hectares dedicated to banana production which has the potential of producing about 32550 tons (The Zimbabwean, 2019). Bananas thrive in the tropical climate, which is found mainly in the Manicaland province. According to Farmers Review Africa (2018), Manicaland generated 81% (396 975 tons) of

total yearly output during the 2017/18 cropping season, with the remaining 19 % (8 800 tons) coming from the remainder of the country (Business Daily, 2019).

Honde Valley, Mutema, Chipinge and Burma Valley have quite a number of banana plantations. According to The Zimbabwean, (2019), Chipinge alone has approximately 1,130 hectares but only a tenth of it is currently being utilized, and the area has three other districts that are producing bananas. Mutema is the most productive, with Burma valley and Honde valley close behind. Matanuska Company in the Burma Valley in Mutare District is the country's top banana grower in Zimbabwe. (Tsiko, 2018).

Apart from the Eastern Highlands, other parts of the country produce bananas on a smaller scale. Production in other agro-ecological regions of the country is less than that of region 1, however irrigation schemes have successfully yielded quality fruits. The Middle Sabi's farm 32 has managed to produce bananas on over 160 hectares of land, positively transforming the lives of A2 farmers (The Herald, 2020).

Mashonaland West 's Charara and Baguta which sit on 80 and 100 hectares respectively have successfully produced export quality bananas including Mazowe in Mashonaland Central provinces of the country. Regardless of there being mostly unfavourable conditions for banana production in new areas as compared to region 1, the new areas have potential of yielding good quality bananas should the limitations be addressed. Scouting for these areas then requires suitability mapping and modelling of the potential areas.

Suitability mapping for bananas involves integration of various types of information such as soil properties, temperature, rainfall, topography, and slope so that suitable maps can be developed. Among the factors that have been observed as causing variations in banana farm productivity in countries such as Uganda and Kenya, they include deteriorating soil fertility, drought occurrences and subsequent reduction in soil moisture (Sabiiti et al., 2018). Climate and soil characteristics have a strong limiting effect on the distribution of plant species (Thuiller, 2013). Relationships between plant species and environmental factors have been studied extensively in plant ecology and a wide range of modelling methods have been used in banana studies (Henderson et al, 2014). These include suitability modelling of banana in Uganda by Sabiti et al., (2018), GIS-based suitability mapping in the Phillipines by Bato, (2018) as well as suitability analysis and projected climate change impact on banana production in India (Ranjitkar et al., 2016). Species distribution models (SDMs) are tools that are used to estimate actual or potential distribution of a species, as a function of the environmental

variables, through quantifying the relationship between plant distribution and these variables using field measured data, auxiliary maps and expert knowledge (Hengl et al., 2009). These therefore can be employed to measure the importance of each environmental factor to the geospatial plant distribution, to predict the distribution of species in areas where field data is unavailable (Elith & Leathwick, 2009), and also to predict the past and future potential distributions, hence, can be used to observe the impact of climate change.

Maximum Entropy (Maxent) is a learning technique that can be used to predict geographical distribution of any spatial phenomena, plants and animals included (Endries, 2001). The main goal of Maxent is to find a probability distribution with the highest entropy (most spread out), within the limitations imposed by the information currently available on the presence of species and the associated environmental variables throughout the research area (Phillips et al. 2008; Elith et al. 2011). Countering the biases that can be brought about when using Maxent is the use of presence-absent modelling method since the models are less susceptible to problems of sample bias (Elith et al., 2010). Maxent contracts presence against background locations where the presence or absence of a species is unmeasured. The background samples (sometimes referred to as pseudo-absence) are desired because according to Phillips & Dudı (2008), Maxent allows one to predict the probability of presence within a particular location. By default, the Maximum Entropy modelling technique uses a method which assumes that a species is equally likely to be anywhere on the targeted landscape that is on the Zimbabwean map, every pixel has the probability of being selected as background (Merow et al., 2006). Maxent then contrasts the environmental conditions (through the use of different climatic layers) at background locations (all the Zimbabwe area) with those at observed presence locations (those with banana production) and then shows the new areas in which banana production can be done.

In this study, Maxent together with ArcGIS were employed to find, according to bioclimatic data, areas that are suitable to support the growth of bananas across the country.

7.4. Materials and Methods

7.4.1. Study Area

The study area covered the whole of Zimbabwe for suitability modelling and some parts of the country for sample collection.

7.4.2. Environmental Variability

7.4.2.1. Bioclimatic data for suitability modelling

The bioclimatic data used were downloaded from Harmonized World Soil Database v 1.2 https://www.fao.org/soils-portal/soil-survey/soil-maps-and-databases/harmonized-world-soil-database-v12/en/ which is a 30 arc-second raster database with over 15 000 different soil mapping units and the WorldClim database as shown in Table 3.0. The common climate model (CCSM4) was used for sourcing environmental data and a 30 arc-second spatial resolution was adopted. Unification of the geographic coordinates was done as GCS_WGS_1984. ArcGIS v 10.8 extract by mask tool was employed in cutting and extracting the environmental data. The range of data included was then converted to ASII format before being used.

The bioclimatic variables (temperature, precipitation, land cover, drainage, dam distance, river distance and elevation) that corresponded to the banana distribution points were extracted and imported into Maxent v 3.4.4. (Phillips, S. J. 2017). http://biodiversityinformatics.amnh.org/open_source/maxent/.

7.4.2.2. Data preparation

Country level data was extracted from world raster files
https://freegeographytools.com/2007/fao-world-climate-data.

7.4.2.3. Data Collection.

Banana production data was collected from production areas across the country. The data included GPS coordinates, banana varieties, and hectarage of the plantations as indicated in Table 7.1. The data was utilized in the mapping of production areas within the country.

Table 7. 1.Geographical locations of banana producing areas in Zimbabwe

Location	Farm Name	GPS coordinates	Banana Variety	Hectares
Chinhoyi	Baguta	-17.2949, 30.2113	Sweet Williams	100
	Pamene	-18.36053, 29.8152	Williams	5
Kariba	Charara	-16.55878, 28.9561	-	80
Chipinge	Makata Banana Pvt Ltd	-20.16948, 32.3658	Sweet Williams Apollo	120

	Farm 32	-20.263997, 32.3658	Williams	160
	Avontuur Extension	-20.25299, 32.8114	Sweet Williams Grand Naine Nandi Grand Negro Chico Delta Asdia	-
	Joppa	-20.0854, 32.6018	Sweet Williams	153.15
	Roscommon Estatate	-20.0087, 32.8004	Sweet Williams	-
Chibuwe	-	-20.5412, 32.3953	Williams	30
Mutasa	Chiteme	-18.33078, 32.9095	Dwarf Cavendish	60
Chimanimani		-20.08998, 32.6248	Williams	-
Mutare		-18.8598, 32.493	Grand Naine	-
Burma Valley	Fangundu Farm	-19.1452, 32.685	Chinese Cavendish	36
Honde Valley	Mupangwa Project	-18.3308, 32.9095	Sweet WiIIiams Asidia	32
Mazowe		-17.5069, 30.9747		
Chiredzi		-21.03335, 31.6796	Giant Cavendish	
Zaka		-20.3367, 31.4635	Williams	

7.4.3. Model Construction

The distribution points and bioclimatic data were imported into Maxent v 3.4.4. The bioclimatic variables were then evaluated using the Jackknife test.

7.4.3.1. Accuracy of model prediction

Utilizing the receiver operating characteristic area under the curve, the Maxent model's prediction accuracy was evaluated. An average of training and test AUC of 0.938 and 0.976 respectively with a standard deviation of 0.03 was achieved after 10 replication trials as indicated in Figure 7.1. According to the data, there is a 93% chance that a randomly chosen presence point will be found in a raster cell with a probability value that represents a species' occurrence as opposed to a randomly created point. The maximum possible AUC for models utilizing presence-only data is typically lower than those involving presence-absence data (Wiley et al. 2003; Phillips et al. 2008), and it is also smaller for species that are defined by a wider range of environmental circumstances (Phillips et al. 2004). While the accuracy in this

case is much better than a random prediction, this value of AUC may also be due to the inherent properties of modelling using presence-only data as well as the species' vast habitat range when modelled using variables individually rather than weighted contribution (Tesfaye et al. 2002). As indicated here, 1-specificity (fractional predicted area) indicates the percentage of all pixels projected to have sufficient conditions supporting the species, while sensitivity corresponds to the proportion of test localities properly predicted present (1-extrinsic omission rate) (Phillips et al. 2008; Phillips 2017).

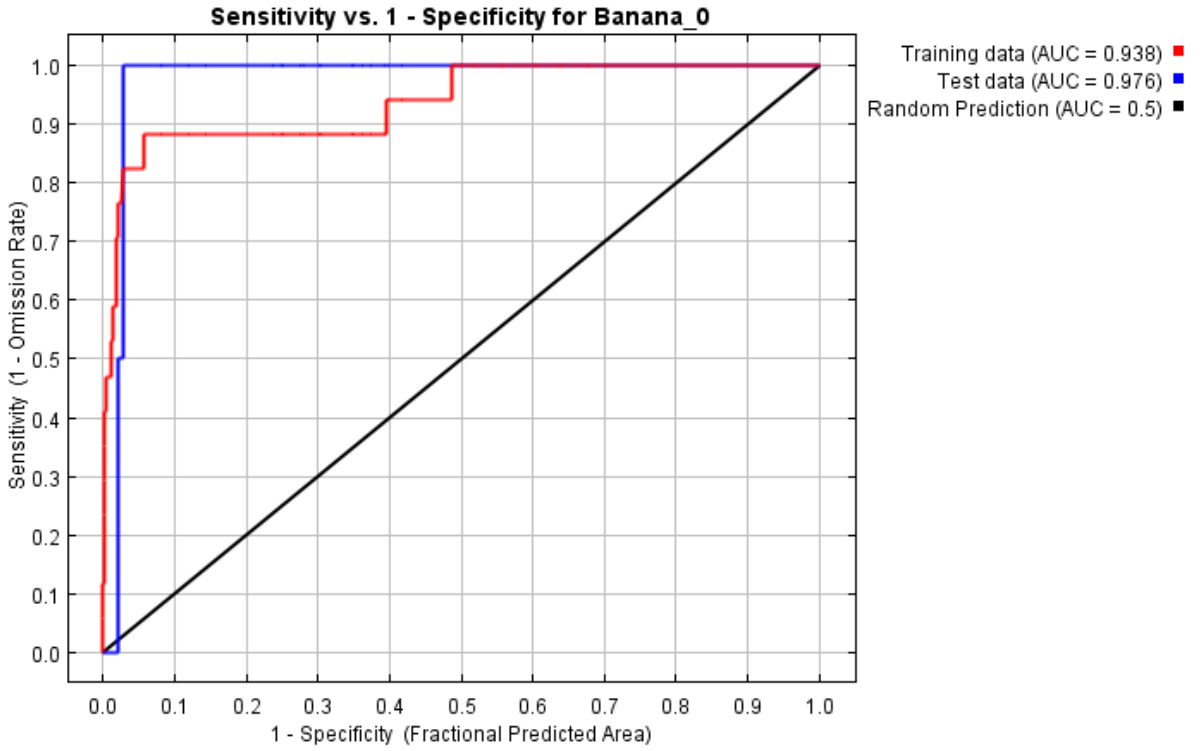

Figure 7. 1.Sensitivity vs 1-specificity test for banana suitability modelling

7.4.3.2. Accuracy assessment of model performance

The accuracy of the model prediction was evaluated using the receiver operating characteristics (ROC) area under the curve (AUC), a popular and reliable method of model evaluation. A total of 20 samples from independent test data were used. AUC describes a model's performance at all potential levels, regardless of any specific threshold. A perfect classifier achieves an AUC of 1, whereas a value of 0.5 denotes a random prediction. The model quantifies how a random positive and negative instance are correctly sorted by the classifier (with random ordering in the case of ties). AUC is represented by showing sensitivity on the y-axis and 1-specificity on the x-axis for all potential thresholds (Phillips et al. 2008; Girma et al. 2016).

Sensitivity displays the fraction of test locations that were accurately predicted to be present (1-extrinsic omission rate), whereas 1-specificity is the fraction of all pixels that were projected to contain the species' ideal circumstances. Alternatively, 1-specificity represents a false positive rate whereas sensitivity represents a true positive rate (commission error). Which means that the higher the AUC, the higher the sensitivity in distinguishing variations between x-axis values and y-axis values. In contrast, the ROC curve appears to be useless when presence-only data is employed (as in Maxent). Absences are necessary because, without them, there would be no source of unfavourable examples to compare specificity against.

Instead of distinguishing between presences and absences, this problem can be resolved by utilizing a different categorization technique to separate presences from chance.

7.5. Results and Discussion

7.5.1. Distribution Areas

Banana plantations are mainly found in regions 1 and 2 in the Manicaland province of the country (Figure 7.2). Manicaland province is characterized by a tropical climate hence the high distribution. Banana plantations were found in Chiredzi, Zaka, Mazowe, Harare, Mutare, Honde Valley, Nyanga, Chipinge, Chinhoyi, and Kariba. Production analysis of banana production reflects areas such as Kariba and Chinhoyi in the Mashonaland West which are characterized by high temperatures and low rainfall.

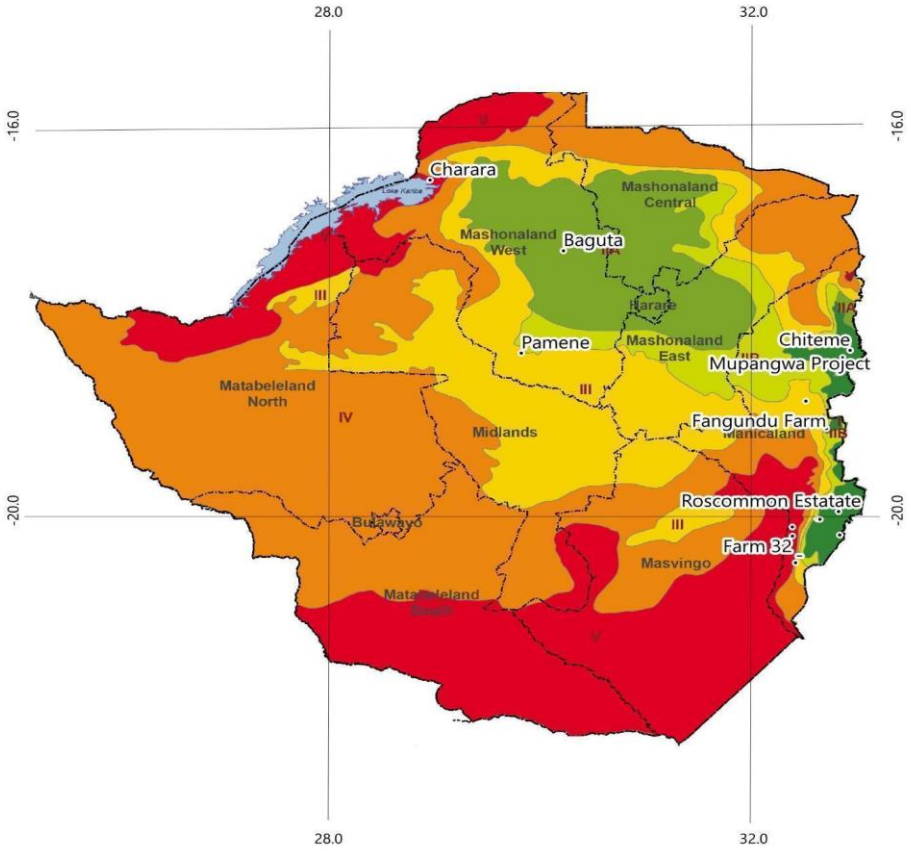

Figure 7. 2.Geo-spatial distribution of banana production areas in Zimbabwe

Kariba has Charara, a banana plantation comprising 80 hectares of land whilst Chinhoyi has Baguta as well as Pamene with 100 and 5 hectares of land under banana production respectively. This, therefore, reviewed that the presence of a source of water that can sustain banana production through irrigation can qualify as a potential banana production area. Water reservoirs such as dams, lakes as well as rivers can be used in sustainable banana production expansion. Improving agricultural practices and productivity through irrigation is one of the most useful strategies for alleviating poverty and improving rural communities as the majority of the underprivileged depend directly or indirectly on agriculture (Moyo et al., 2017).

7.5.2. Suitability Modelling for potential banana producing areas

Maxent modelling was used in the prediction of new potential banana farming areas. The model was visualised using ArcGIS. Based on the bioclimatic data, the outputted model showed AUC values greater than 0.9 indicating a good prediction ability. Distinction of suitable areas from unsuitable areas was established using an automatic generated threshold. Low suitable areas

ranged from 0-0.46 (red), medium suitable areas ranged from 0.46-0.77 (yellow-green) and high suitable areas ranged from 0.77-1.

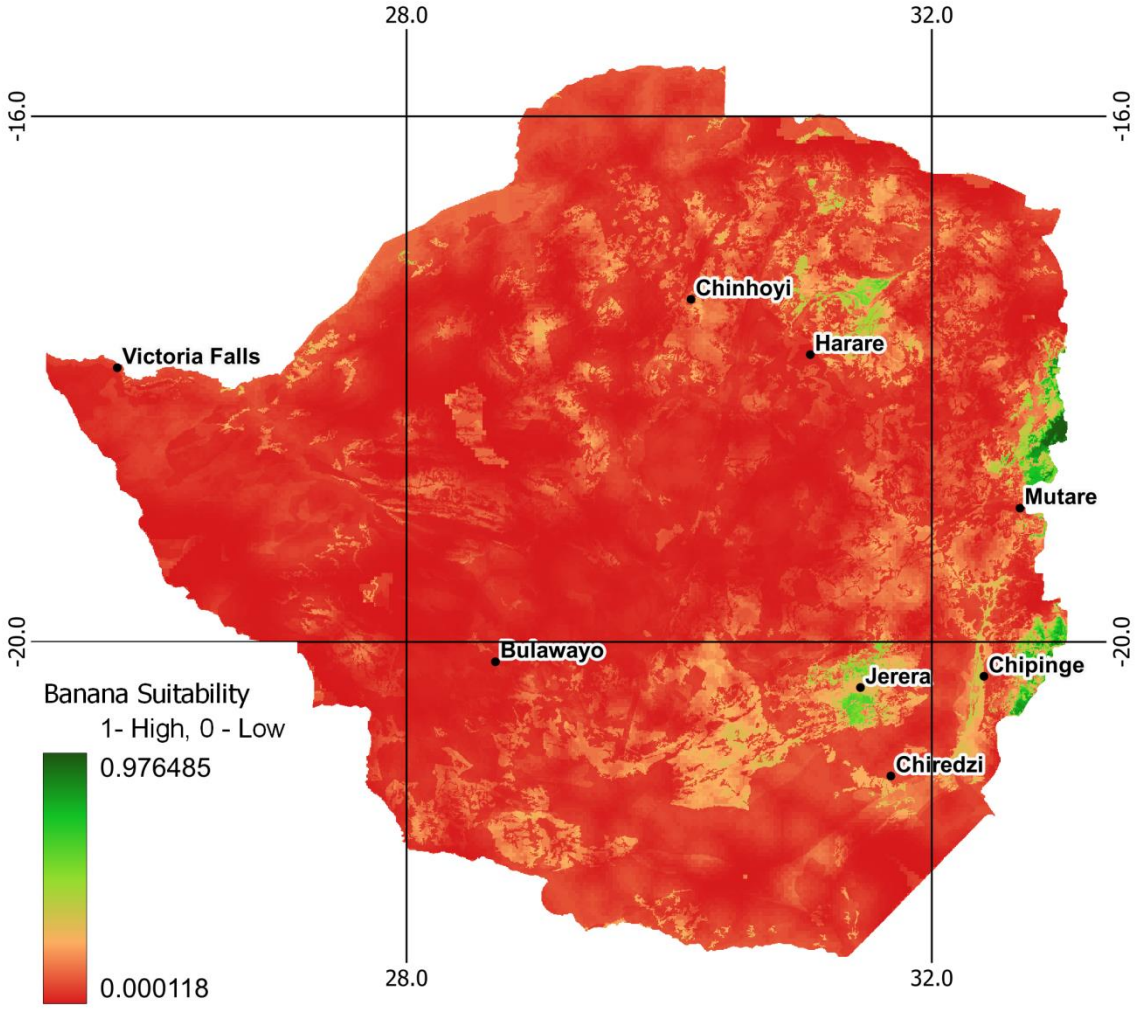

Figure 7. 3.Suitability model for banana production new potential areas.

The spatial distribution of bananas in Zimbabwe follows the Eastern Highlands climatic conditions. Matabeleland North and South, Midlands, and Masvingo are characterized as low-suitability areas. The probability of success of banana production in these areas was found in the range of 0-0.46 as indicated in Figure 7.3. The Manicaland and Mashonaland provinces contain regions of higher probability for successful banana production hence can be expanded and utilised in production.

7.5.2.1. Climatic factors influencing banana suitability modelling

The five bioclimatic variables used in Maxent modelling are known to have an influence in the distribution and thriving of bananas in any environment. However, to counteract the absence or limited precipitation, the presence of dams and rivers at a site was factored in such that irrigation can pose as an alternative. Bananas require high rainfall and adequate temperatures therefore they tend to thrive well in tropical temperatures. The results obtained from the Jackknife test indicated that precipitation was the most influential factor as shown in Figure 7.4.

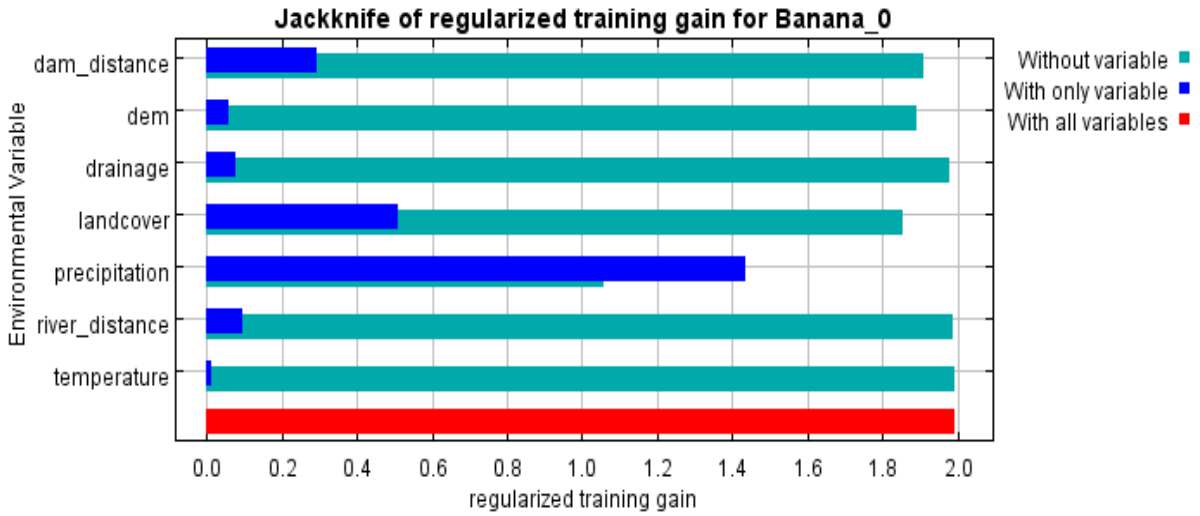

Figure 7. 4.Jackknife of regularized training gain for banana suitability modelling

The environmental variable that had the highest gain when used alone was precipitation, which therefore appeared to have the most useful information on its own. It contributed 78.4% to the model whilst land cover contributed 15.1%. Dams, elevation, rivers and temperature contributed less than 50% to the model. The environmental variable that decreased the model gain the most when eliminated was precipitation, which therefore appeared to have the most information that wasn't present in the other environmental variables.

Table 7. 2.Bioclimatic factors and their percentage contribution to the suitability model

Bioclimatic Factor	% contribution	Permutation importance
Precipitation	78.4	49.4
Land cover	15.1	23.7
Dam distance	3.7	11.2
Elevation	2.1	9.4
Drainage	0.6	6.2
River distance	0.1	0.1
Temperature	0	0

7.5.2.2. Major environmental predictors determining banana plantations distribution

The main use of species distribution modelling is to objectively determine which variable or variables are most important for forecasting the species under study. In order to do this, the relative percent contribution is analysed through a Jackknife significance test (applied to the training and test data) were used. The test is a resampling test where the bias and standard error of a predicted variable is tested against smaller samples derived from the entire data set. The importance of this test lies in its ability to estimate variations and remove correlation biases. Comparatively, precipitation has contributed the most, followed by the land cover, dam distance, elevation, drainage river distance and temperature. Temperature, however, has contributed the least. Precipitation has supplied the biggest gain to the model when used alone, showing that it has the most valuable information on its own, according to the training and test data's Jackknife importance score.

Temperature, was the variable that both had the most impact on the model's gain when it was excluded and had the greatest predictive power for the distribution of the independent test data. Temperature therefore seems to be the primary predictor based on its relative contribution and Jackknife importance test since it provides the most important information that is absent from the other predictor variables.

Response curves of individual predictors also offer more detailed and practical information that describes the distribution of the species. These graphs illustrate how each predictor's variation affects the projected probability of the species' distribution while maintaining the

average sample value for all other variables. The curves display the mean response of the 10 replicate Maxent runs in red. The predictors which contributed the most to the Maxent model are represented by their response distanced curves in Figure 7.5.

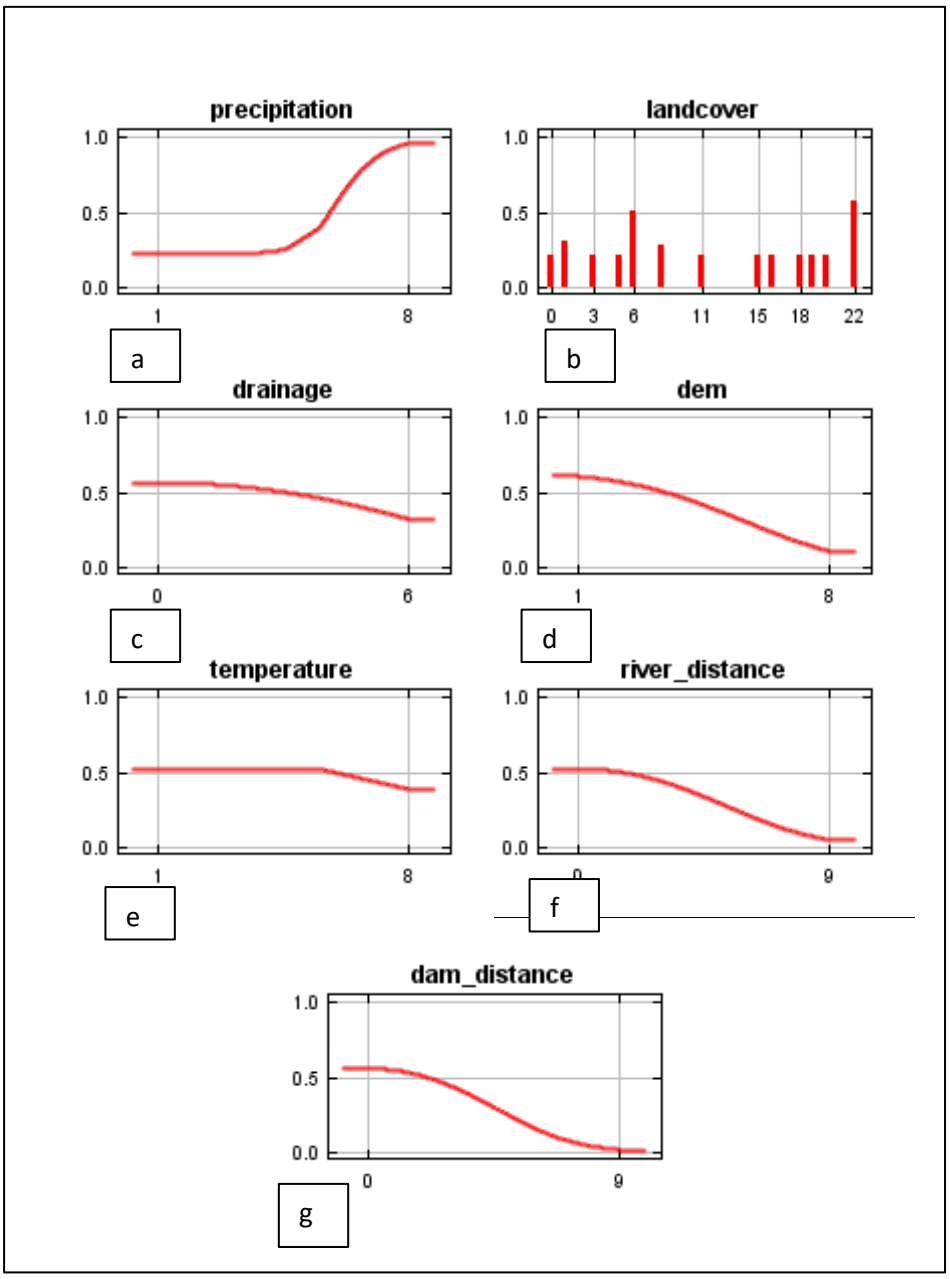

Figure 7. 5.Distanced-response curves in banana suitability modelling

The distanced response curves showed significance of each variable from testing point or site. The precipitation graph indicated that it becomes more significant as the distance away from the test site increases (Carpenter et al., 1993) (Rahmati et al., 2016). At approximately 4 km away from the site, its significance was noted. Land cover was significant in the first 5 km where there is a sharp increase. Significance was reduced as the distance from the site increased. The sharp rise at 6 and 22 km indicates a landmark that has been encountered away from the site. The drainage output graph showed significance in the first 0-5 km from the site. The temperature remained constant for the first 6 km from the testing site. The further away

from the site, the less significant it became. Elevation, like river and dam distance, was significant on site. The further away from the site, the less significant they become.

A total of approximately 4757.112 km^2 country-wide (including the already existing plantations) were found to be suitable for banana production. Currently Zimbabwe utilizes approximately 45 000 hectares of land under banana production which has, according to statistics, produced an annual tonnage of 189 499 tons as of 2021. The 2021 tonnage translated to USD $ 85, 274, 550 million at a price of USD $450/ton. Utilization of the new suitable land which is approximately 385 000 hectares of land would then mean a 8.5-fold increase in tonnage which translates to USD $ 729, 571, 150 million dollars annually. An increase in banana production in Zimbabwe is a sure way of creating self-sustaining communities and livelihoods.

7.6. Conclusion

The study focused on mapping out banana production areas in Zimbabwe as well as modelling new production areas using Maxent. The use of elevation (DEM), temperature, precipitation, land cover, drainage, dams and river distances, and soil data were available allowed the generation of a suitability model map that can help the farming, breeding, and scholar communities as well as funders and the government to assess the expansion of banana production within the country. A total of approximately 385 000 hectares of land is available for potential banana production. More work is needed for the success of banana production expansion in Zimbabwe and this includes implementation of irrigation schemes in suitable areas as well as allocation of the available land towards subsistence as well as commercial banana production.

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Chapter 8

8.1. General Discussion, Conclusion and Recommendations

The results of this present study showed that identifying varieties and cultivars allows genetic and phenotypic analysis of each which in turn paves way for genetic conservation and improvement programs in the banana industry. Characterization and cataloguing of the bananas that are being produced will help people across disciplines including farmers, breeders, academia as well as the food industry with knowledge on each banana cultivar and varieties thereby making informed decisions. The presence of a bio-banked gene pool opens up opportunities for more research on *Musa* species in Zimbabwe. Identification through genome-targeted sequencing allowed cultivars' evolutionary and phylogenetic analysis to be inferred and as a result some local names like the *Nzarayapera* cultivar were clarified. Morphological characterization of the cultivars resulted in plant distinct descriptors being used for differentiation and complimenting molecular characterization. The identified genomic groups showed a significant difference in their growth characteristics under a controlled environment. The AAA genome bananas showed high proliferation and low browning percentage and therefore are highly recommended for in vitro propagation. The B genome in *Musa* contributes to a slow growth and browning rate. This therefore means that the BB, AB hybrid and ABB bananas have a higher phenolic composition.

Even though the B genome bananas were less preferred in in-vitro propagation, the study has also shown that they give the best banana flour. At the green mature developmental stage, these bananas possess high fibre content, high starch quantity and low moisture content as compared to the Cavendish (AAA) group. Regardless of their genomic groups, banana production can be extrapolated to new areas. However, a change in climatic conditions poses minimal changes in the life cycle of the plant and therefore the extent of adaptation requires consistent monitoring. The study showed that a total of approximately 385 000 hectares of arable land is available for potential banana production expansion in Zimbabwe. With precipitation being the main limiting factor in banana optimal growth, the study included the modelling of a suitability map which showed presence or access to water sources that can support irrigation schemes.

More work is therefore needed for the success of banana production expansion in Zimbabwe and this includes implementation of irrigation schemes in the suitable areas as well as allocation of the available land towards subsistence as well as commercial banana production.

The availability of a suitability model for banana production expansion allows for exploration of virgin land that can be utilized for banana production in the suitable parts of the country. Micro-propagation of banana plants coupled with virus indexing will help eliminate diseases and viruses from locally grown bananas as well as being a source of clean planting material. Established banana tissue culture therefore reduces the importation of plantlets into the country at the same time creating a self-sufficient ecosystem in banana farming. Value addition of bananas through the production of flour and flour-based products helps reduce farmer losses and fight non-communicable diseases such as diabetes and obesity. These efforts together with others across the country help establish and conserve banana cultivars and varieties in Zimbabwe.

Appendices

Appendix 1: Analysis of variance for the height of Musa shoots in-vitro for a period of eight weeks

Analysis of variance for shoot development

week 1

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
AAA	3	0.3	0.1	2.89E-34
ABB	3	0	0	0
AB Hybrid	3	0	0	0
BB	3	0	0	0

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.0225	3	0.0075	1.04E+32	1.0701E-126	4.066181
Within Groups	5.78E-34	8	7.22E-35			
Total	0.0225	11				

Week 2

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
AAA	3	2.15	0.716667	0.010833
ABB	3	1.35	0.45	0.0025
AB Hybrid	3	2.15	0.716667	0.010833
BB	3	0.85	0.283333	0.005833

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.409167	3	0.136389	18.18519	0.000623636	4.066181
Within Groups	0.06	8	0.0075			
Total	0.469167	11				

Week 3

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
AAA	3	6.25	2.083333	0.005833
ABB	3	3.6	1.2	0
AB Hybrid	3	4.45	1.483333	0.000833
BB	3	1.5	0.5	0.0025

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3.888333	3	1.296111	565.5758	1.19131E-09	4.066181
Within Groups	0.018333	8	0.002292			
Total	3.906667	11				

Week 4

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
AAA	3	7.55	2.516667	0.000833
ABB	3	5.6	1.866667	0.013333
AB Hybrid	3	5.1	1.7	0
BB	3	2.05	0.683333	0.005833

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	5.184167	3	1.728056	345.6111	8.43215E-09	4.066181
Within Groups	0.04	8	0.005			
Total	5.224167	11				

Week 5

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
AAA	3	8.8	2.933333	0.000833
ABB	3	7.2	2.4	0.0025
AB Hybrid	3	6.65	2.216667	0.000833

BB	3	5.65	1.883333	0.000833		

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.734167	3	0.578056	462.4444	2.65308E-09	4.066181
Within Groups	0.01	8	0.00125			
Total	1.744167	11				

week 6

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
AAA	3	9.4	3.133333	0.013333
ABB	3	8	2.666667	0.000833
AB Hybrid	3	7.15	2.383333	0.000833
BB	3	6.3	2.1	0

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.747292	3	0.582431	155.3148	1.98426E-07	4.066181
Within Groups	0.03	8	0.00375			
Total	1.777292	11				

week 7

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
AAA	3	10.2	3.4	0
ABB	3	8.4	2.8	2.96E-31
AB Hybrid	3	7.6	2.533333	0.003333
BB	3	7	2.333333	0.000833

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.933333	3	0.644444	618.6667	8.33547E-10	4.066181
Within Groups	0.008333	8	0.001042			
Total	1.941667	11				

week 8

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
AAA	3	11.8	3.933333	0.003333
ABB	3	10.1	3.366667	0.003333
AB Hybrid	3	8.7	2.9	0
BB	3	7.45	2.483333	0.000833

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3.497292	3	1.165764	621.7407	8.1726E-10	4.066181
Within Groups	0.015	8	0.001875			
Total	3.512292	11				

Appendix 2.: Analysis of variance for moisture content, crude fibre and ash content composition in green banana flour

Analysis of variance for proximate analysis

Moisture Content

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Musa ABB	3	30.84	10.28	0.0004
Musa Acuminata	3	32.07	10.69	1E-04
Musa balbisiana	3	31.07	10.35667	0.000133
Musa AB hybrid	3	30.95	10.31667	0.000133

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.320558	3	0.106853	557.4928	1.26E-09	4.066181
Within Groups	0.001533	8	0.000192			

Total	0.322092	11				

crude fiber

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Musa ABB	3	4.85	1.616667	0.000133
Musa Acuminata	3	2	0.666667	0.000233
Musa balbisiana	3	4.62	1.54	0
Musa AB hybrid	3	4.54	1.513333	3.33E-05

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.799492	3	0.599831	5998.306	9.59E-14	4.066181
Within Groups	0.0008	8	1E-04			
Total	1.800292	11				

Ash content

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Musa ABB	3	3.99	1.33	0.0004
Musa Acuminata	3	7.08	2.36	0
Musa balbisiana	3	3.7	1.233333	0.000233
Musa AB hybrid	3	3.76	1.253333	3.33E-05

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2.677958	3	0.892653	5355.917	1.51E-13	4.066181

Within Groups	0.00133 3	8	0.00016 7
Total	2.67929 2	11	

Appendix 3.: Analysis of variance for colour, aroma and texture of green banana flour

Colour

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Musa ABB	3	24.5	8.166666667	0.000233333
Musa Acuminata	3	24.86	8.286666667	3.33333E-05
Musa balbisiana	3	22.51	7.503333333	3.33333E-05
Musa AB hybrid	3	21.54	7.18	0.0001

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2.528092	3	0.842697222	8426.972222	2.46E-14	4.066181
Within Groups	0.0008	8	1E-04			
Total	2.528892	11				

Aroma

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Musa ABB	3	19.94	6.646667	3.33E-05
Musa Acuminata	3	19.33	6.443333	3.33E-05
Musa balbisiana	3	19.57	6.523333	3.33E-05
Musa AB hybrid	3	19.56	6.52	0.0001

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.063667	3	0.021222	424.4444	3.73E-09	4.066181
Within Groups	0.0004	8	5E-05			
Total	0.064067	11				

Texture

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Musa ABB	3	19.45	6.483333	3.33E-05
Musa Acuminata	3	21.57	7.19	0.0001
Musa balbisiana	3	19.44	6.48	0.0001
Musa AB hybrid	3	19.5	6.5	1E-04

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.1102	3	0.370067	4440.8	3.19E-13	4.066181
Within Groups	0.000667	8	8.33E-05			
Total	1.110867	11				

Appendix 4. Correlation coefficients for crude fibre, moisture content and ash content

	Moisture Content	*Ash Content*	*Crude Fiber*
Moisture Content	1		
Ash Content	0.96666259	1	
Crude Fiber	-0.99217539	-0.9822395	1

Appendix 5. Consumer Sensory evaluation questionnaire

Consumer Sensory Evaluation Questionnaire

Welcome to banana flour sensory evaluation. The samples presented are safe and have been prepared in a food laboratory. The presenters of the samples will provide you with all the necessary information before giving you the samples for testing. Please ask for any information that you need to ask before you carry out the test.

Consent/Indemnity

I do agree that I have been well informed about the samples and accept to do the test on my own will. (Please tick?)

Yes:……. No:……

Instructions

You have been given 4 coded flour samples to evaluate. Please evaluate the samples and indicate how much you like or dislike the sample in terms of the provided properties. Please use the **rating numbers** provided to indicate how much you like or dislike the samples. Please tick (√) your level of like/dislike in the boxes provided.

Sample Code:……..

Rating Scale (1-5)	1. Dislike very much	2. Dislike slightly	3. Neither like nor dislike	4. Like slightly	5. Like very much
Color					
Texture					
Aroma					

Would you buy this flour in-store?.....

THANK YOU FOR YOUR PARTICIPATION!

Appendix 6. BLAST identification from ITS sequences

Sample sequence	Blast Similar Sequence	Method of identification	Similarity %	Ambiguity %	Length (bp)	GC content
Bk01_ITS4_G04_3730XL	Musa hybrid cultivar AB Group	Mega blast	84.39	15.61	734	57.59
Bk02_ITS4_H04_3730XL	*Musa acuminata* subsp. *Malaccensis* *AAA Group*	Mega blast	99.52	0.48	733	63.57

Bk03_ITS4_A05_373 0XL	*Musa acuminata* subsp. *Malaccensis* *AAA Group*	Mega blast	99.68	0.32	849	60.54
Bk04_ITS4_B05_373 0XL	*Musa acuminata* subsp. *Malaccensis* *AAA Group*	Mega blast	93.50	6.5	958	57.10
Bk05_ITS4_C05_373 0XL	*Musa balbisiana* B genome	Mega blast	93.35	6.65	781	63.00
Bk06_ITS4_D05_373 0XL	*Musa balbisiana* *B genome*	Mega blast	99.52	0.48	630	63.97
Bk07_ITS4_E05_373 0XL	*Musa acuminata* subsp. *Malaccensis* *AAA group*	Mega blast	99.84	0.16	866	61.89
Bk08_ITS4_F05_373 0XL	*Musa acuminata* subsp. *Malaccensis* AAA Group	Mega blast	99.68	0.32	744	63.84
Bk09_ITS4_G05_373 0XL	*Musa acuminata* subsp. *Malaccensis*	Mega blast	99.84	0.16	738	63.01

Bk10_ITS4_G02_3730XL	*Musa hybrid* *AB Group*	Mega blast	87.83	12.17	384	58.33
Bk11_ITS4_A06_3730XL	*Musa balbisiana* *B genome*	Mega blast	99.52	0.48	820	63.41
Bk12_ITS4_B06_3730XL	*Musa acuminata* subsp. *Malaccensis* AAA Group	Mega blast	99.52	0.48	796	62.94

Appendix 7.BLAST identification from ITS 1 sequence

Sample sequence	**Blast Similar Sequence**	**Method of identification**	**Similarity %**	**Ambiguity %**	**Length (bp)**	**Gc content**
Bk01_ITS1_C03_3730XL	Musa Acuminata AAA Group	Mega blast	84.32	15.68	628	60.71
Bk02_ITS1_D03_3730XL	*Musa acuminata* subsp. *Malaccensis* *AAA Group*	Mega blast	99.36	0.64	625	63.36
Bk03_ITS1_E03_3730XL	*Musa acuminata* subsp. *Malaccensis*	Mega blast	99.84	0.16	627	63.48

	AAA Group					
Bk04_ITS1_F03_373 0XL	*Musa acuminata* *AAA Group*	Mega blast	84.47	15.53	625	60.96
Bk05_ITS1_G03_373 0XL	*Musa ABB* ABB group	Mega blast	99.68	0.32	622	63.99
Bk06_ITS1_H03_373 0XL	*Musa ABB* *ABB Group*	Mega blast	99.68	0.32	626	63.74
Bk07_ITS1_A04_373 0XL	*Musa acuminata* subsp. *Malaccensis* *AAA group*	Mega blast	99.84	0.16	628	63.38
Bk08_ITS1_B04_373 0XL	*Musa acuminata* subsp. *Malaccensis* AAA Group	Mega blast	99.84	0.16	618	63.11
Bk09_ITS1_C04_373 0XL	*Musa acuminata* subsp. *Malaccensis*	Mega blast	99.52	0.48	625	63.52
Bk10_ITS1_D04_373 0XL	*Musa acuminata* subsp. *Malaccensis*	Mega blast	99.84	0.16	624	63.30

Bk11_ITS1_E04_373 0XL	*Musa ABB* *ABB Group*	Mega blast	99.52	0.48	630	63.65
Bk12_ITS1_F04_373 0XL	*Musa acuminata* subsp. *Malaccensis* AAA Group	Mega blast	98.07	1.93	625	63.52

Appendix 8. ITS4 NCBI BLAST Reference sequences

ITS 4 NCBI BLAST Reference Sequences used for sample identification
1. BK01_ITS4 >MF496114.1 Musa hybrid cultivar isolate MN005 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence
2. BK02_ITS4 >HG996476.1 Musa acuminata subsp. malaccensis strain Doubled-haploid Pahang (DH-Pahang) genome assembly, chromosome: A10 Wild malaysian banana (*Musa acuminata* subsp. *malaccensis*) is a subspecies of dwarf banana (*Musa acuminata*)
3. BK03_ITS4 >HG996476.1 Musa acuminata subsp. malaccensis strain Doubled-haploid Pahang (DH-Pahang) genome assembly, chromosome: A10 Wild malaysian banana (*Musa acuminata* subsp. *malaccensis*) is a subspecies of dwarf banana (*Musa acuminata*)
4. BK04_ITS4 >KT257609.1 Musa acuminata subsp. malaccensis isolate SS&JS 137 Pa Ranong 2 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
5. BK05_ITS4 >MT159447.1 Musa balbisiana voucher PBL 29681 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

6. BKO6_ITS4 >MT159446.1 Musa balbisiana voucher PBL 29677 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence
7. BK07_ITS4 >HG996476.1 Musa acuminata subsp. malaccensis strain Doubled-haploid Pahang (DH-Pahang) genome assembly, chromosome: A10
8. BK08_ITS4 >HG996476.1 Musa acuminata subsp. malaccensis strain Doubled-haploid Pahang (DH-Pahang) genome assembly, chromosome: A10
9. BK09_ITS4 >HG996476.1 Musa acuminata subsp. malaccensis strain Doubled-haploid Pahang (DH-Pahang) genome assembly, chromosome: A10
10. BK10_ITS4 >MF496114.1 Musa hybrid cultivar isolate MN005 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence
11. BK11_ITS4 >MT159446.1 Musa balbisiana voucher PBL 29677 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence
12. BK12_ITS4 >HG996476.1 Musa acuminata subsp. malaccensis strain Doubled-haploid Pahang (DH-Pahang) genome assembly, chromosome: A10

Appendix 9. Conference Presented Abstracts

Conference Presented Abstracts

1. Abstract of the 5th Cut International Conference 2023, Chinhoyi, Zimbabwe

Banana Cultivars in Zimbabwe: A Catalogue System

Beaton Kumbirai*[1], Kuri Farai[2], Mupfiga Upenyu[3], Chikwambi Zedias[1]

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Thematic group 7: Biodiversity Conservation, climate change, and sustainable development- Agro-ecological innovations and biodiversity conservation

ABSTRACT

Banana production in Zimbabwe has proved to be more viable compared to commonly grown field and horticultural crops. Being a perennial crop, banana offers food security making it a favorable crop for developing countries. Surveys of banana production showed that production in Zimbabwe is mainly done by smallholder farmers in the Manicaland province under contract system while large scale is being carried by plantations dotted across the country. The research was fuelled by the unavailability of proper documentation of the currently produced banana cultivars origin, genetic information and nutritional analysis such that germplasm conservation and improvements can be employed. Banana germplasm was collected from local farmers across the country in the form of young sword suckers and the GPS of the collection sites was captured and mapped out. Germplasm was established on a 1000 m^2 piece of research land. For over 24 months, the germplasm was monitored, maintained and recorded following molecular and morphological analysis, fruit evaluation and *in-vitro* propagation. Seven distinct cultivars resulted from the analysis and were catalogued. The catalogue serves as an identification system that creates a reservoir of knowledge about each plant's performance. It provides preliminary information on the genetic, morphological and yield characteristics of the seven cultivars. It is intended to serve as a useful guide in the identification of banana cultivars and in the selection of cultivars for further evaluation by researchers.

Keywords: Banana, food security, gene-bank, catalogue, genetic conservation,

2. Abstract of the 5th Cut International Conference 2023, Chinhoyi, Zimbabwe

Nutrient and flour properties analysis of the banana fruits bio-banked at Chinhoyi University farm

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Thematic group 1: Sustainable food systems, indigenous knowledge systems, innovation and industrialization- Food security, SDGs and industrialization

ABSTRACT

Bananas are staple food to some of the developing countries hence it is of paramount importance to evaluate their nutrition. Researchers concur that the proximate analysis of banana pulp dry matter provides valuable information on nutritional composition. Banana flour is a gluten free starchy nutrition that contributes greatly to gluten intolerance problems. The use of green bananas in the production of flour has since been employed in the country to mitigate post-harvest losses as well as generate revenue. This study was aimed at evaluating the proximate compositions of established banana germplasm at the Chinhoyi University of Technology owned Hunyani Farm. Germplasm was characterized into five Musa genome groups namely *Musa acuminata, Musa acuminata maleccensis, Musa Balbisiana, Musa ABB* and *Musa AB hybrid*. Starch, carbohydrates and sugars were assessed and found present in all the samples as revealed by the qualitative tests. Proximate analysis of the samples were carried out in triplicates. The percentage moisture content proximate analysis showed that *Musa ABB, AB hybrid* and *balbisiana* cultivars have lower percentages 68.32 %, 68.90 % and 65.80 % respectively as compared to *Musa Acuminata (73.76 %)* and *Musa acuminata malaccensis (72.95 %)*. A higher ash content was also noted in *Musa AB* hybrid and *Balbisiana which* both recorded 1.25 % and 1.10 % respectively. Crude fibre percentage content was highest in *Musa AB* hybrids (1.60 %) following *Musa balbisiana (1.54 %) and Musa ABB (1.50* %). Results suggested that culinary bananas are more suited for flour production as they have lower glycemic index than the Cavendish group.

Keywords: Banana, nutritional value, proximate analysis, flour starch, value addition.

Appendix 10. Publications

Review Paper

The Thesis generated the following review paper:

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Banana production in Zimbabwe: an analysis from a biotechnological pespective

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Abstract
Globally, banana (*Musa Spp.)* is ranked fourth in terms of gross value production following rice, wheat and maize and its production in Zimbabwe is ranked 57 amongst 132 producing countries. Banana farming in Zimbabwe has been found to produce more profit as compared to maize and tomatoes as far as the dollar to ton ratio is concerned and therefore can be a major contributor to the economy of Zimbabwe. The review serves to troubleshoot and addressed the unavailability of proper documentation of the currently produced banana cultivars' origin, genetic information and nutritional analysis such that germplasm conservation and improvements can be employed. The fruit requires an identification system for breeding purposes and assessments, which would then pose as an easy way of knowing characteristics, strengths and weaknesses of a particular cultivar before production. Apart from knowing the genetic characteristics of bananas, its geo-spatial distribution requires assessment. Banana production in Zimbabwe is concentrated in the warm and humid areas of Natural Region but similar climatic conditions are found in areas dotted across Zimbabwe. Using species distribution models, cultivars can be mapped to new environments mimicking the original production conditions.

Keywords: *Musa Spp.* germplasm, genetic characterization, gene pool, morphological characterization, species distribution models

Preprint

Kumbirai Beaton[1], Zedias Chikwambi[1], Ezekia Svotwa[2], Upenyu Mupfiga[3]. BANANA PRODUCTION IN ZIMBABWE: AN ANALYSIS FROM A BIOTECHNOLOGICAL PERSPECTIVE. Authorea. April 04, 2022. DOI: 10.22541/au.164909002.27016069/v1

Research Papers

1. Beaton, K., Mazadza, A. & Chikwambi, Z. Identification of Zimbabwe's locally grown banana (*Musa* Spp.) cultivars using morphology and genome-targeted sequencing. *J Genet Eng Biotechnol* **21**, 118 (2023). https://doi.org/10.1186/s43141-023-00562-

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Identification of Zimbabwe's locally grown banana (*Musa* Spp.) cultivars using morphology and genome-targeted sequencing

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Abstract

Background Banana production is increasingly under threat due to harsh weather conditions as a result of climate change and different diseases. As such there is a need for the preservation and the characterization of the banana cultivar population for the purposes of crop improvement. The identification of collected banana germplasm in Zimbabwe was conducted based on the Inter-transcribed spacer region as well as morphology. The study was conducted with the aim of distinguishing one cultivar from another towards genetic conservation as well as banana improvement.

Results ITS 1 and ITS 4 region targeting primers were used to amplify the DNA from twelve cultivars as well as sequence. Blast results identified five *Musa* groups which are *Musa balbisiana (BB), Musa ABB, Musa AB hybrid, Musa acuminata (AAA)*, and *Musa acuminata subsp. Malaccensis (AA).* Phylogenetic analysis was done on the sequences under study and a maximum likelihood tree was generated to determine relationships between the sequences. Further identification was done using the inflorescence, bract, and male bud and fruit characteristics of each cultivar complementing the molecular evaluation.

Conclusion Genetic and morphological identification of locally grown bananas was therefore successful. An important step towards identifying pure lines suitable for breeding.

Keywords Banana, Target sequencing, Genetic diversity, Inter transcribed spacer

2. Suitability modelling of banana production in Zimbabwe using MaxEnt. **Journal of Basic and Applied Ecology**. Beaton Kumbirai[1], Manyuchi Tatenda[2], Mupfiga Upenyu[3], Chikwambi Zedias[1]
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The thesis generated three publications.