Characterisation, identification and integration of drought tolerance in banana (*Musa* spp.)

By

Moureen Nansamba

MSc Plant Sciences (Plant breeding and Genetic resources), Wageningen University, The Netherlands

BSc Horticulture, Makerere University, Uganda

A Thesis Submitted in Fulfilment of the Requirements for the Degree of Doctor of Philosophy (PhD) in Plant Breeding

Discipline of Plant Breeding

School of Agricultural, Earth and Environmental Sciences
College of Agriculture, Engineering and Science
University of KwaZulu-Natal
Republic of South Africa

December 2024

Thesis Abstract

Banana (*Musa* spp.) is one of the major staple foods grown in the subtropical and tropical parts of the world. Given the increasing drought occurrences in sub-Saharan Africa and high susceptibility of banana to water stress, the improvement of cultivars through breeding offers the most effective and long-term solution to protect the crop against the daunting effects of climate change. Therefore, the objectives of this study were: (1) to assess the effects of drought on banana production and identify management strategies deployed by farmers (2) to determine the response of a diverse set of *Musa* genotypes to water stress based on phenotypic and physiological traits and select promising genotypes for use in banana drought tolerance breeding (3) to analyse the hybridisation success of the two selected drought-tolerant candidate male parental lines based on pollination success, seed production, embryo recovery and embryo germination rates (4) to determine the genetic relationships and diversity among 55 banana genotypes using DArT-based SNP markers and (5) to determine the variability of water usage and assess the growth behaviour and transpiration responses of secondary banana hybrids and their parental lines to declining soil water content in function of vapour pressure deficit and light intensity.

A baseline participatory study involving 120 banana farmers selected from eight major banana growing districts in the cattle corridor of Uganda showed that the majority of the farmers were small-scale holders utilizing intercropping systems and growing mostly East African Highland (EAHB) cooking-type bananas. Fifteen drought stress effects were reported, with reduced bunch weight (90%) being the most prevalent. Amongst the different drought mitigation practices, mulching (56%) was used the most, although irrigation (10%) was the most effective option, but the high cost of water pumps and water scarcity limited its deployment. The extent of deployment of mitigation practices was mostly low (72%), with farmers applying only 1-3 practices.

The second study evaluated 16 genetically diverse banana genotypes in the screen-house at the National Agricultural Research Laboratories, Uganda, under well-watered and water stress conditions. Water stress significantly reduced the plant height (PH), total leaf area (TLA), number of functional leaves (FL), total dry matter (TDM), chlorophyll content (CC) and relative water content (RWC). Genotype by water treatment interaction effects

were significant for TLA, PH, FL, number of new leaf cigars (LC) and RWC ($p < 0.01$; $p < 0.05$). The water use efficiency (WUE) of 12 genotypes increased under water stress conditions. Stomatal conductance was also affected by the genotype x water treatment interaction ($p < 0.05$). 'ITC.0987' was the most tolerant, whilst among the improved diploids, 'TMB2x9722-1' had the least TDM reduction and the highest WUE, and 'TMB2x9172' showed the least decrease in RWC and highest root-shoot ratio (RSR) under stress conditions. Thus, 'ITC.0987', 'TMB2x9722-1' and 'TMB2x9172' are essential drought-tolerant candidates that may be utilized in breeding.

In the third study, pollination success, seed set rate, embryo recovery and germination success were determined for crosses between four tetraploid *Musa* hybrids, including '660K-1', '917K-2', '1201K-1' and '222K-1' (female parents) with two diploid drought-tolerant candidates, 'TMB2x9722-1' and 'TMB2x9172' (male parents). Seed set ($p < 0.05$), embryo recovery ($p < 0.001$) and embryo germination ($p < 0.001$) were significantly affected by female-male parent interaction effects. Crosses with 'TMB2x9172' exhibited relatively higher pollination success, seed set, and embryo recovery than those with 'TMB2x9722-1'. Thus, 'TMB2x9172' was the more ideal male parent for generating progeny populations for subsequent drought tolerance studies.

The fourth study determined the genetic relationships and diversity among 55 *Musa* spp. genotypes using 1551 high-quality DArTseq-based SNP markers. The study population comprised breeding lines, *Musa* progenies, wild species and landraces. Neighbour-joining (NJ) cluster analysis divided the population into two major clusters, with one large cluster consisting of all the breeding lines, progenies, 'Mpologoma' (AAA) and 'Calcutta-4' (AA) and a smaller cluster consisting of '*Musa balbisiana'* and three landraces, all with a 'B' genome. We zoomed into the genetic relationships of 16 selected *Musa* progenies and their drought-sensitive female ('917K-2') and tolerant male ('TMB2x9172') parents and identified sub-clusters of uniquely and genetically similar progenies. Principal component analysis was consistent with the NJ tree and predicted that three progenies, 'NM101F1', 'MNK-17-11' and 'MNK-17-12', were more genetically identical to their male parent, while the rest of the progenies were more identical to the female parent. Analysis of molecular variance showed that 88% and 12% of the total genetic variation were within and between the gene pools, respectively. The SNP markers had a mean proportion of

polymorphic loci and observed heterozygosity of 0.65 and 0.36, respectively, indicating the existence of substantial genetic diversity across the study population.

The fifth study investigated the water usage, plant growth and transpiration rates (E_{rate}) of 18 *Musa* spp. hybrids, including 16 progenies and their two parental genotypes. Traits recorded were duration of soil water content (SWC) decline from 2.1 g g^{-1} (-0.01 MPa) to 0.7 g g^{-1} (-1.92 MPa), functional total leaf area (TLA), leaf damage, and transpiration rates (E_{rate}). E_{rates} were modelled in relation to SWC, vapour pressure deficit (VPD) and light intensity according to the Jarvis-Stewart model. The number of days taken for SWC to drop to 0.7 g g-1 (-1.92 MPa) differed significantly ($p < 0.05$) among the genotypes. The TLA, leaf damage and E_{rate} were significantly affected by the genotype, water treatment and genotype x water treatment interaction effects. The female parent ('917K-2') and four triploid progenies, 'MNK-16-3', 'MNK-16-16', 'MNK-16-8' and 'MNK-17-5', exhibited good growth potential under optimum conditions, but their growth was significantly reduced during short periods of water stress (17-22 days). The male parent ('TMB2x9172') and two diploid progenies, 'MNK-17-11' and 'MNK-17-12'' took longer to deplete their soil moisture (28-35 days) and sustain relatively good growth under water stress conditions. 'MNK-16-2', 'MNK-16-4', 'MNK-16-5' and 'MNK-17-6' (all triploids) had relatively good growth under both well-watered and stress conditions but relatively fast soil water depletion. Genotype-specific critical thresholds (SWC_{crit}) were observed, implying varying stomatal and or hydraulic control levels. Based on the daily E_{rate} model, 'MNK-17-6' had the highest SWC_{crit} (4.92 g g^{-1}, -0.0002 MPa), indicating a conservative, drought-avoiding response to a declining SWC. Among the eight genotypes assessed under gradual increase in VPD and light, 'MNK-17-4' and 'MNK-17-12' had the fastest and slowest E_{rate} increases, respectively.

In conclusion, drought is a major threat to sustainable banana production in the East African Great Lakes region. Therefore, there is an urgent need to develop and deploy varieties with improved tolerance to drought. Also, farmers need to prioritise preventive drought mitigation practices. The selected drought-tolerant candidate banana genotypes (diploids) are recommended for further field testing and future use in crosses as male parents to generate larger segregating populations and or improve drought tolerance in cultivars that are susceptible but possess desirable agronomic and taste attributes, e.g. the

EAHBs. The causes of low hybridisation success and poor embryo germination in crosses with the drought-tolerant candidate, ‘TMB2x9722-1’, should be investigated to enhance its utilisation in banana drought tolerance crossbreeding. The study also recommends crossing ‘TMB2x9172’and ‘TMB2x9722-1’ to generate even more drought-tolerant hybrids. Understanding the genetic relations of the progenies is valuable in providing insight into the genetic diversity underlying the phenotypic diversity, especially regarding their responses to water stress. The great phenotypic variation observed in the water usage, growth, and transpiration behaviour of the tested progenies in relation to the fluctuating environment suggests that the suitability of a given progeny will depend on the prevailing drought scenario. The relatively drought-tolerant diploid progenies, ‘MNK-17-11’ and ‘MNK-17-12’, should be further evaluated for their growth and yield potential under optimum and drought field conditions. The contrasting phenotypic behaviour among genetically similar progenies highlighted the significance of the environmental impact on a plant’s phenotypic expression. Despite the challenges posed by the complexity of banana drought tolerance, this work illustrates that banana drought tolerance improvement through crossbreeding is possible.

Declaration 1: Plagiarism

I, **Moureen Nansamba**, declare that.

1. The research reported in this thesis, except where otherwise indicated, is my original research.
2. This thesis has not been submitted for any degree or examination at any other university.
3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
    a. Their words have been re-written but the general information attributed to them has been referenced
    b. Where their exact words have been used, then their writing has been placed in italics and inside quotation marks and referenced.
5. This thesis does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the thesis and in the References sections.

Signed:

Moureen Nansamba

As the candidate's supervisor, I agree to the submission of this thesis

Prof Julia Sibiya (Supervisor)

Dr Robooni Tumuhimbise (Co-supervisor)

Declaration 2: Publications

The following chapters have been published in peer reviewed journals.

Chapter 2

1. **Nansamba, M.,** J. Sibiya, R. Tumuhimbise, D. Karamura, J. Kubiriba, and Karamura E. 2020. Breeding banana (*Musa* spp.) for drought tolerance: A review. Plant Breed. 139:685–696. https://doi.org/10.1111/pbr.12812

Chapter 3

2. **Nansamba, M.,** J. Sibiya, R. Tumuhimbise, W. Ocimati, E. Kikulwe, et al. 2022. Assessing drought effects on banana production and on-farm coping strategies by farmers — a study in the cattle corridor of Uganda. Clim. Change 173:21. https://doi.org/10.1007/s10584-022-03408-w

Chapter 4

3. **Nansamba, M.,** J. Sibiya, R. Tumuhimbise, D. Karamura, J. Ssekandi, et al. 2023. Response of banana (*Musa* spp.) to drought stress based on phenotypic and physiological traits. J. Crop Improv. 37:751-775. https://doi.org/10.1080/15427528.2022.2148313

Acknowledgments

This thesis summaries all the hard work that has gone into this research for the last couple of years. I have had the opportunity to work with a great team of people from Uganda, South Africa and Belgium. To say the least, I could never have completed this research successfully without their support. As such, I would like to express my heartfelt gratitude to the following individuals and institutions.

My supervisors, Prof Julia Sibiya (University of KwaZulu-Natal) and Dr Robooni Tumuhimbise (National Agricultural Research Organisation, Uganda), for their continuous encouragement, supervision, technical guidance and support.

Dr Sebastien Carpentier of the Alliance of Bioversity International and CIAT and Katholieke University, Leuven (KU-Leuven), for his unwavering and immense technical input and guidance, supervision and administrative support, especially for Chapter 7, which was performed at the Bioversity International phenotyping facility in Belgium.

The team at the Laboratory for Tropical Crop Improvement, KU-Leuven, specifically Clara Gambart, Poi Verwilt, Loïck Derette, Daan van Gils and Scott Lemmens, for their unwavering assistance during my experiments at KU-Leuven. Catherine Breton for her assistance with bioinformatics procedures.

The Consultative Group for International Agricultural Research (CGIAR) program on Roots, Tubers and Bananas (Biotech Project: B100821 C100414) for funding this research and my academic fees.

The Belgian Development Cooperation for providing financial support for my travel, stay and research activities at KU-Leuven.

Joseph Ssekandi, Naboth Oyesigye, Elias Oyesigye, Isaac Ninsiima, Isabel Mubangizi and Osbert Atubeho for their immense contribution towards my farm visits, laboratory, field and screen-house experiments.

Dr Eldad Karamura and Dr Deborah Karamura. Your experience and passion for banana research, were inspiring and I am lucky to have had you both as my supervisors. Thank

you for your continuous guidance, even during your retirement. I couldn't have had better mentors to start my research career with.

The National Agricultural Research Laboratories at Kawanda, Uganda, for allowing me to perform my screen-house and laboratory experiments at their facilities.

A special thank you to my parents and siblings for their continuous prayers and unconditional support through it all.

Above all, I would like to the Almighty God for His constant strength and provision in this unforgettable experience. Thank You for being with me every step of the way.

Dedication

This thesis is dedicated to my beloved parents, Mr & Mrs Lubega and my siblings Olivia, Nicholas, Michael, Stella and Prossy.

Table of Contents

List of tables

List of figures

Appendices

Abbreviations and acronyms

ADMX	Admixed genotypes
AFLP	Amplified fragment length polymorphism
CC	Chlorophyll content
CTAB	Cetyltrimethylammonium bromide method
DArT	Diversity Arrays Technology
DArT P/L	Diversity Arrays Technology Pty Ltd
DArTseq	Diversity Arrays Technology sequencing
DH	Double haploid
EAGL	East African Great Lakes region
EAHB	East African highland bananas
ECAGC	East and Central Africa banana germplasm collection
E_{rate}	Transpiration rate
ET	Evapotranspiration
EU	European Union
FAO	Food and Agricultural Organization
FL	Number of functional leaves
GIGWA	Genotype Investigator for Genome-Wide Analyses
IITA	International Institute of Tropical Agriculture
INIBAP	International Network for the Improvement of Banana and Plantain

ITC	International transit centre
KU-Leuven	Katholieke University, Leuven
LC	Number of new leaf cigars
MGIS	*Musa* Germplasm Information System
NARL	National Agricultural Research Laboratories, Uganda
NARO	National Agricultural Research Organization, Uganda
PAR	Photosynthetic Active Radiation
PCA	Principal component analysis
PH	Plant height
QTL	Quantitative trait loci
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
RMSE	Root-mean-square error
RSR	Root: shoot ratio
RWC	Relative water content
SC	Stomatal conductance
SNP	Single nucleotide polymorphism
SSR	Simple sequence repeats
SWC	Soil water content
SWC_{crit}	Threshold soil water content at which stomatal closure starts

SWC_{slope}	Slope of transpiration rate reduction
SWC_{wilt}	Soil water content at which water uptake is ceased
SWP	Soil water potential
TDM	Total dry matter
TLA	Total leaf area
VPD	Vapor pressure deficit
WAI	Weighted average index
WS	Water-stress treatment
WUE	Water use efficiency
WW	Well-watered treatment

Chapter 1: Introduction to thesis

1.1 Background

Banana (*Musa* spp.) ranks among the world's top ten food commodities (Anders et al., 2021), with an estimated annual production volume of 135.1 million tonnes harvested from a total global acreage of 5.94 million ha (FAOSTAT, 2022). It is mainly grown in tropical and subtropical regions and consumed in various forms, including cooked (cooking banana), beverage (juice/beer banana), or eaten ripe and raw (dessert banana) (Karamura, 1998; Fungo and Pillay, 2011; Nansamba et al., 2022). Processed banana products include banana chips, starch and flour but their markets are still underdeveloped. Major banana exporting regions include Latin America (Ecuador, Costa Rica, Guatemala and Colombia), Asia (Philippines) and the Caribbean (FAO, 2023). In Africa, Côte d'Ivoire and Cameroon are the two leading exporters of bananas. Currently, all global banana export varieties belong to the Cavendish group and are valued at approximately US$12.8 billion (FAOSTAT, 2021). In developing countries, the crop is mainly grown for domestic consumption in both urban and rural areas.

Bananas are grown in nearly all tropical countries in Africa. Of particular interest is the East African Great Lakes (EAGL) region, which hosts some of the largest banana production systems on the continent. In fact, in 2021, the EAGL region constituted about 51.1% of Africa's banana production according to FAOSTAT estimates. Banana production in this region is dominated mainly by smallholder farmers who rely on the crop for their food and income source (Lynam, 2000; Gambart et al., 2020). In this region, the East African Highland Bananas (EAHBs, *Musa* AAA genome), believed to have originated from Southeast Asia and Indochina (Simmonds, 1962), comprise most of the bananas cultivated. For instance, in Uganda, EAHBs are a major daily energy source for over 13 million people (Bill and Melinda Gates Foundation, 2014) and are grown by at least 75% of the farmers (Gold et al., 2002; Tumuhimbise et al., 2018). Karamura et al. (2012) estimated the region's banana annual per capita consumption to range between 250 and 600 kg. The EAHBs were introduced into the EAGL region around 2500-3000 years ago, and the region has since become the secondary centre of diversity for the EAHBs (AAA genome) (Simmonds, 1966). The current diversity originates from relatively few meiosis events, followed by somaclonal mutations and continuous selection by farmers (Sardos et al., 2016a).

Despite its importance, banana productivity in the EAGL region remains below (<30 t ha^{-1} $year^{-1}$) the potential (>70 t ha^{-1} $year^{-1}$) (Tushemereirwe et al., 2001; van Asten et al., 2005) due to several biotic and abiotic constraints. Among the banana pests, the banana weevil *Cosmopolites sordidus* (Germar) and parasitic nematodes (*Pratylenchus coffeae* and *Radopholus similis*) are the most destructive (Gowen et al., 2005; Ocan et al. 2008). Major diseases of banana include fusarium wilt (*Fusarium oxysporum* f.sp. *cubense*) (Kangire et al., 2000), banana bacterial wilt (*Xanthomonas campestris* pv. *musacearum*) (Tripathi et al., 2013; Ocimati et al., 2014) and black Sigatoka (also known as black leaf streak disease) (*Mycosphaerella fijiensis*) (Barekye et al., 2009). One of the key solutions to tackle some of the above biotic challenges is developing disease and pest resistant varieties through crossbreeding (Tenkouano et al., 2003; Tumuhimbise et al., 2019; Madalla et al., 2022a). For instance, the National Agricultural Research Organization (NARO) of Uganda bred and released four improved cultivars ('NAROBan1', 'NAROBan2', 'NAROBan3', and 'NAROBan4') with resistances to black Sigatoka, banana weevil and nematodes, three of the country's major pests and diseases (Tumuhimbise et al., 2018). Moreover, these banana hybrids produce high yields (54.9 - 68.8 t/ha/year) and possess desirable consumer taste attributes such as a soft, yellow and tasty pulp. The same breeding program released earlier a 'matooke' hybrid called 'Kiwangaazi', which is also resistant to nematodes, black Sigatoka and banana weevils (Nowakunda et al., 2015). Other efforts to improve host-plant resistance and key agronomic attributes have come from a joint research partnership between NARO and the International Institute of Tropical Agriculture (IITA) (Madalla et al., 2022a; 2022b).

Regarding abiotic stresses, drought is the leading factor constraining banana production in the East African highlands (Taulya et al., 2006; van Asten et al., 2011; Uwimana et al., 2021; Nansamba et al., 2022). EAHBs have a high water usage, low water use efficiency, and a weak drought avoidance mechanism (van Wesemael et al., 2019; Eyland et al., 2021). While it is predicted that among all the roots, tubers and banana crops, bananas will exhibit the highest growth in demand and supply under future climates (Petsakos et al., 2019; Manners et al., 2021), increasing rainfall variability and vapour pressure deficits caused by rising atmospheric temperatures pose a significant risk for its production and yield (Varma and Bebber, 2019; Abdoussalami et al., 2023). EAHB bananas have received much attention with respect to breeding for improved pest and disease resistance and high yield but much effort is still required to enhance the crop's resilience to abiotic stresses (Nowakunda et al., 2015; Madalla

et al., 2022a), including drought stress. This calls for research efforts geared towards the development of improved banana varieties with adaptability to limited rainfall conditions. In the EAGL region and globally, recurrent and severe droughts associated with unpredictable rainfall patterns or seasonal rainfall variability, emanate from climate change and extreme weather changes (Hepworth and Goulden, 2008; Mohammed et al., 2022). To offset the devasting impacts of climate change on crop production (and hunger), investments in agricultural research, water management and rural infrastructure are required (Sulser et al., 2021).

1.2 Problem statement

Drought occurs when the soil water supply (from rainfall and/or irrigation) is inadequate to meet the crop's transpiration needs (Tuberosa et al., 2012). Depending on the variety and geographical location, the banana crop requires mean annual rainfall ranging between 1200 mm and 2690 mm for optimal growth (Carr, 2009; Ravi et al., 2013). However, major banana production systems, such as the East African highlands, lie within marginal areas (characterised by seasonal droughts) where bananas are mainly grown by small-scale farmers with limited financial resources to set up irrigation systems. Given that crop production in this region is completely rainfed, banana cultivation is greatly threatened in the face of climate change phenomena, including water scarcity, increasing temperatures and erratic weather patterns (Sabiti et al., 2018; Girvetz et al., 2019; Vandamme et al., 2022). Agnolucci et al. (2020) have already reported the negative impacts of rising temperatures on the yields of several crop species across countries. With climate change, frequent and more severe droughts are expected in the Sub-Saharan African region (Owoyesigire et al., 2016; Sabiti et al., 2018; Chari and Ngcamu, 2022). It is also projected that rainfall variability and atmospheric vapor pressure deficits will increase due to increasing temperatures, thereby negatively impacting production potential and yield gains (Rippke et al., 2016; Varma and Bebber, 2019; Abdoussalami et al., 2023). For instance, Sabiti et al. (2018) predicted that soil water deficits resulting from expected temperature rises and increased evaporative demand could retard future banana production in specific areas in Uganda. Moreover, in Central and Southwest Uganda, where banana is a major staple, drought reduces the bunch weight by 9% for every 100 mm yr^{-1} reduction in rainfall (van Asten et al., 2011). Other studies have also shown how seasonal

drought contributes immensely to the crop's failure to attain optimal growth and substantial yield losses (Wairegi et al., 2010; Uwimana et al., 2021).

It is critical to note that bananas have a long crop cycle of 12-18 months (depending on the cultivar), which exposes the crop to longer and /or more drought events (Ravi et al., 2013). Moreover, drought affects various banana growth stages, including the early vegetative stage, floral primordial initiation, flowering and bunch/finger development (Robinson and Alberts, 1986; Stevens et al., 2020). During the early stages, drought significantly reduces leaf production and the growth in plant height, plant girth and leaf area (Delfin et al., 2016; Uwimana et al., 2020; Eyland et al., 2022). In mature plants, drought stress delays the flowering time and bunch maturity and negatively impacts yield traits such as bunch weight, number of hands and number of fruits (Goenaga and Irizarry, 2000; Van Asten et al., 2011; Uwimana et al., 2020; Nkoulou et al., 2023). Considering that EAHBs are a primary staple food and an income generating commodity for millions of people in the EAGL region, drought indeed, poses a major threat to sustainable banana production, food- and income security. So far, no breeding program has purposely developed and released EAHB hybrids that are tolerant to drought.

Large collections of genetically diverse bananas are maintained *ex-situ* and *in-situ* by the International *Musa* Germplasm Transit Centre (ITC) and twelve banana breeding programmes spread across the world. The banana germplasm collection centre of East and Central Africa, hosted in Mbarara, Uganda, is one of the largest *ex-situ* conservation centres. The *Musa* Germplasm Information System (MGIS) indicates that over 440 accessions, including local and exotic types, are currently maintained in this germplasm collection. Among these accessions are diploids, some of which have been utilized in banana pest and disease resistance breeding (weevil, nematodes and black Sigatoka) (Nowakunda et al. 2015; Tumuhimbise et al. 2018) but have not been assessed for their response to water stress and hence their potential use in banana drought tolerance improvement is yet to be established.

The lack of banana cultivars in the EAGL region that are tolerant to drought conditions prompted several researchers to initiate screening and evaluation of different EAHB accessions under water stress conditions. (Taulya et al., 2006; Kayongo et al., 2015; Stevens et al., 2020). However, only a few accessions have been evaluated, given the huge investment (in time, space, financial and human resources) required to characterize a more extensive set of cultivars.

So far, over 115 different EAHB varieties consisting of a wide phenotypic diversity have been described (Tugume et al., 2002; Karamura et al., 2012; Perrier et al., 2019), and only a fraction of these accessions has been evaluated for their reaction to water stress. Therefore, the diversity (regarding drought tolerance) within several EAHB cultivars is not yet known. Further, literature on the inheritance of genomic regions controlling drought stress tolerance and phenotype–genotype associations in banana is very scanty. Sampangi-Ramaiah et al. (2023) identified five major quantitative trait loci (QTLs) on chromosomes 2, 5 and 8 for three drought tolerance-related traits, including leaf cuticular wax, adaxial stomatal density and leaf water retention capacity. Unfortunately, the F1 hybrids/ individuals used in this study were developed by crossing two wild banana relatives (*M. acuminata* ssp. *burmannicoides* Colla –'Calcutta-4' and *M. balbisiana* collection *'Bee hee kela'*), which potentially harbour and most likely transferred several undesirable agronomic and taste attributes to their offspring. This has made their deployment and adoption by farmers difficult, despite some of them being drought tolerant. Although wild crop relatives naturally possess beneficial breeding traits, they also often contain undesirable agronomic traits which are inherited by the offspring (Iskra-Caruana et al., 2014; Prohens et al., 2017). Moreover, information on the transfer of favourable drought tolerance alleles/ genes from cultivars with desirable agronomic and taste qualities (e.g., EAHBs or hybrids with an EAHB genetic background) to their offspring is lacking.

1.3 Rationale of this study

The increasing adverse effects of drought on banana productivity in the East African highlands pose a huge threat to sustainable banana production, food security and agricultural incomes. It is, therefore, crucial that more research efforts be directed towards enhanced drought stress management. Effective drought stress management solutions lie in breeding banana varieties that are adapted to drought stress and better cultivation and crop management practices. Therefore, involving farmers early in the breeding process in the form of participatory baseline studies will provide insight into the variability (in terms of drought sensitivity levels, i.e., highly sensitive, moderately tolerant, very tolerant, or not affected at all) within the genetically diverse banana gene pool (set of cultivars) that farmers maintain. Moreover, since participatory investigations require fewer financial resources, they offer a cheaper alternative for determining and documenting the variability within the large set of cultivars grown by farmers. The participatory baseline study will also be critical in identifying coping practices that banana

growers deploy to mitigate drought effects on-farm, the extent of deployment and the specific purpose for each practice. The knowledge from this systematic preliminary study will not only be crucial in supporting the improvement of farmer drought-coping practices but also inform more targeted research efforts geared towards improved banana drought tolerance and stress management.

While proper cultural and crop management practices can narrow the gap between the actual and maximum crop yields, the genotype determines the potential under specific climatic conditions (Negin and Moshelion, 2017). Drought-tolerant genotypes will sustain growth and bring forth reasonable yield even during limiting water conditions. The introduction of drought tolerance genes into farmer-preferred cultivars through crossbreeding requires prior selection of appropriate male parent candidates. Screening of the genetically diverse set of banana accessions maintained at the ECAGC in Uganda will be critical in identifying drought-tolerant candidates that can be utilized as male parents in mainstream banana genetic improvement initiatives. Successful selection for drought-tolerant candidates could be achieved through selection for morphological and physiological traits, including attributes pertaining to plant growth, plant water status and plant function (Ravi et al., 2013).

Selected candidates, particularly improved diploids, could be crossed with other accessions (e.g., improved EAHB tetraploids) to develop superior progenies with combined taste, agronomic and drought tolerance superiority. Utilization of improved diploid candidates in crosses will minimize the co-inheritance with poor taste and agronomic traits which are often harboured in wild banana relatives (Iskra-Caruana et al., 2014; Prohens et al., 2017). However, the usability of the selected diploid candidate in banana drought tolerance breeding will depend on the hybridization success after crossing it with a female fertile accession. Therefore, the efficiency of selected drought-tolerant candidates (used as male parents) in producing progenies through crossbreeding needs to be established in pre-breeding initiatives. This will facilitate the identification of the actual causes of low hybridization success due to the male candidate, if any, followed by the development of solutions for overcoming such breeding barriers to enhance its usability in future banana drought improvement.

The progenies developed from *Musa* spp. crosses with tolerant candidates need to be thoroughly evaluated for their reaction to water stress conditions before deployment to farmers. Given that drought hardly ever occurs by itself in nature and varies over time and location, the

growth potential and transpiration responses of progenies to drought should be assessed in relation with prevailing environmental factors such as light and vapor pressure deficit (VPD) (calculated from temperature and relative humidity). Construction of genotype-specific transpiration response models in conjunction with VPD, light and soil water potential will contribute immensely to successful breeding for unpredictable field conditions and hence enable the identification of improved genotypes suited for specific drought scenarios i.e., short, medium, and long drought periods.

Knowledge of plant genetic diversity and the association between the phenotype and genetic markers is critical for crop improvement. Diversity studies in banana have utilized both morphological and molecular markers (Sardos et al., 2016b; Nyine et al., 2017; Hinge et al., 2022). However, morphological markers are greatly affected by environmental variance, which lowers the selection efficiency during the development of improved cultivars. On the contrary, molecular markers are stable and not affected by environmental conditions since their determination is largely automated. Among the several molecular markers that have been used in banana research over the years are Diversity Array Technology (DArT) markers (Jaccoud et al., 2001; Risterucci et al., 2009; Hippolyte et al., 2010; Mbanjo et al., 2012; D'Hont et al., 2012; Sardos et al., 2016a). The DArT-based SNP markers not only provide an opportunity to assess the genetic diversity among EAHB populations and exotic banana germplasm (Sardos et al., 2016a; Nyine et al., 2019) but also offer sufficient variation to distinguish between closely related genotypes. As such, DArT markers will be very useful in determining the genotypic variation among *Musa* progenies sharing the same parental lines. Furthermore, understanding the relationship between the genotype and phenotype is crucial for identifying genotypes carrying favourable alleles/ genes that are correlated to drought tolerance as well as determine the potential of the drought-tolerant male candidate(s) to transfer favourable genes/alleles to their offspring(s).

1.4 Aim

The overall goal of this research study was to contribute to the improvement of East African Highland bananas through identification and introgression of drought tolerance genes into the EAHB populations.

1.5 Specific objectives

The specific objectives of the research were:

i) To assess the effects of drought on banana production and identify management strategies deployed by farmers.
ii) To determine the response of a diverse set of *Musa* genotypes to water stress based on phenotypic and physiological traits and select promising genotypes for use in banana drought tolerance breeding
iii) To analyse the hybridisation success of the two selected drought-tolerant candidate male parental lines based on pollination success, seed production, embryo recovery and embryo germination rates and to generate genotypes for use in subsequent genetic drought tolerance analyses.
iv) To determine the genetic relationships and diversity among 55 banana genotypes using DArT-based SNP markers.
v) To determine the variability of water usage and assess the growth behaviour and transpiration responses of secondary banana hybrids and their parental lines to a declining soil water content in function of vapour pressure deficit and light intensity.

1.6 Research hypothesis

The hypotheses of this study were:

i) Farmers in Uganda are aware of the impacts of drought on the growth and yield of locally grown banana varieties, variability within the cultivars and have devised management practices to mitigate the drought stress.
ii) Phenotypic traits and physiological traits significantly vary among *Musa* spp. genotypes under well-watered and water stress conditions and depend on the plant growth stage.
iii) The hybridisation success of two selected drought-tolerant candidate male parental lines varies significantly.
iv) Despite the narrow genetic diversity within the EAHB gene pool, a meiotic recombination will lead to the segregation of reactions of transpiration responses and resulting growth to declining soil water content and fluctuating light and vapor pressure deficit.

v) Valuable genetic diversity exists within the study's test *Musa* spp. genotypes for drought tolerance.

1.7 Outline of the thesis

This thesis consists of eight different but interconnected chapters. The first chapter (1) is a general introduction to the thesis while Chapter 2 is written as a separate review paper. Chapters 3-7 are written as separate research papers, each with a journal paper format. Chapter 8 covers the general overview and implications of the study's findings. There are some inevitable overlaps and repetitions of information and references throughout the chapters because they have been formatted as distinct journal research papers. The Crop Science Society of America (CSSA) referencing system was applied, except for the published chapters, which followed the specific journal's recommended style.

Therefore, the thesis outline is as follows:

1. Chapter 1: Introduction to thesis.
2. Chapter 2: A review of the literature.
3. Chapter 3: Assessing drought effects on banana production and on-farm coping strategies by farmers – a study in the cattle corridor of Uganda.
4. Chapter 4: Response of banana (*Musa* spp.) to water stress based on phenotypic and physiological traits.
5. Chapter 5: Evaluation of hybridization success of two improved drought-tolerant diploid bananas (*Musa* spp.).
6. Chapter 6: Genetic relationships and diversity analysis of banana germplasm using Diversity Array Technology (DArT)-based SNP markers: implications for banana drought tolerance breeding.
7. Chapter 7: Differential growth and transpiration response among banana hybrids - hope for banana drought tolerance breeding?
8. Chapter 8: General overview and implications of the study.

References

Abdoussalami, A., Z. Hu, A.R.T. Islam, and Z. Wu. 2023. Climate change and its impacts on banana production: a systematic analysis. Environ. Dev. Sustain. 1-30.

Agnolucci, P., C. Rapti, P. Alexander, V. De Lipsis, R.A. Holland, et al. 2020. Impacts of rising temperatures and farm management practices on global yields of 18 crops. Nature Food. 1:562-571.

Anders, S., W. Cowling, A. Pareek, K.J. Gupta, S.L. Singla-Pareek, and C.H. Foyer. 2021. Gaining acceptance of novel plant breeding technologies. Trends Plant Sci. 26:575-87.

Barekye, A., P. Tongoona, J. Derera, and W.K. Tushemereirwe. 2009. Variation in diploid (AA) and tetraploid (AAAA) banana populations for black Sigatoka resistance and agronomic traits. Afr Crop Sci J. 9:509–516.

Bill and Melinda Gates Foundation. 2014. Multi-crop value chain Phase II Tanzania/Uganda cooking banana.

Carr, M.K.V. 2009. The water relations and irrigation requirements of banana (*Musa* spp.). Exp. Agric. 45:333–371.

Chari, F., and B.S. Ngcamu. 2022. Climate change and its impact on urban agriculture in Sub-Saharan Africa: A literature review. Environ. Socio-Econ. Stud. 10: 22-32.

D'Hont, A., F. Denoeud, J.M. Aury, F.C. Baurens, F. Carreel, et al. 2012. The banana (*Musa acuminata*) genome and the evolution of monocotyledonous plants. Nature 488:213-217.

FAOSTAT. 2022. Food and Agriculture Organization of the United Nations Database of agricultural production. FAO Statistical Databases. http:www.fao.org/faostat/ Accessed 12 July 2024.

FAO. 2023. Banana Market Review 2022. Food and Agriculture Organization, Rome, Italy.

Fungo, R., and M. Pillay. 2011. β-Carotene content of selected banana genotypes from Uganda. Afr J. Biotechnol. 10:5423–5430.

Gambart, C., R. Swennen, G. Blomme, J.C. Groot, R. Remans, and W. Ocimati. 2020. Impact and opportunities of agroecological intensification strategies on farm performance: A case study of banana-based systems in Central and South-western Uganda. Front. Sustain Food Syst. 4:87.

Girvetz, E., J. Ramirez-Villegas, L. Claessens, C. Lamanna, C. Navarro-racines, et al. 2019. Future climate projections in Africa: where are we headed? P.15-27, *In* T. Rosenstock, A. Nowak, and E. Girvetz, eds. The Climate-Smart Agriculture Papers: Investigating the

business of a productive, resilient and low emission future. Springer Nature, Cham, Switzerland.

Goenaga, R., and H. Irizarry. 2000. Yield and quality of banana irrigated with fractions of class A pan evaporation on an oxisol. Agron J. 92:1008–1012.

Gold, C.S., Kiggundu, A., Abera, A.M.K, and Karamura, D. 2002. Diversity, distribution and farmer preference of *Musa* cultivars in Uganda. Exp. Agric. 38: 39-50.

Gowen, S.C., P. Quénéhervé, and R. Fogain. 2005. Nematode parasites of bananas and plantains, p. 611-643, *In* M. Luc, R.A. Sikora, and J. Bridge, eds. Plant parasitic nematodes in subtropical and tropical agriculture. 2nd ed. CAB International, Wallingford, UK.

Hepworth, N., and M. Goulden. 2008. Climate change in Uganda: Understanding the implications and appraising the response. LTS International, Edinburgh, UK.

Hinge, V.R., I.M. Shaikh, R.I. Chavhan, A.S. Deshmukh, R.M. Shelake, et al. 2022. Assessment of genetic diversity and volatile content of commercially grown banana (*Musa* spp.) cultivars. Sci. Rep. 12:7979.

Iskra-Caruana, M., M. Chabannes, P.O. Duroy, and E. Muller. 2014. A possible scenario for the evolution of banana streak virus in banana. Virus Res. 186:155–162.

Jaccoud, D., K. Peng, D. Feinstein, and A. Kilian. 2001. Diversity arrays: a solid state technology for sequence information independent genotyping. Nucleic Acids Res. 29: e25.

Kangire, A., E.B. Karamura, C. Gold, and M.A. Rutherford. 2000. Fusarium wilt of banana in Uganda, with special emphasis on wilt-like symptoms observed on East African Highland cooking cultivars (*Musa* spp., AAA). Acta Hortic. 540:343–353.

Karamura, D.A. 1998. Numerical taxonomic studies of the East African Highland Bananas (*Musa* AAA-East Africa) in Uganda. PhD thesis. University of Reading, London, UK.

Karamura, D.A., E. Karamura, and W. Tinzaara. 2012. Banana cultivar names, synonyms and their usage in East Africa. Bioversity International, Kampala, Uganda.

Kayongo, S.N., J.M. Sebuliba, and K. Nyombi. 2015. Responses of East African highland banana (EAHB-AAA) cultivars to drought stress. Uganda J. Agric. Sci. 16:55-81.

Lynam, J.K. 2000. Market development and production potential for banana and plantain. Acta Hortic. 540:39-53.

Madalla, N.A., C. Massawe, M. Shimwela, D. Mbongo, G. Kindimba, et al. 2022a. TARIBAN1, TARIBAN2, TARIBAN3, and TARIBAN4 ‘Matooke’ cooking banana cultivars for the Great Lakes region of Africa. HortScience. 57:1588-1592.

Madalla, N.A., R. Swennen, A. F. Brown, C. Massawe, M. Shimwela, et al. 2022b. Yield stability of East African Highland cooking banana 'Matooke' hybrids. J. Amer. Soc. Hort. Sci. 147:334–348.

Manners, R., E. Vandamme, J. Adewopo, P. Thornton, M. Friedmann, et al. 2021. Suitability of root, tuber, and banana crops in Central Africa can be favoured under future climates. Agr Syst. 193:103246.

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

Mohammed, U.D., S.A. Legesse, A.B. Berlie, and M.A. Ehsan. 2022. Climate change repercussions on meteorological drought frequency and intensity in South Wollo, Ethiopia. Earth Syst. Environ. 6:645-55.

Nansamba, M., J. Sibiya, R. Tumuhimbise, W. Ocimati, E. Kikulwe, et al. 2022. Assessing drought effects on banana production and on-farm coping strategies by farmers—A study in the cattle corridor of Uganda. Clim. Change. 173:21.

Negin, B., and M. Moshelion. 2017. The advantages of functional phenotyping in pre-field screening for drought-tolerant crops. Funct. Plant Biol. 44:107–118.

Nkoulou, L.F.M., L.A.T. Ninla, D. Cros, G. Martin, Z. Ndiang, et al. 2023. Analysis of genetic diversity and agronomic variation in banana sub-populations for genomic selection under drought stress in southern Benin. Gene. 859:147210.

Nowakunda, K., A. Barekye, R.T. Ssali, J. Namaganda, W.K. Tushemereirwe, et al. 2015. 'Kiwangaazi' (syn 'KABANA 6H') Black Sigatoka nematode and banana weevil tolerant 'Matooke' hybrid banana released in Uganda. HortScience. 50:621–623.

Nyine, M., B. Uwimana, R. Swennen, M. Batte, A. Brown, et al. 2017. Trait variation and genetic diversity in a banana genomic selection training population. PLoS One. 12: e0178734.

Ocan, D., H.H. Mukasa, P.R. Rubaihayo, W. Tinzaara, and G. Blomme. 2008. Effects of banana weevil damage on plant growth and yield of East African *Musa* genotypes. J. Appl Biosci. 9:415.

Ocimati, W., G.V. Nakato, K.K.M. Fiaboe, F. Beed, and G. Blomme, G. 2014. Incomplete systemic movement of *Xanthomonas campestris* pv. *musacearum* and the occurrence of latent infections in Xanthomonas wilt-infected banana mats. Plant Pathol. 64:81–90.

Owoyesigire, B., D. Mpairwe, P. Ericksen, and D. Peden. 2016. Trends in variability and extremes of rainfall and temperature in the cattle corridor of Uganda. Uganda J. Agric Sci 17:231–244.

Perrier, X., C. Jenny, F. Bakry, D. Karamura, M. Kitavi, et al. 2019. East African diploid and triploid bananas: a genetic complex transported from South-East Asia. Ann. Bot. 123:19-36.

Petsakos, A., S.D. Prager, C.E. Gonzalez, A.C. Gama, T.B Sulser, et al. 2019. Understanding the consequences of changes in the production frontiers for roots, tubers and bananas. Glob. Food Secur. 20:180-188.

Prohens, J., P. Gramazio, M. Plazas, H. Dempewolf, B. Kilian, et al. 2017. Introgressiomics: a new approach for using crop wild relatives in breeding for adaptation to climate change. Euphytica, 213: 1-9.

Ravi, I., S. Uma, M.M. Vaganan, and M.M. Mustaffa. 2013. Phenotyping bananas for drought resistance. Front. Physiol. 4:9.

Rippke, U., J. Ramirez-Villegas, A. Jarvis, S.J. Vermeulen, L. Parker, et al. 2016. Timescales of transformational climate change adaptation in sub-Saharan African agriculture. Nat. Clim. Change. 6:605-609.

Risterucci, A.M., I. Hippolyte, X. Perrier, L. Xia, V. Caig, et al. 2009. Development and assessment of diversity arrays technology for high-throughput DNA analyses in Musa. Theoret. Appl. Genet. 119:1093–1103.

Robinson, J.C., and A.J. Alberts. 1986. Growth and yield responses of banana (cultivar 'Williams') to drip irrigation under drought and normal rainfall conditions in the subtropics. Sci. Hortic. 30:187–202.

Sabiiti, G., J.M. Ininda, L.A. Ogallo, J. Ouma, G. Artan, et al. 2018. Adapting agriculture to climate change: suitability of banana crop production to future climate change over Uganda. Limits to climate change adaptation. 175-190.

Sampangi-Ramaiah, M.H., K.S. Shivashankara, A. Rekha, R.H. Laxman, E. Dayanandhi, and K.V. Ravishankar. 2023. High-density GBS-based genetic linkage map construction and QTL identification associated with leaf cuticular wax, adaxial stomatal density and leaf water retention capacity in banana. Sci. Hortic. 321:112205.

Sardos. J., X. Perrier, J. Doležel, E. Hřibová, P. Christelová, et al. 2016a. DArT whole genome profiling provides insights on the evolution and taxonomy of edible banana (*Musa* spp.). Ann. Bot. 118:1269-78.

Sardos, J., M. Rouard, Y. Hueber, A. Cenci, K.E. Hyma, et al. 2016b. A genome-wide association study on the seedless phenotype in banana (*Musa* spp.) reveals the potential of a selected panel to detect candidate genes in a vegetatively propagated crop. PLoS One. 11:p.e0154448.

Simmonds, N.W. 1962. The evolution of bananas. Longman, London, UK.

Simmonds, N.W. 1966. Bananas. 2nd ed. Longmans, London, UK.

Stevens, B., J. Diels, A. Brown, S. Bayo, P.A. Ndakidemi, and R. Swennen. 2020. Banana biomass estimation and yield forecasting from non-destructive measurements for two contrasting cultivars and water regimes. Agronomy. 10:1435.

Sulser, T., K.D. Wiebe, S. Dunston, N. Cenacchi, A. Nin-Pratt, et al. 2021. Climate change and hunger: Estimating costs of adaptation in the agrifood system. Food policy report. International Food Policy Research Institute (IFPRI). Washington DC, USA.

Taulya, G., P.J.A. van Asten, S. Okech, and C. Gold. 2006. Effect of drought on the yields of an East African highland banana (cv. 'Kisansa') in Uganda. The proceedings of the 23rd Soil Science Society of East Africa conference. Masaka, Uganda.

Tenkouano, A., D. Vuylsteke, J. Okoro, D. Makumbi, R. Swennen, and R. Ortiz. 2003. Registration of TMB2x5105-1 and TMB2x9128-3 diploid banana hybrids with good combining ability, partial resistance to black Sigatoka and resistance to nematodes. HortScience. 38:468–472.

Tripathi, L., J.N. Tripathi, W.K. Tushemereirwe, G. Arinaitwe, and A. Kiggundu. 2013. Transgenic bananas with enhanced resistance against Xanthomonas wilt disease. Acta Hortic. 974:81–90.

Tuberosa, R. 2012. Phenotyping for drought tolerance of crops in the genomics era. Front. Physiol. 3:347.

Tugume, A.K., G.W. Lubega, and P.R. Rubaihayo. 2002. Genetic diversity of East African Highland bananas using AFLP. Infomusa. 11:28–32.

Tumuhimbise, R., A. Barekye, J. Kubiriba, K. Akankwasa, I.K. Arinaitwe, et al. 2018. New high-yield cooking banana cultivars with multiple resistances to pests and diseases ('NAROBan1','NAROBan2','NAROBan3', and 'NAROBan4') released in Uganda. HortScience. 53:1387-1389.

Tumuhimbise, R., H. Buregyeya, J. Kubiriba, W.K. Tushemereirwe, A. Barekye, et al. 2019. 'NABIO808' (Syn. 'NAROBAN5'): A tasty cooking banana cultivar with resistance to pests and diseases. Crop Breed. Appl. Biotechnol. 19:491-495.

Tushemereirwe, W.K., D. Karamura, H. Ssali, D. Bwamiki, I. Kashaija, et al. 2001. Bananas (*Musa* spp), *In* J.K. Mukiibi Joseph, ed. Agriculture in Uganda, vol. 11 (crops). Fountain Publishers, Technical Centre for Agricultural and Rural Cooperation/National Agricultural Research Organization, Kampala, Uganda.

Uwimana, B., Y. Zorrilla-Fontanesi, J. van Wesemael, H. Mduma, A. Brown, et al. 2020. Effect of seasonal drought on the agronomic performance of four banana genotypes (*Musa* spp.) in the East African Highlands. Agronomy. 11:4.

Van Asten, P.J.A., C.S. Gold, J. Wendt, D. De Waele, S.H.O. Okech, et al. 2005. The contribution of soil quality to yield and its relation with other banana yield loss factors in Uganda, p. 100-115, Proceedings of a workshop held on Farmer participatory testing of IPM options for sustainable banana production in Eastern Africa. Bioversity International, Montpellier, France.

Van Asten, P.J.A., A.M. Fermont, and G. Taulya. 2011. Drought is a major yield loss factor for rainfed East African Highland Banana. Agric. Water Manage. 98:541–552.

Vandamme, E., R. Manners, J. Adewopo, G. Thiele, M. Friedmann, and P. Thornton. 2022. Strategizing research and development investments in climate change adaptation for root, tuber and banana crops in the African Great Lakes Region: A spatial prioritization and targeting framework. Agric. Syst. 202:103464.

Varma, V., and D.P. Bebber. 2019. Climate change impacts on banana yields around the world. Nat. Clim. Change. 9:752–757.

Wairegi, L.W.I., P.J.A. van Asten, M.M. Tenywa, and M.A. Bekunda. 2010. Abiotic constraints override biotic constraints in East African highland banana systems. Field Crops. Res. 117, 146–153.

Chapter 2[1]: A review of the literature

Abstract

Drought is a major abiotic stress affecting banana production worldwide, leading to yield losses of up to 65%. Consequently, numerous efforts to understand and mitigate drought effects that include developing tolerant crop varieties are ongoing in several banana breeding programs. The breeding efforts, however, have been greatly slowed down by inherent banana problems (polyploidy and male or female sterility) and complexity of drought tolerance (reportedly controlled by several genes). This review summarizes the pertinent research findings on water requirements of banana for its proper growth and productivity, symptoms of drought-sensitive varieties and field management strategies to cope with drought stress. The coping strategies deployed by drought tolerant cultivars include high assimilation rates and water retention capacity as well as minor losses in leaf area and gaseous exchange. Reduced bunch weight, leaf chlorosis, wilting and choking are underlined to be directly associated with drought susceptibility. Integration of conventional, molecular breeding and biotechnological tools as well as exploitation of the existing banana genetic diversity presents a huge opportunity for successful banana improvement.

Keywords: banana water requirement, drought stress, drought tolerance, molecular markers, *Musa* spp., phenotyping

[1] This chapter was published in the journal, Plant Breeding: Nansamba, M., J. Sibiya, R. Tumuhimbise, D. Karamura, J. Kubiriba, and Karamura E. 2020. Breeding banana (*Musa* spp.) for drought tolerance: A review. Plant Breed. 139:685–696. https://doi.org/10.1111/pbr.12812.

2.1 Introduction

Banana (*Musa* spp.) is a major staple and commercial crop with a global production of 113.9 million tons (FAOSTAT, 2017). It is grown in several countries, mainly in the warm and humid tropical regions of the world with abundant rainfall, including Africa, Latin America, Caribbean, Asia and Pacific. In Africa, over 70 million people derive 25% of their dietary energy from banana and plantain (Edward and Fredy, 2012). Bananas are consumed in various forms including raw, cooked, baked, steamed, or fermented (Fungo and Pillay, 2011). However, their production in major growing regions is greatly affected by a complex of biotic and abiotic stresses, ultimately threatening the livelihoods of smallholder farmers in the developing world. Prominent biotic stresses include pests such as banana weevil (*Cosmopolites sordidus*) and nematodes (*Pratylenchus coffeae* and *Radopholus similis*) (Gold et al., 2004; Ocan et al., 2008; Speijer, 1999), while diseases include banana bacterial wilt (caused by *Xanthomonas campestris* pv. *musacearum*) (Tripathi et al., 2013; Ocimati et al., 2014), black leaf streak disease (caused by *Mycosphaerella fijiensis*) (Marín et al., 2003; Barekye et al., 2009), fusarium wilt (caused by *Fusarium oxysporum* f.sp. *cubense*) (Ploetz, 2015) and banana bunchy top virus disease (causal agent, banana bunchy top virus), (Niyongere et al., 2012). Among the abiotic stresses, salinity (Ravi and Uma, 2011), heat, low soil fertility (Bekunda and Woomer, 1996; Wairegi et al., 2010) and drought (Taulya et al., 2006; van Asten et al., 2011) are the most prevalent.

As a result of climate change, adverse effects of drought have become more pronounced in the tropics and subtropics of the world (Ravi et al., 2013). Plants experience drought stress when the amount of water in the soil is insufficient to meet their transpiration demand (Tossi et al., 2022). For optimal production, bananas require a minimum of 100 mm of evenly distributed rainfall every month (Robinson and Sauco, 2010). Bananas are very sensitive to drought, which may cause yield reductions of up to 65% when the annual rainfall falls below 1,100 mm per annum (van Asten et al., 2011). Therefore, banana varieties which can produce reasonable yield with less water offer the most promising alternative to protecting the banana crop against daunting drought effects.

Drought tolerance is a complex trait whose expression is controlled by many genes and environmentally varies over location and time, which complicates the development of a standard for drought (Ravi et al., 2013). Despite these challenges, the banana gene pool is very

diverse and hence presents a great opportunity for the enhancement of complex quantitative traits, including drought tolerance. Currently, the *Musa* International Transit Centre (ITC) in Belgium hosts and maintains over 1,500 *Musa* accessions in vitro including cultivated banana varieties, wild relatives, and improved hybrids (Swennen et al., 2011). These accessions require screening as only a fraction of the collection has been assessed in vitro for reaction to drought stress (Vanhove et al., 2012). From this germplasm collection, appropriate male and female parents can be selected and crossed to produce segregating populations from which drought-tolerant banana genotypes can be obtained. The challenge, however, is that banana is by nature a clonally propagated crop and every attempted successful cross results in new genotypes, often accompanied by co-inheritance with undesirable growth and fruit characteristics (Ramirez et al., 2011). Consequently, fixation of drought tolerance in banana through conventional breeding would require several generations of backcrossing. Co-inheritance of undesirable genes on the other hand can be minimized using genetic modification techniques, whereby only the desirable genes are inserted into the genome of the genotype which requires improvement (Tripathi et al., 2010). The objective of this review is to provide a summary of research efforts that have been directed towards understanding and breeding for drought tolerance in bananas. The following research questions will be addressed in the review: (a) "What are the physiological, biochemical and molecular changes that occur in banana under conditions of moisture stress?", (b) "What is the molecular basis of drought tolerance in banana?" and (c) "What prospects do the current research findings, technology advancements and banana genetic diversity present for banana drought tolerance improvement?".

2.2 The banana root system

The banana plant is a C_3 monocotyledon plant that can grow up to 15 m in wild species and 2 to 8 m in cultivated bananas (Karamura and Karamura, 1995; Swennen and Vuylsteke, 2001). The roots arise from the cambium layer of the corm (true stem) and emerge in bundles of four roots, forming an adventitious root system with no individual root as the primary root (Skutch, 1932; Vásquez, 2003; Carr, 2009). These adventitious roots spread horizontally in the soil, reaching lengths of about 3 to 4 m (Swennen and Vuylsteke, 2001; Carr, 2009). However, 70 % of these roots are found in the top 20 to 45 cm soil layers and grow within a radius of 1 m around the pseudostem (Draye et al., 2003; Sebuwufu et al., 2004; Carr, 2009). A previous report has shown that only 1.2% of these roots explore deeper soil layers (< 1 m) (Belalcázar

et al. (2003). Root hairs and lateral roots emerge at the tips of the adventitious roots, facilitating the uptake of water and nutrient uptake (Belalcázar et al., 2003; Draye et al., 2003). Although the banana root system is similar across genotypes, diversity in morphology exists amongst wild and edible bananas (Sebuwufu et al., 2004; Blomme et al., 2005).

2.3 Water requirements for banana growth

Banana is a large perennial fruit herb which requires at least 1,300 mm of rainfall per year for optimal growth (Mustaffa and Kumar, 2012). However, major production systems such as the East African highland banana are often small-scale, completely rainfed, and irrigation is not practiced. The area experiences bimodal rainfall patterns with an average of 900–1,100 mm per year and includes larger parts of Eastern Rwanda, south-western districts of Uganda and the western Kagera Region of Tanzania (van Asten et al., 2011). Moreover, such rains are unevenly distributed, which makes bananas prone to drought stress, thereby limiting production in large areas of Eastern Africa. In addition, with the changing climate, longer and more severe dry spells can be anticipated in eastern Africa (Hulme et al., 2001), and these will ultimately lead to increased moisture stress, which affects banana productivity and production. Accordingly, over 65 per cent of global commercial and /or export banana production is supplemented with irrigation (Carr, 2009).

Irrigation needs may differ between locations due to environmental variations which are influenced by temperature, latitude and elevation, as well as seasonality and rainfall amounts and distribution during the growing season. Crop water requirements may also be influenced by the crop type and growth stage, with advanced stages requiring more water than the initial development stages (Brouwer and Heibloem, 1986). Irrigation requirements of horticultural crops like melon, green beans, watermelon, and pepper have been estimated using drainage lysimeters which measure evapotranspiration (Orgaz et al., 2005). Similarly, banana evapotranspiration has been estimated using the pan evaporation method (Goenaga and Irizarry, 2000).

Table 2.1 summarizes the different irrigation treatments formerly investigated on banana, which can be used as references in drought tolerance studies. Although a substantial amount of research has been carried out to determine the water requirements of drip-irrigated bananas, specific information on the amount of irrigation to be applied is still lacking. Nonetheless,

Goenaga and Irizarry (1998) recommended a pan factor of 1.0 (100% evapotranspiration) as the adequate water consumption for optimum banana production after reporting a significant improvement in banana yield components of the mother plant and two ratoon crops.

Table 2.1 Previously used irrigation regimes in banana

Irrigation treatment	Source
0.25 to 1.25 ET[a] in increments of 0.25	Goenaga and Irizarry (2000)
40%, 65%, 85% and 100% Standard maximum ET	Shongwe et al. (2008)
33%, 66%, 99%, and 120% (ET)	Coelho et al. (2013)
25%, 50%, 75%, 100%, 125% and 150% (ET)	D'Albuquerque et al. (2013)
0%, 50%, and 100% (ET)	Fandika et al. (2014)
0.5, 0.75 and 1 (ET)	Ali et al. (2015)

[a]ET, evapotranspiration from a class A pan

2.4 Phenotypic symptoms of drought-sensitive banana cultivars

Drought effects on banana are manifested at various growth stages including early vegetative stage, floral primordial initiation, flowering and bunch /finger development (Robinson and Alberts, 1986; Anon, 2008). However, the intensity of this damage depends on the growth stage of the plant and duration of stress (Ravi et al., 2013). Drought-sensitive banana cultivars exhibit characteristic symptoms both externally and internally (Uma et al., 2002; Mahouachi, 2007). Such symptoms may either be common across all sensitive genotypes or are genotype-specific. Internal symptoms are manifested as physiological and biochemical changes which occur at cell level (Surendar et al., 2013b). The most prominent external symptoms include leaf folding (Stevens et al., 2020a), wilting and /or drying of leaves resulting from overheating and dehydration of cells (Ravi and Vaganan, 2016) and a significant reduction in bunch yield (van Asten et al., 2011). For instance, when banana plants were deprived of water at flowering for four weeks, a reduction in the bunch weight, fruit length and circumference was observed at harvest in cultivars ‘Robusta’, ‘Karpuravalli’ and ‘Rasthali’ (Anon, 2008). Such significant decline in crop yield may be attributed to water stress at flower initiation which reduces the number of fruits and hands, whereas if drought stress occurs after flower initiation, only fruit

filling will be affected (Goenaga and Irizarry, 1998; Stevens et al., 2020; Uwimana et al., 2020). A significant reduction in the plant's photosynthetic rate (during water stress conditions), which is greatly influenced by the leaf chlorophyll content as well as the closure of the stomata also contributes to the crop yield reduction (Flexas and Medrano, 2002). On the other hand, drought stress imposed at the juvenile stage was shown to cause significant reductions in transpiration rates and plant growth traits, including leaf area, pseudostem height, pseudostem girth, fresh and dry biomass, and leaf emergence (Ravi et al., 2013; van Weasel et al., 2019; Eyland et al., 2020; 2022; 2023; Thingnam et al., 2023). In field-grown plants, seasonal drought significantly slowed vegetative growth (i.e. plant height, number of suckers produced and plant girth), delayed flowering time and bunch maturity and had a negative impact on the yield traits (Uwimana et al., 2020).

Water stress results in reduced production and increased breakdown of chlorophyll in leaves, which is manifested as leaf senescing or chlorosis (Dekov et al., 2000). Moreover, a correlation between leaf area and yield suggests the importance of leaf area and chlorophyll content as major determinants of the harvestable yield (Surendar et al., 2013a). A decline in photosynthesis also results in reduced biomass production and allocation (Mahouachi, 2009; Delfin et al., 2016) to major plant tissues like the pseudostem, which weaken and eventually snap or collapse. Other drought susceptibility symptoms such as stunted growth, choking, and formation of leaf petiole rosette (Figure 2.1) become more apparent when severe water stress occurs throughout the entire crop cycle. Screening banana germplasm in the field is important as it unveils the reaction of assessed genotypes under stress conditions which are consistent with reality in nature. However, this requires the use of large rainout shelters, screening in multiple locations, lengthy observations due to the long crop cycle (10–12 months), controlled water application as well as large human and monetary resources required.

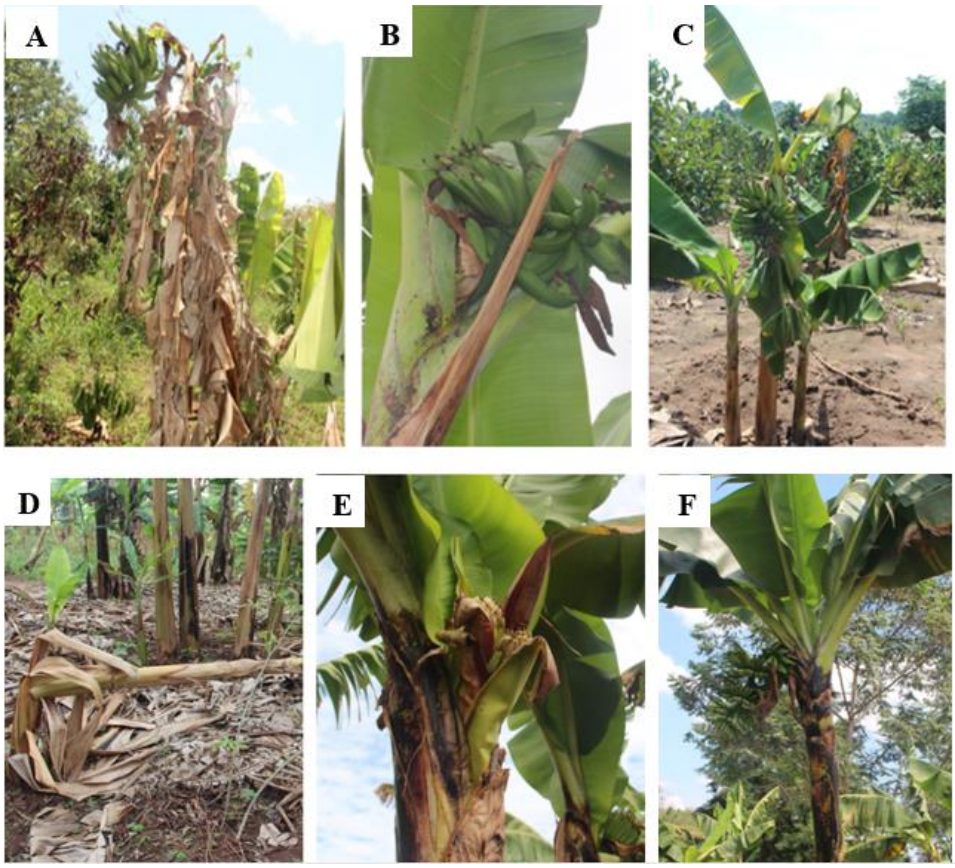


Figure 2.1 Symptoms of drought stress on banana: (A) wilting and drying of leaves (B) reduced bunch and finger size (C) stunted growth (D) snapping of weak pseudostem (E) choking and (F) formation of petiole rosette

2.5 Cultural practices for management of drought stress

Drought tolerance in plants could be achieved by incorporating research technologies such as genetic engineering, screening of large amounts of germplasm (followed by identification of tolerant genotypes and breeding for tolerance), marker-assisted selection, and exogenous application of hormones and osmoprotectants to seed or growing plants (Farooq et al., 2009). Banana farmers, on the other hand, cope with drought stress by practicing the following management practices to replenish and maintain soil moisture to levels optimum for banana production.

2.5.1 Mulching

Mulching, as a cultural management option, is often practiced in semi-arid regions and rainfed agricultural systems as a water-saving technique that regulates soil temperature, maintains soil moisture and reduces soil evaporation (Zegada-Lizarazu et al., 2011; Li et al., 2013; Zribi et al., 2015; Shaikh et al., 2023). Mulching also improves the soil structure and porosity, thereby enabling increased root development, which allows more water and nutrient uptake by the plant (El-Beltagi et al., 2022). Additionally, mulch insulates the soil, protecting soil organisms and preventing damage and desiccation of plant roots (Krader et al., 2019). When organic mulch degrades, it enhances the soil's organic matter and consequently increases the water-holding capacity of that soil (Kader et al., 2017, 2019; El-Beltagi et al., 2022). Common mulch options used in East African highland banana systems include dried weeds, grass (*Pennisetum purpureum*) and other crop or banana plant residues, e.g. banana peelings, pseudostems and pruned dry or fresh leaves (Bekunda, 1999; Wairegi et al., 2010; Kisakye et al., 2022; den Braber et al., 2024).

2.5.2 Irrigation

As indicated in Section 2.2 above, banana plantations require a large amount of water for irrigation. Supplementary irrigation is particularly vital during floral primordial initiation and development, flowering, and one-month post-flowering to ensure successful bunch emergence and fruit filling (Ravi and Vaganan, 2016). Moreover, increased irrigation boosts banana yield and quality (Fandika, et al., 2014; Stevens et al., 2020b). Large-scale commercial banana farmers supplement the available rainfall with drip or sprinkler irrigation, while for small-scale growers (form the majority and are often resource-limited), irrigation is often constrained by water scarcity and cost of water (Blum, n.d.; Nansamba et al., 2022) as well as limited access to irrigation facilities (Kabunga, et al., 2012). Even those smallholder growers that irrigate their plantations, the irrigation intensity remains rather low and less frequent (Kabunga et al., 2012). Supplementary irrigation, in many environments, can be made more effective if the water is applied before planting (Blum, n.d.). Pre-planting irrigation allows the crop to have a sufficient water supply early in the season, thereby ensuring its proper establishment and growth in spite of unexpected rainfall variations. As the atmospheric conditions become warmer and drier,

irrigation is required to maintain a high moisture content while ensuring free movement of water molecules within the soil for easy uptake by plant roots (Robinson, 2000).

2.6 Phenotyping under drought stress conditions

2.6.1 Physiological analyses

Considering that drought is becoming more and more prevalent in various parts of the world, several authors have demonstrated the physiological changes which occur in banana during such water stress conditions (Bananuka et al., 1999; Surendar et al., 2013b). These physiological changes affect processes such as stomatal conductance, water retention capacity, water-use efficiency (WUE) and photosynthesis. Drought-sensitive cultivars show significant reductions in plant dry mass due to reduced biomass production (Mahouachi, 2009), whereas their tolerant counterparts have inbuilt mechanisms for drought tolerance such as high assimilation rates and water retention capacity with minor losses in leaf area and gaseous exchange (Bananuka et al., 1999). Similar findings were reported from an experiment conducted under glasshouse conditions in the Philippines (Delfin et al., 2016). The study concluded that genotypes with the highest WUE, root volume, total plant biomass production and root dry weight were drought-tolerant. On the other hand, stomatal conductance and photosynthesis are significantly reduced under limiting moisture (Thomas and Turner, 2001).

Inasmuch as prolific vegetative growth is a good indicator of better tolerance to drought stress, it is not satisfactory as a sole parameter since a healthy plant absorbs more moisture from the soil and will at times forfeit its inner water balance to allow growth, which ultimately intensifies the stress and reduces development of the final yield (Passioura, 2012). Therefore, one approach is to take both plant growth and efficient use of water during water stress into account. Transpiration efficiency (TE) has been assessed and found to be a useful indicator of drought tolerance in cereal crops including barley, rice and wheat (Anyia et al., 2007; Cabrera-Bosquet et al., 2007; Haefele et al., 2009). In banana, however, only a few studies have assessed the correlation between TE and growth. For instance, Kissel et al. (2015) demonstrated an increase and clear genotypic variation in the transpiration efficiency for six banana cultivars (‘Mpologoma’, ‘Mbwazirume’, ‘Sukali Ndiizi’, ‘Kayinja’, ‘Cachaco’ and ‘Yangambi Km5’) when stressed. Nonetheless, selection for high transpiration efficiency did not result in selection of cultivars with slower vegetative growth under watered and mild moisture stress

imposition. Therefore, selection of banana cultivars based on the TE during stress conditions could serve as a quick tool for selecting drought-tolerant candidates under controlled conditions, thereby reducing the amount of time required for screening and selection under field conditions. There is, thus, a need to evaluate TE for more genotypes which are currently maintained by different banana breeding programmes. It is important, though, to note that a high TE during moisture stress does not necessarily imply drought tolerance for that cultivar or crop (Jones, 2014). A cultivar is only regarded as drought-tolerant if it has a high water-use efficiency accompanied by a minor reduction in growth and yield.

Several studies have reported banana genotypes with the "B" genome such as AAB and ABB to be more drought-tolerant than those entirely based on the "A" genome, for example 'Cavendish' AAA (Robinson, 1996; Robinson and Sauco, 2010). This drought tolerance has been imputed to the belief that the *Musa balbisiana* (*M. balbisiana*) originated from drier parts of South Asia including lower Himalayan ranges (Tenkouano, 2006; Janssens et al., 2016; Mertens et al., 2021) unlike *Musa acuminata* (*M. acuminata*), whose origin is the humid forest regions of South-East Asia (Kissel et al., 2015). In a field study by Ravi and Uma (2011), several *M. acuminata* diploids exhibited high susceptibility to drought, which was manifested by bunch choking, fewer hands, ill-filled fruits and no seed set. Conversely, water deficit conditions had little impact on fruit and seed development of *M. balbisiana* cultivars. Under controlled conditions, Thomas et al. (1998) reported a higher sensitivity to leaf-air vapour pressure deficit in the cultivar 'Williams' (AAA) than cultivars 'Bluggoe' (ABB) and 'Lady Finger' (AAB). Likewise, an *in vitro* assessment of banana varieties showed that ABB varieties had the least reduction in growth under slight osmotic stress (Vanhove et al., 2012). As such the B genome may contain drought tolerance genes which can be identified, isolated and introduced in drought-sensitive farmer preferred cultivars.

2.6.2 Biochemical analyses

Drought stress elicits a series of biochemical responses including mechanisms of susceptibility to osmotic or moisture stress, transformation of stress signals to cellular signals, transfer of cell signals to the nucleus and transcriptional control of moisture stress-induced genes, resulting in tolerance to the water deficit (Bray, 1997; Blum, 2017). For instance, drought stress specifically induces the synthesis and build-up of organic solutes such as proline, free amino acids, total

soluble proteins, and carbohydrates, which act as osmolytes (Lacerda et al., 2001; Surendar et al., 2013b). Such osmolytes, for example proline, may regulate the osmotic balance of the cell thereby countering the osmotic stress induced by drought (Hayat et al., 2012). Osmotic adjustment safeguards major plant cell structures such as chloroplasts and cell membranes (Martìnez et al., 2004). This was corroborated by Surendar et al. (2013c), who concluded that the higher yield observed in tolerant banana genotypes may have been due to higher water and osmotic potential, resulting from increased epicuticular wax, proline, and free amino acids during stress conditions. On the other hand, accumulated total sugars serve as an important energy source under severe stress in corn (Pimentel, 1999). These biochemical compounds can be used as suitable indices to rapidly select drought-tolerant candidates among existing banana cultivars and hybrids.

Drought stress enhances the production of reactive oxygen species (ROS) (including alkoxy radicals, OH, HO_2 and O_2^-), which impair proteins, lipids, carbohydrates, and DNA, and thereby causing oxidative stress (Gill and Tuteja, 2010). To maintain the integrity of cellular structures, such active species of O_2^- are eliminated by a catalase (an antioxidant enzyme), which in turn eliminates the hydrogen peroxide produced during plant metabolic processes (Surendar et al., 2013a). Literature sources have reported the continuous production and scavenging of ROS molecules by many antioxidative defence mechanisms during drought stress (Foyer and Noctor, 2000). In fact, a close association between drought tolerance and an increment in antioxidant enzyme activity under conditions of drought stress has been observed in wheat genotypes by Sairam et al. (1997). Considering ROS scavenging reduces oxidative stress caused by moisture deficit, we presume that the enhancement of such antioxidative processes would improve drought tolerance in banana.

2.6.3 Molecular analyses for drought tolerance in bananas

Drought stress triggers the expression of several genes and transcription factors influencing different plant resistance mechanisms including escape, avoidance, tolerance, and acclimatization (Farooq et al., 2009). Such molecular factors are responsible for the production of biochemical compounds which maintain or restore the integrity of plant cells during moisture deficit conditions. Several studies provide insight into the molecular basis of drought tolerance in important cereal crops such as wheat (Ibrahim et al., 2012; Ahmad et al., 2014),

rice (Shim et al., 2018) and maize (Liu et al., 2013; Wang et al., 2016). In bananas, transcriptomic changes in drought-tolerant (*cv*. 'Saba') and sensitive (*cv*. 'Grand Naine') cultivars have been monitored and compared using mRNASeq under well-watered and water stress conditions (Muthusamy et al., 2016). Among the upregulated and /or downregulated differentially expressed genes (DEGs), several genotype-specific gene expression patterns were observed for drought stress in both cultivars. Such unique gene expression profiles observed in tolerant cultivars could represent candidate drought stress tolerance genes considering their stress association in plants is already known. Also, notable are transcription factors, namely the banana NAC transcription factor (*Musa*SNAC1) which imparts drought tolerance by regulating stomatal closure and hydrogen peroxide content after binding to the CGT[A/G] motif in regulatory region of many stress-related genes (Negi et al., 2018). Table 2.2 summarizes the different protein /gene families that have been associated with tolerance to drought and other abiotic constraints through molecular characterization and genome wide analysis. Understanding the molecular basis for drought tolerance will provide required genetic information influencing drought resilience in banana.

2.7 Breeding for drought tolerance in banana

Crop improvement involves the creation, selection and fixation of resistance or tolerance against biotic and abiotic constraints into superior plant phenotypes, which meet the needs of farmers and consumers. The wide genetic diversity within *Musa* germplasm is an important source for disease and pest resistance genes, good agronomic performance and tolerance to abiotic stresses, hence an indispensable resource for banana enhancement. However, identification and characterization of this genetic diversity and putative resistance genes, and utilization of this genetic variation to improve the inherently female and /or male sterile and clonally propagated crop can be a challenge (Ssali, 2016). For banana improvement, conventional crossbreeding and non-conventional breeding approaches including molecular breeding techniques have been deployed (Chen et al., 2011).

Table 2.2 Protein families and their role in drought and other abiotic stresses affecting banana.

Protein /Gene family	Specific protein /Gene	Tolerance to which abiotic stress	Role during stress	Source
Heat shock protein (HSP)	*HSP20*	Drought, oxidative stress	Maintains homeostasis by suppressing protein aggregation	Vanhove et al. (2012)
Aquaporin (AQP)	*MaPIP1;1*	Drought, salt	Reduces membrane injury, improves ion distribution and maintains osmotic balance	Xu et al. (2014)
	MaPIP1;2	Drought, cold, salt	Lowers malondialdehyde levels, elevated proline and relative water content and higher photosynthetic efficiency	Sreedharan et al. (2013)
	MaTIP1;2 (Promoter)	Drought, salt	Controls the flow of water and other small molecules through biological membranes	Song et al. (2018)
Copper Chaperone Gene	*MaCCS* gene	Drought, copper, heat, cold and light	Delivers copper to its target *Cu/ZnSODs*. Function is not yet so clear.	Feng et al. (2016)
Calmodulin-binding transcription activator (*CAMTA*)	*M. acuminata* CAMTA1 (*MuCAMTA1*) transcription factor	Drought	No systematic study has been conducted yet on *MuCAMTA* transcription factors to identify their role in drought stress in banana plants.	Meer et al. (2019)
Leucine zipper (*bZIP*) transcription factor gene family	*MabZIP* genes	Drought, cold, salt	They participate in stress signalling, but their function is yet to be confirmed in banana	Hu et al. (2016)
Sugars Will Eventually be Exported Transporters (*SWEET*)	*M. acuminata MaSWEET* genes	Drought, cold, salt	Promote early sugar transport to improve fruit quality and enhance stress tolerance in banana.	Miao et al. (2017)
Constitutive Banana cDNA	*M. acuminata* root hair defective 3 (*MaRHD3*)	Drought	ROS scavenging enhanced lateral root branching and root hair density.	Wonga et al. (2018)
	ATPase and heat shock proteins	Drought	Involved in metabolism, responses to stress, growth and development.	Mattos-Moreira et al. (2018)

2.7.1 Genetic diversity in banana

The two principal progenitors of present-day edible bananas are *M. acuminata* Colla, (AA) and *M. balbisiana* Colla, (BB), whose centre of origin is believed to be in South and South-East Asia and Pacific countries (Simmonds, 1962; Ravi and Uma, 2011). These were later introduced to the tropical and subtropical climatic zones, where the crop is now a major food crop. The existing large diversity of banana is a result of natural interspecific hybridization of *M. acuminata* and *M. balbisiana* species, thus forming various genomic groups like diploids (AA, AB, BB), triploids (AAA, AAB, ABB, BBB) and tetraploids (such as AAAB and AABB) (Simmonds, 1966). Over the years, humans have domesticated, selected and perpetuated useful banana genotypes. Such useful diversity includes good agronomic performance as well as resistance or tolerance to several biotic and abiotic stresses (Singh and Uma, 2000). Large collections of banana germplasm including progenitors, wild relatives, landraces and hybrids are currently assembled and maintained in situ and ex-situ by twelve banana breeding programmes spread all over the world. Although only a few species have been domesticated, wild relatives of banana consist of at least 75 species, which originate between India and the Pacific, specifically in the humid tropical forests (Manimaran et al., 2018). From these germplasm collections, drought-tolerant candidates can be selected and used to introgress drought tolerance in commercially important but susceptible bananas.

2.7.2 Screening banana germplasm for drought tolerance

For a long time, breeders have focused on breeding for resistance to banana pests and diseases (Aguilar-Morán, 2013; Nowakunda et al., 2015), with drought tolerance only considered an important trait along with these traits. Currently, drought is gaining importance and has been considered a primary production constraint by both farmers and researchers. Intrinsic crop-based challenges such as long crop cycle, large green canopy and shallow root system predispose banana to drought stress (Robinson, 1996). Consequently, breeding programs like that of India, the National Research Centre for Banana (NRCB), Mexico, the Centro de Investigacion Cientifica del Yucatan (CICY) and Australia (Turner, 2005) have initiated the screening of germplasm for drought. For instance, NRCB has screened 112 out of 340 genotypes for their response to soil moisture shortage under field conditions (Ravi and Uma, 2011). On the other hand, researchers at Katholieke Universiteit, Leuven, Belgium, have

focused on developing models for in vitro screening of *Musa* diversity for drought tolerance via proteomics (Vanhove et al., 2012), after which, candidates need to be validated at plant level. Large amounts of germplasm have been screened for specific phenotypic traits related to plant growth (plant height, dry matter content, leaf emergence rate, leaf area index, root: shoot ratio), plant water status (water-use efficiency, relative water content, water retention capacity) and plant function (such as stomatal conductance, quantification of photosynthetic pigments) (Uma and Sathiamoorthy, 2002; Delfin et al., 2016). However, there is need for more experimentation at different ecological sites to obtain more reliable information regarding drought-sensitive and tolerant accessions. Thereafter, crossbreeding could be utilized to produce drought-tolerant genotypes. To date, the utilization of identified tolerant candidates in banana drought tolerance improvement using conventional crossbreeding has not yet been reported.

2.7.3 Application of molecular markers to improve drought tolerance in banana

Over the years, research programs have deployed several methods for banana improvement, including conventional crossbreeding, marker-assisted selection, genetic engineering, induced mutation breeding, protoplast fusion and selecting somaclonal variants (Chen et al., 2011). Marker-assisted selection is a molecular breeding technique which is becoming popular because it offers the possibility of significantly reducing the amount of time taken for banana improvement when using conventional crossbreeding, which requires one and a half years for the crop to complete its growth cycle and produce new plantlets (Pillay et al., 2002). In major cereal crops, genetic markers which co-inherit with specific target traits have been linked to drought tolerance (Agrama and Moussa, 1996; Courtois et al., 2000; Galeano et al., 2012; Sabouri et al., 2018). For instance, various genetic analyses have linked several markers to major effect quantitative trait loci (QTLs) of grain yield, root morphology, leaf rolling and withering degree, under conditions of drought stress (Champoux et al., 1995; Yue et al., 2006; Han et al., 2018; Tabkhkar et al., 2018). In common bean, QTL mapping revealed twenty-two QTLs responsible for leaf temperature, chlorophyll production, days to flowering as well as traits related to yield and biomass production under stress and watered conditions (Briñez et al., 2017). Such phenotypic traits are often associated with drought tolerance in plants.

In banana, however, molecular markers have mostly been used for germplasm characterization. For example, assessment of genetic diversity within *Musa* genotypes has been done using random amplified polymorphic DNA markers (RAPDs) (Pillay et al., 2001; Poerba and Ahmad, 2010; Hasan and Khasim, 2018; Singh et al., 2021; Karuwal et al., 2024; Pillay, 2024), restriction fragment length polymorphism markers (RFLPs) (Raboin et al., 2005; Singh et al., 2021), microsatellites / simple sequence repeats (SSRs) (Karamura et al., 2016; Pierre et al., 2019; Šimoníková et al., 2020; Mertens et al., 2021; 2022), amplified fragment length polymorphism markers (AFLPs) (Ude et al., 2003; Ahmad et al., 2014; Safhi et al., 2023) and diversity array technology markers (DArTs) (Risterucci et al., 2009; Ssali, 2016; Sardos et al., 2022; Martin et al., 2023). To our knowledge, no banana breeding programme has developed or screened existing mapping populations with such molecular markers and linked them to drought tolerance. 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 (Pillay et al., 2002; Ortiz and Swennen, 2014). Such screening would permit tagging of specific molecular markers to drought tolerance in bananas. Moreover, marker-assisted selection eliminates environmental effects during selection, especially when screening for multigenic traits like drought tolerance (Mwadzingeni et al., 2016).

The Banana Genome Hub developed by the French Agricultural Research Centre for International Development (CIRAD) and Bioversity International centralizes databases of genetic and genomic data for the *M. acuminata* crop (https://banana-genome-hub.southgreen.fr). It is a valuable resource in banana research as it provides data on the whole genome sequences coupled with gene structures and families, gene product information metabolism, transcriptomics (expressed sequence tags (ESTs), RNASeq), molecular markers (SSR, DArT, SNP) and genetic maps (Droc et al., 2022). The ESTs can be used in finding genes, mapping the genome and identification of coding regions in genomic sequences (Fulton et al., 2002; Passoss et al., 2012; Nahas et al., 2020; Haq et al., 2023) as well as developing genetic maps and markers, or to detect functional genes (Pillay et al., 2012; Satrio et al., 2019; Saha et al., 2020; Kumar et al., 2022). The increasing number of EST databases in different plant species, including *Musa*, is important for developing genetic markers based on ESTs (Ssali, 2016; Jiang et al., 2023). Furthermore, genetic markers and maps developed from useful

sequences can be used in identifying and potentially cloning QTLs and genes of agricultural and biological significance.

2.7.4 Integration of biotechnology to improve drought tolerance

Molecular biology and plant tissue culture techniques are applied to enhance the improvement of banana. As such, biotechnology utilizes applications of cell biology such as embryo culture for *in vitro* seed germination (Uma et al., 2011; Kaya et al., 2020; Kallow et al., 2020, 2022), micropropagation for rapid multiplication of banana germplasm (Khaskheli et al., 2021; Subrahmanyeswari and Gantait, 2022) and genetic engineering using cell suspensions (Tripathi et al., 2017; Dong et al., 2020; Uma et al., 2023; Handayani et al., 2024). However, the application of genetic engineering requires prior identification and isolation of valuable genes. Since the beginning of the 21st century, beneficial genes have been instituted into banana with focus on genes conferring resistance to Fusarium wilt (Paul et al., 2011; Sunisha et al., 2020; Rocha et al., 2021; Wang et al., 2021), black leaf streak disease (Vishnevetsky et al., 2011; Portal et al., 2012; Kovács et al., 2013; Onyilo et al., 2018), nematodes (Roderick et al., 2012; Tripathi et al., 2013) and Xanthomonas wilt (Namukwaya et al., 2012; Tripathi et al., 2014, 2017; Adero et al., 2021) and genes responsible for improved provitamin A content in the banana fruit (Paul et al., 2017; 2018; Dale et al., 2020) and modulation of fruit ripening (Wu et al., 2023). So far, genetic modification techniques, including Agrobacterium-mediated transformation and particle bombardment/ biolistic-mediated transformation of banana, have yielded stable transgenic lines (Becker et al., 2000; Daniels et al., 2018; Tripathi et al., 2019; Namanya et al., 2020; Dale et al., 2020) and hence can now be deployed to introduce drought tolerance genes in cell lines of drought-sensitive banana cultivars. Similarly, advancements in the efficiency of transformation and regeneration of embryogenic cell suspensions (Wong et al., 2008; Dale et al., 2020) could be exploited to ensure effective banana drought tolerance improvement. However, the above traditional transgenic approaches have been argued to pose potential adverse risks on non-target organisms, food safety concerns like allergenicity and toxicity, unintended antibiotic resistance, evolution of pest and weed resistance and environmental risks connected to possible gene flow (Yaqoob et al., 2016; Mackelprang et al., 2020; Zhao et al., 2022; Caradus, 2023).

Modern transgenic approaches such as RNA interference (RNAi) and genome editing technologies, specifically CRISPR/Cas9, have been deployed in crop research to potentially address the numerous biosafety concerns of genetic engineering (Petrick et al., 2013; Tripathi et al., 2022; Feng et al., 2023). RNAi technology is considered a safe option given that it is based on host-delivered double-stranded (ds)RNA, and hence, no new protein is produced in the transgenic plant (Casacuberta et al., 2015; Fletcher et al., 2020). In banana, RNAi technology has been demonstrated in multiple disease resistance studies (Paul et al., 2011; Shekhawat et al., 2012; Magambo et al., 2016; Tripathi et al., 2019). More recently, Mwaka and colleagues (2023) reported enhanced resistance to nematodes (*Radopholus similis*) through host-delivered RNAi. Transgenic EAHB plants with dsRNA constructs against five nematode genes, including *Rps13*, chitin synthase (*Chs-2*), *Unc-87*, *Pat-10* or beta-1,4-endoglucanase (*Eng1a*), on average, showed less root damage and lower nematode multiplication than the non-transgenic controls. On the other hand, CRISPR/Cas9 technology has been used to knock out, silence and activate genes of interest (Tripathi et al., 2022). For instance, CRISPR/Cas9 has been applied to improve resistance to banana bacterial wilt and banana streak virus (BSV) by inactivating the endogenous banana streak virus (eBSV) found in the 'B' genome of plantain (Tripathi et al., 2019; 2021). In addition to enhancing disease resistance, CRISPR/Cas9-mediated genome editing has also been used to increase the shelf life (Hu et al., 2021) and banana fruit quality (Kaur et al., 2020).

The utilization of genetic modification and genome editing in banana research could ameliorate a deeper understanding of the morphophysiological and genetic bases of drought tolerance in banana and enable the identification of useful putative QTL/gene sequences influencing drought tolerance. Failure to incorporate such biotechnology approaches may not only delay the introgression of drought tolerance genes into banana germplasm but also hinder further understanding of the molecular factors influencing drought tolerance in banana.

2.8 Concluding remarks and future directions

Global banana production is threatened by recurrent drought spells due to its long growth duration. Minimal efforts have been directed towards developing drought-tolerant banana cultivars as most research has only gone as far as understanding physiological changes which occur in banana during drought stress, with fewer biochemical and molecular analyses done.

Screening efforts have stopped at assessing and reporting drought-tolerant and sensitive cultivars under controlled and field conditions. Therefore, there is a need to develop drought-tolerant cultivars through conventional breeding or identifying candidate genes and integrating them in susceptible cultivars. Systematic studies are required to confirm the role of already identified putative genes and transcription factors such as *M. acuminata* CAMTA1 (*Mu*CAMTA1) and *MabZIP* genes in banana drought tolerance. Such molecular factors have potential applications in the genetic improvement of susceptible banana cultivars. Similarly, further refining of phenotypic tools and methodologies to be followed in the field and under controlled conditions is still required. Recent technological developments such as genetic engineering, high-throughput precision phenotyping and marker-assisted selection should also be exploited to hasten improvement of drought tolerance in banana as they permit thorough and precise selection of drought-tolerant candidates. Moreover, genome editing using the CRISPR/Cas9 technology, a type of genetic engineering, can be used to activate drought tolerance genes in the banana genome and silence or knock out genes associated with drought susceptibility.

References

Adero, M.O., E.D. Syombua, and R. Oduor. 2021. Genetic engineering of banana against bacterial diseases. J. Plant Sci. 9:253-256.

Agrama, H.A.S., and M.E. Moussa. 1996. Mapping QTLs in breeding for drought tolerance in maize (*Zea mays* L.). Euphytica. 9:89–97.

Aguilar-Morán, J.F. 2013. Improvement of Cavendish banana cultivars through conventional breeding. Acta Hortic. 986:205–208.

Ahmad, F., R. Megia, and Y.S. Poerba. 2014. Genetic diversity of *Musa balbisiana* Colla in Indonesia based on AFLP Marker. HAYAT J. Biosci. 21:39–47.

Ahmad, M.Q., S.H. Khan, A.S. Khan, A.M. Kazi, and S. Basra. 2014. Identification of QTLs for drought tolerance traits on wheat chromosome 2A using association mapping. Int J. Agric. Biol. 16: 862–870.

Ali, A.B.M., N.A. Elshaikh, Y. Shuang-En, A.K. Basheer, M. Alnail, et al. 2015. Correlation between water deficiency, yield components and crop productivity of banana. J. Environ Agric Sci. 4:11–20.

Anon. 2008. Annual Report. National Research Centre for Banana (ICAR), Trichy, India.

Anyia, A.O., J.J. Slaski, J.M. Nyachiro, D.J. Archambault, and P. Juskiw. 2007. Relationship of carbon isotope discrimination to water use efficiency and productivity of barley under field and greenhouse conditions. J. Agron. Crop Sci. 193:313–323.

Bananuka, J.A., P.R. Rubaihayo, and M.M. Tenywa. 1999. Reactions of *Musa* genotypes to drought stress. Afr. Crop Sci J. 7: 333–339.

Barekye, A., P. Tongoona, J. Derera, and W.K. Tushemereirwe. 2009. Variation in diploid (AA) and tetraploid (AAAA) banana populations for black Sigatoka resistance and agronomic traits. Afr. Crop Sci J. 9: 509–516.

Becker, D.K., B. Dugdale, M.K. Smith, R.M. Harding, and J.L. Dale. 2000. Genetic transformation of Cavendish banana (*Musa* spp. AAA group) cv. Grand Nain via microprojectile bombardment. Plant Cell Rep. 19:229–234.

Bekunda, M. 1999. Farmers' responses to soil fertility decline in banana-based cropping systems of Uganda. Managing Africa's soils No.4. Russell Press, Nottingham, UK.

Bekunda, M.A., and P.L. Woomer. 1996. Organic resource management in banana-based cropping systems of the Lake Victoria basin, Uganda. Agriculture Ecosyst. Environ. 59:171–180.

Belalcázar, S.C., F.E. Rosales, and L.E. Pocasangre. 2003. Development and formation of plantain roots (*Musa* AAB Simmonds), p. 75-82, *In* D.W. Turner, and F.E. Rosales, eds. Banana root system: towards a better understanding for its productive management. Proceedings of an international symposium held in San José, Costa Rica, 3-5 November 2003. International Network for the Improvement of Banana and Plantain (INIBAP), Montpellier, France.

Blomme, G., K. Teugels, I. Blanckaert, G. Sebuwufu, R. Swennen, and A. Tenkouano. 2005. Methodologies for root system assessment in bananas and plantains (*Musa* spp.), p. 43:57, *In* D.W. Turner, and F.E. Rosales, eds. Banana Root System: Towards a Better Understanding for its Productive Management. International Network for the Improvement of Banana and Plantain (INIBAP), Montpellier, France.

Blomme, G., W. Ocimati, J. Groot, J. Ntamwira, L. Bahati, D. Kantungeko, et al. 2018. Agroecological integration of shade- and drought-tolerant food/feed crops for year-round productivity in banana-based systems under rain-fed conditions in Central Africa. Acta Hortic. 1196:41–54.

Blum, A. (n.d.). The Mitigation of drought stress. https://plantstress.com/drought-mitigation/ Accessed 12 December 2019.

Blum, A. 2017. Osmotic adjustment is a prime drought stress adaptive engine in support of plant production. Plant, Cell. Environ. 40:4–10.

Bray, E.A. 1997. Plant responses to water deficit. Trends Plant Sci. 2:48–54.

Briñez, B., J.M.K.C. Perseguini, J.S. Rosa, D. Bassi, J.G.R. Gonçalves, et al. 2017. Mapping QTLs for drought tolerance in a SEA 5 x AND 277 common bean cross with SSRs and SNP markers. Genet. Mol.. Biol. 40:813–823.

Brouwer. C., and M. Heibloem. 1986. Irrigation water management: Irrigation water needs. Rome: Food and Agriculture Organization. http://www.fao.org/docrep/S2022E/S2022E00.htm# . Accessed 21 November 2019.

Cabrera-Bosquet, L., G. Molero, J. Bort, S. Nogués, and J.L. Araus. 2007. The combined effect of constant water deficit and nitrogen supply on WUE, NUE and Δ13C in durum wheat potted plants. Ann. Appl. Biol. 151:277–289.

Caradus, J.R. 2023. Intended and unintended consequences of genetically modified crops–myth, fact and/or manageable outcomes? New Zeal J. Agr Res. 66:519-619.

Carr, M.K.V. 2009. The water relations and irrigation requirements of banana (*Musa* spp.). Exp. Agric. 45:333–371.

Casacuberta JM, Y. Devos, P. Du Jardin, M. Ramon, H. Vaucheret, et al. 2015. Biotechnological uses of RNAi in plants: risk assessment considerations. Trends. Biotechnol. 33:145-147.

Champoux, M.C., G. Wang, S. Sarkarung, D.J. Mackill, J.C. O'Toole, et al. 1995. Locating genes associated with root morphology and drought avoidance in rice via linkage to molecular markers. Theor. Appl. Genet. 90:969–981.

Chen, Y.F., X.M. Dai, Q. Gong, X. Huang, W. Xiao, et al. 2011. Non-conventional breeding of banana (*Musa* spp.). Acta Hortic. 897:39–46.

Coelho, E.F., R.C. de Oliveira, and A.J.M. Pamponet. 2013. Water requirements of terra-type banana under coastal tableland conditions. Pesqui. Agropecu. Bras. 48:1260–1268.

Courtois, B., G. McLaren, P.K. Sinha, K. Prasad, R. Yadav, and L. Shen. 2000. Mapping QTLs associated with drought avoidance in upland rice. Mol. Breed. 6:55–66.

D'Albuquerque, J., E.R. Gomes, V.F. de Sousa, and A.P. de Sousa. 2013. Water requirement and levels of irrigation of banana FHIA-18 in the semiarid region of Piauí state. IRRIGA. 18:756–767.

Dale, J., W. Tushemereirwe, and R. Harding. 2020. Genetic modification of bananas: the long road to farmers' fields, p. 339-366, *In* Achieving sustainable cultivation of bananas. Burleigh Dodds Science Publishing, Cambridge, UK.

Dekov, I., T. Tsonev, and I. Yordanov. 2000. Effect of water stress and high temperature stress on structure and activity of photosynthetic apparatus of *Zea mays* and *Helianthus annuus*. Photosynthetica. 38:361–366.

Delfin, E.F., E.T.M. Ocampo, F.M. Cueva, O.P. Damasco, F. de la Cruz, et al. 2016. Response of wild and edible *Musa* spp. seedlings to limiting moisture stress. Philipp J. Crop Sci. 41:1–12.

den Braber, H., G. van de Ven, J. van Heerwaarden, W. Marinus, E. Ronner, et al. 2024. What shapes yields of East African Highland banana? An explorative study from farmers' fields. Eur J. Agron. 156:127141.

Draye, X., F. Lecompte, and L. Pagès. 2003. Distribution of banana roots in time and space: new tools for an old science, p. 58-74, *In* D.W. Turner and F.E. Rosales, eds. Banana root system: towards a better understanding for its productive management. Proceedings of an international symposium held in San José, Costa Rica, 3-5 November 2003. International Network for the Improvement of Banana and Plantain (INIBAP), Montpellier, France.

Dong, T., F.C. Bi, Y.H. Huang, G.M. Deng, H.J. Gao, et al. 2020. Highly efficient biolistic transformation of embryogenic cell suspensions of banana via a liquid medium selection system. HortScience. 55:703-708.

Droc, G., G. Martin, V. Guignon, M. Summo, G. Sempéré, et al. 2022. The banana genome hub: a community database for genomics in the Musaceae. Hortic Res. 9:p.uhac221.

Edward, E., and B. Fredy. 2012. Banana Market. Food and Resource Economics Department Series No. FE901. University of Florida/IFAS extension, Florida, USA.

El-Beltagi, H.S., A. Basit, H.I. Mohamed, I. Ali, S. Ullah, et al. 2022. Mulching as a sustainable water and soil saving practice in agriculture: A review. Agronomy. 12:1881.

Eyland, D., C. Breton, J. Sardos, S. Kallow, B. Panis, et al. 2020. Filling the gaps in gene banks: Collecting, characterizing, and phenotyping wild banana relatives of Papua New Guinea. Crop Sci. 61:137–149.

Eyland, D., N. Luchaire, L. Cabrera-Bosquet, B. Parent, S.B. Janssens, et al. 2022. High-throughput phenotyping reveals differential transpiration behaviour within the banana wild relatives highlighting diversity in drought tolerance. Plant, Cell. Environ. 45:1647–1663.

Eyland, D., C. Gambart, R. Swennen, and S. Carpentier. 2023. Unravelling the diversity in water usage among wild banana species in response to vapour pressure deficit. Front. Plant Sci. 14:1068191.

Fandika, I.R., D. Kadyampakeni, B.M.L. Mwenebanda, and T.M. Magombo. 2014. Banana irrigation management and optimization: A comparative study of researcher-managed and farmer-managed irrigated banana production in Shire Valley, Malawi. Afr. J. Agric. Res. 9:2687–2693.

FAOSTAT. 2017. Food and Agricultural Organization (FAO). http://www.fao.org/faostat/en/#data/QC . Accessed 4 May 2019.

Farooq, M., A. Wahid, N. Kobayashi, D. Fujita, and S.M.A. Basra. 2009. Plant drought stress: Effects, mechanisms and management. Agron. Sustain. Dev. 29:185–212.

Feng, X., F. Chen, W. Liu, M. Thu, Z. Zhang, et al. 2016. Molecular characterization of *MaCCS*, a novel copper chaperone gene involved in abiotic and hormonal stress responses in *Musa acuminata* cv. Tianbaojiao. Int J. Mol Sci. 17:441.

Feng, H., W. Chen, S. Hussain, S. Shakir, V. Tzin, F. Adegbayi, et al. 2023. Horizontally transferred genes as RNA interference targets for aphid and whitefly control. Plant Biotechnol J. 21:754-768.

Fletcher, S.J., P.T. Reeves, B.T. Hoang, and N. Mitter. 2020. A perspective on RNAi-based biopesticides. Front. Plant Sci. 11:508089.

Flexas, J., and H. Medrano. 2002. Drought-inhibition of photosynthesis in C3 plants: Stomatal and non-stomatal limitations revisited. Ann. Bot. 89:183–189.

Foyer, C.H., and G. Noctor. 2000. Oxygen processing in photosynthesis: Regulation and signaling. New Phytol. 146:359–388.

Fulton, T., R. van der Hoeven, N.T. Eannetta, and S.D. Tanksley. 2002. Identification, analysis and utilization of conserved ortholog set (COS) markers for comparative genomics in higher plants. Plant Cell. 14:1457–1467.

Fungo, R, and M. Pillay. 2011. β-Carotene content of selected banana genotypes from Uganda. Afr. J. Biotechnol. 10:5423–5430.

Galeano, C.H., A.J. Cortés, A.C. Fernández, Á. Soler, N. Franco-Herrera, et al. 2012. Gene-based Single Nucleotide Polymorphism Markers for genetic and association mapping in common bean. BMC Genet. 13:48.

Gill, S.S., and N. Tuteja. 2010. Reactive oxygen species and antioxidant machinery in abiotic tolerance in crop plants. Plant Physiol. Biochem. 48:909–930.

Goenaga, R., and H. Irizarry. 1998. Yield of banana growth with supplemental drip irrigation on an ultisol. Exp. Agric. 34:439–448.

Goenaga, R., and H. Irizarry. 2000. Yield and quality of banana irrigated with fractions of class A pan evaporation on an oxisol. Agron J. 92:1008–1012.

Gold C.S., G.H. Kagezi, G. Night, and P.E. Ragama. 2004. The effects of banana weevil, *Cosmopolites sordidus* (Germar), damage on highland banana growth, yield and stand duration in Uganda. Ann. Appl. Biol. 145:263–269.

Govindappa, M., Pallavi, and C. Seenappa. 2014. Importance of mulching as a soil and water conservative practice in fruit and vegetable production-Review. Int J. Agric Innov. Res. 3:1014–1017.

Haefele, S.M., J.D.L.C. Siopongco, A.A. Boling, B.A.M. Bouman, and T.P. Tuong. 200). Transpiration efficiency of rice (*Oryza sativa* L.). Field Crops Res. 111:1–10.

Han, B., J. Wang, Y. Li, X. Ma, S. Jo, et al. 2018. Identification of quantitative trait loci associated with drought tolerance traits in rice (*Oryza sativa* L.) under PEG and field drought stress. Euphytica. 214:74.

Handayani, T., A. Maharijaya, Y. Wahyu, Sobir and Witjaksono. 2024. Induction, growth, and characteristics of embryonic cell suspension culture of wild bananas (*Musa acuminata* ssp.). In Vitro Cell. Dev Biol.- Plant. 60:1-15.

Hasan, S.A., and S.M. Khasim. 2018. Evaluation of RAPD analysis to differentiate the genotypes of 11 *Musa* cultivars. J. Biotechnol. Biochem. 4:29-36.

Hayat, S., Q. Hayat, M.N. Alyemeni, A.S. Wani, J. John Pichtel, and A. Ahmad A. 2012. Role of proline under changing environments – A review. Plant Signal. Behav. 7:1456–1466.

Haq, S., R.B. Singh, V. Gupta, M.D. Mahendrakar, S.L. Kothari, and S. Kachhwaha. 2023. Metabolic pathway responsive gene encoding enzyme anchored EST–SSR markers based genetic and population assessment among Capsicum accessions. Genet. Resour. Crop Evol. 70:1767-1783.

Hu, W., L. Wang, W. Tie, Y. Yan, Z. Ding, et al. 2016. Genome-wide analyses of the *bZIP* family reveal their involvement in the development, ripening and abiotic stress response in banana. Sci. Rep. 6:30203.

Hu, C., O. Sheng, G. Deng, W. He, T. Dong, et al. 2021. CRISPR/Cas9-mediated genome editing of *MaACO1* (aminocyclopropane-1-carboxylate oxidase1) promotes the shelf life of banana fruit. Plant Biotechnol. J. 19:654–656.

Hulme, M., R. Doherty, T. Ngara, M. New, and D. Lister. 2001. African Climate Change: 1900–2100. Clim. Res. 17:145–168.

Ibrahim, S.E., A. Schubert, K. Pillen, and J. Léon. 2012. QTL analysis of drought tolerance for seedling root morphological traits in an advanced backcross population of spring wheat. Int J. AgriSci. 2:619–629.

Janssens, S.B., F. Vandelook, E. De Langhe, B. Verstraete, E. Smets, et al. 2016. Evolutionary dynamics and biogeography of Musaceae reveal a correlation between the diversification of the banana family and the geological and climatic history of Southeast Asia. New Phytol. 210:1453-1465.

Jiang, H., M. Waseem, Y. Wang, S. Basharat, X. Zhang, et al. 2023. Development of simple sequence repeat markers for sugarcane from data mining of expressed sequence tags. Front. Plant Sci. 14:1199210.

Jones, H.G. 2014. Plants and Microclimate: A quantitative approach to environmental plant physiology. 3rd ed. Cambridge University Press, Cambridge, UK.

Kabunga, N.S., T. Dubois, and M. Qaim. 2012. Yield effects of tissue culture bananas in Kenya: Accounting for selection bias and the role of complementary inputs. J. Agric Econ. 63:444–464.

Kader MA, M. Senge, M.A. Mojid, and K. Nakamura. 2017. Mulching type-induced soil moisture and temperature regimes and water use efficiency of soybean under rain-fed condition in central Japan. Int Soil Water Conserv Res. 5:302–308.

Kader, M.A., A. Singha, M.A. Begum, A. Jewel, F.H. Khan, et al. 2019. Mulching as water-saving technique in dryland agriculture. Bull. Natl Res Cent. 43:1-6.

Kallow, S., K. Longin, N.F. Sleziak, S.B. Janssens, F. Vandelook, et al. 2020. Challenges for ex situ conservation of wild bananas: seeds collected in Papua New Guinea have variable levels of desiccation tolerance. Plants. 9:1243.

Kallow, S., M. Garcia Zuluaga, N. Fanega Sleziak, B. Nugraha, A. Mertens, et al. 2022. Drying banana seeds for ex situ conservation. Conservation physiology. 10:p.coab099.

Karamura, E.B., and D.A. Karamura. 1995. Banana morphology - part II: the aerial shoot', p. 190-205, *In* S. Gowen, ed. Bananas and plantains. World Crop Series, The Netherlands.

Karamura, D., M. Kitavi, M. Nyine, D. Ochola, W. Ocimati, et al. 2016. Genotyping the local banana landrace groups of East Africa. Acta Hortic. 1114:67–74.

Karuwal, R.L., Kasiamdari, R. and Daryono, B.S., 2024. Genetic variation of Fei banana (*Musa troglodytarum* l.) in Maluku islands using RAPD markers. SABRAO J. Breed. Genet. 56(1).

Kaya, E., F.V.D. Souza, J.A. dos Santos-Serejo, and S. Galatali. 2020. Influence of dehydration on cryopreservation of *Musa* spp. germplasm. Acta Bot. Croat. 79:99-104.

Kaur, N., A. Alok, Shivani, P. Kumar, N. Kaur, et al. 2020. CRISPR/Cas9 directed editing of lycopene epsilon-cyclase modulates metabolic flux for β-carotene biosynthesis in banana fruit. Metab. Eng. 59:76–86.

Khaskheli, A.J., M. Ali, S.Z.H. Shah, Z.F. Memon, S. Awan, et al. 2021. Initiation, proliferation, and improvement of a micropropagation system for mass clonal production of banana through shoot-tip culture. J. Plant Biotechnol. 48:86-92.

Kisaakye, J., H. Fourie, S. Haukeland, J. Kisitu, S. Nakimera, et al. 2022. Endophytic non-pathogenic *Fusarium oxysporum*-derived dual benefit for nematode management and improved banana (*Musa* spp.) productivity. Agriculture. 12:125.

Kissel, E., P. van Asten, R. Swennen, J. Lorenzen, and S.C. Carpentier. 2015. Transpiration efficiency versus growth: Exploring the banana biodiversity for drought tolerance. Sci. Hortic. 185:175–182.

Kovács, G., L. Sági L, G. Jacon, G. Arinaitwe, J-P. Busogoro, et al. 2013. Expression of a rice chitinase gene in transgenic banana (‘Gros Michel’, AAA genome group) confers resistance to black leaf streak disease. Transgenic Res. 22:117–130.

Kumar, S., J. Bhati, A. Saha, S.B. Lal, P.K. Pandey, et al. 2022. CerealESTDb: a comprehensive resource for abiotic stress-responsive annotated ESTs with predicted genes, gene ontology, and metabolic pathways in major cereal crops. *Frontiers in Genetics*, *13*, p.842868.

Lacerda, C.F., J. Cambraia, M. Cano, and H.A. Ruiz. 2001. Plant growth and solute accumulation and distribution in two sorghum genotypes under NaCl stress. Rev. Bras. Fisiol. Veg. 13:270–284.

Li, R., X. Hou, Z. Jia, Q. Han, X. Ren, et al. 2013. Effects on soil temperature, moisture, and maize yield of cultivation with ridge and furrow mulching in the rainfed area of the Loess Plateau, China. Agric. Water Manag. 116:101–109.

Liu, S., X. Wang, H. Wang, H. Xin, X. Yang, et al. 2013. Genome wide analysis of *ZmDREB* genes and their association with natural variation in drought tolerance at seedling stage of *Zea mays* L. PLoS Genet. 9(9):e1003790.

Mackelprang, R., and P.G. Lemaux. 2020. Genetic engineering and editing of plants: an analysis of new and persisting questions. Annu. Rev. Plant Biol. 71:659-687.

Magambo, B., K. Harjeet, G. Arinaitwe, S. Tendo, I.K. Arinaitwe, et al. 2016. Inhibition of cell death as an approach for development of transgenic resistance against Fusarium wilt disease. Afric J. Biotechnol. 15:786‑797.

Mahouachi, J. 2007. Growth and mineral nutrient content of developing fruit on banana plants (*Musa acuminata* AAA, 'Grand Nain') subjected to water stress and recovery. J. Hortic. Sci. Biotechnol. 82:839–844.

Mahouachi, J. 2009. Changes in nutrient concentrations and leaf gas exchange parameters in banana plantlets under gradual soil moisture depletion. Sci. Hortic. 120:460–466.

Manimaran, P., M.R. Sanjay, P. Senthamaraikannan, M. Jawaid, S.S. Saravanakumar, and R. George. 2018. Synthesis and characterization of cellulosic fibre from red banana peduncle as reinforcement for potential applications. J. Nat. Fibres 16:768–780.

Marin. D.H., R.A. Romero, M. Guzman, and T.B. Sutton. 2003. Black Sigatoka: An increasing threat to banana cultivation. Plant Dis. 87:208–222.

Martin, G., A. Cottin, F.C. Baurens, K. Labadie, C. Hervouet, et al. 2023. Interspecific introgression patterns reveal the origins of worldwide cultivated bananas in New Guinea. The Plant Journal. 113:802-818.

Martínez, J.P., S. Lutts, A. Schanck, M. Bajji, and J.-M Kinet. 2004. Is osmotic adjustment required for water stress resistance in the Mediterranean shrub *Atriplex halimus* L. J. Plant Physiol. 161:1041–1051.

Mattos-Moreira, L.A., C.F. Ferreira, E.P. Amorim, C.P. Pirovani, E.M. de Andrade EM, et al. 2018. Differentially expressed proteins associated with drought tolerance in bananas (*Musa* spp.). Acta Physiol. Plantarum 40:60.

Meer, L., S. Mumtaz, A.M. Labbo, M.J. Khan, and I. Sadiq. 2019. Genome-wide identification and expression analysis of calmodulin-binding transcription activator genes in banana under drought stress. Sci Hortic. 244:10–14.

Mertens, A., Y. Bawin, S. Vanden Abeele, S. Kallow, T. Vu Dang, et al. 2021. Genetic assessment of Vietnamese *Musa balbisiana* populations. PLoS One. 16:e0253255.

Mertens, A., Y. Bawin, S. Vanden Abeele, S. Kallow, R. Swennen, et al. 2022. Phylogeography and conservation gaps of *Musa balbisiana* Colla genetic diversity revealed by microsatellite markers. Genet. Resour. Crop Evol. 69:2515-2534.

Miao, H., P. Sun, Q. Liu, Y. Miao, J. Liu, et al. 2017. Genome-wide analyses of *SWEET* family proteins reveal involvement in fruit development and abiotic/ biotic stress responses in banana. Sci. Rep. 7:3536.

Mustaffa, M.M., and V. Kumar. 2012. Banana production and productivity enhancement through spatial, water and nutrient management. J. Hortic Sci. 7:1–28.

Muthusamy, M., S. Uma, S. Backiyarani, M.S. Saraswathi, and A. Chandrasekar. 2016. Transcriptomic changes of drought-tolerant and sensitive banana cultivars exposed to drought stress. Front. Plant Sci 7:1609.

Mwadzingeni, L., H. Shimelis, E. Dube, M.D. Laing, and T.J. Tsilo. 2016. Breeding wheat for drought tolerance: Progress and technologies. J. Integr Agric. 15:935–943.

Mwaka, H.S., L. Bauters, J. Namaganda, S. Marcou, P.N. Bwesigye, et al. 2023. Transgenic East African highland banana plants are protected against *Radopholus similis* through host-delivered RNAi. Int J. Molecul Sci. 24:12126.

Nahas, L.D., A.M. Alsamman, A. Hamwieh, N. Al-Husein, and G. Lababidi. 2020. Characterization of EST-SSR markers in bread wheat EST related to drought tolerance and functional analysis of SSR-containing unigenes. Highlights in BioScience. 3:1-12.

Namanya, P., J. Tindamanyire, S. Buah, J. Namaganda, J. Kubiriba, et al. 2020. Genetically modified bananas for communities of the Great Lakes region of Africa, p. 117-130, *In* V. Andersen, ed. Genetically modified and irradiated food. Controversial issues: Facts versus Perceptions. Academic Press, Cambridge, UK.

Namukwaya, B., L. Tripathi, J.N. Tripathi, G. Arinaitwe, S.B. Mukasa, and W.K. Tushemereirwe. 2012. Transgenic banana expressing *Pflp* gene confers enhanced resistance to Xanthomonas wilt disease. Transgenic Res. 21:855–865.

Nansamba, M., J. Sibiya, R. Tumuhimbise, W. Ocimati, E. Kikulwe, et al. 2022. Assessing drought effects on banana production and on-farm coping strategies by farmers—A study in the cattle corridor of Uganda. Clim. Change. 173:21.

Negi, S., H. Tak, and T.R. Ganapathi. 2018. A banana NAC transcription factor (*Musa*SNAC1) impart drought tolerance by modulating stomatal closure and H_2O_2 content. Plant Mol Biol. 96:457–471.

Niyongere, C., T. Losenge, E.M. Ateke, D. Nkezabahizi, G. Blomme, and P. Lepoint. 2012. Occurrence and distribution of banana bunchy top disease in the Great Lakes Region of Africa. Tree. Forestry Sci. Biotechnol. 6:102–107.

Nowakunda, K., A. Barekye, R.T. Ssali, J. Namaganda, W.K. Tushemereirwe, et al. 2015. ‘Kiwangaazi’ (syn ‘KABANA 6H’) Black Sigatoka nematode and banana weevil tolerant ‘Matooke’ hybrid banana released in Uganda. HortScience. 50:621–623.

Ocan, D., H.H. Mukasa, P.R. Rubaihayo, W. Tinzaara, and G. Blomme. 2008. Effects of banana weevil damage on plant growth and yield of East African Musa genotypes. J. Appl. Biosci. 9:407–415.

Ocimati, W., G.V. Nakato, K.K.M. Fiaboe, F. Beed, and G. Blomme. 2014. Incomplete systemic movement of *Xanthomonas campestris* pv. *Musacearum* and the occurrence of latent infections in Xanthomonas wilt-infected banana mats. Plant Pathol. 64:81–90.

Onyilo, F., G. Tusiime, J.N. Tripathi, L.H. Chen, B. Falk, et al. 2018. Silencing of the mitogen-activated protein kinases (MAPK) Fus3 and Slt2 in *Pseudocercospora fijiensis* reduces growth and virulence on host plants. Front. Plant Sci. 9:291.

Orgaz, F., M.D. Fernandez, S. Bonachela, M. Gallardo, and E. Fereres. 2005. Evapotranspiration of horticultural crops in an unheated plastic greenhouse. Agric. Water. Manag. 72:81–96.

Ortiz, R., and R. Swennen. 2014. From crossbreeding to biotechnology-facilitated improvement of banana and plantain. Biotechnol. Adv. 32:158–169.

Passioura, J.B. 2012. Phenotyping for drought tolerance in grain crops: When is it useful to breeders? Funct. Plant Biol. 39:377–412.

Passos, M.A., V. de Oliveira Cruz, F.L. Emediato, C. de Camargo Teixeira, M.T. Souza Jr, et al. 2012. Development of expressed sequence tag and expressed sequence tag–simple sequence repeat marker resources for *Musa acuminata*. AoB Plants. p.pls030.

Paul, J.Y., D.K. Becker, M.B. Dickman, R.M. Harding, H.K. Khanna, and J.L. Dale. 2011. Apoptosis-related genes confer resistance to fusarium wilt in transgenic ‘Lady Finger’ bananas. Plant Biotechnol J. 9:1141–1148.

Paul, J.Y., H. Khanna, J. Kleidon, P. Hoang, J. Geijskes, et al. 2017. Golden bananas in the field: Elevated fruit pro-vitamin A from the expression of a single banana transgene. Plant Biotechnol J. 15:520–532.

Paul, J.Y., R. Harding, W. Tushemereirwe, and J. Dale. 2018. Banana21: from gene discovery to deregulated golden bananas. Front. Plant Sci. 9:363551.

Petrick, J.S., B. Brower-Toland, A.L. Jackson, and L.D. Kier. 2013. Safety assessment of food and feed from biotechnology-derived crops employing RNA-mediated gene regulation to achieve desired traits: A scientific review. Regul. Toxicol. Pharmacol. 66:167–176.

Perrier, X., C. Jenny, F. Bakry, D. Karamura, M. Kitavi, et al. East African diploid and triploid bananas: a genetic complex transported from South-East Asia. Ann. Bot. 123:19-36.

Pillay, M., E. Ogundiwin, D.C. Nwakanma, G. Ude, and A. Tenkouano. 2001. Analysis of genetic diversity and relationships in East African banana germplasm. Theor. Appl. Genet. 102:965–970.

Pillay, M., J. Hartman, and A. Tenkouano. 2002. Bananas and Plantains: Future challenges in *Musa* breeding, p. 223-252, *In* M.S. Kang, ed. Crop improvement, challenges in the twenty-first century. Food Products Press, New York.

Pillay, M., K. Ashokkumar, A. James, S.J. Kirubakara, R. Miller, R. Ortiz, and E. Sivalingam. 2012. Molecular marker techniques in *Musa* genomic research, p. 70-90, *In* M. Pillay, G. Ude, and C. Kole, eds. Genetics, Genomics, and Breeding of Bananas. CRC Press, Boca Raton, Florida, USA.

Pillay, M. 2024. The genetic homogeneity of Uganda's East African Highland bananas (Mutika/Lujugira) does not match the extensive morphological variation identified in this subgroup. Int J. Plant Biol. 15:267-280.

Pimentel, C. 1999. Water relations in two hybrids of corn under two cycles of water stress. Pesq. Agropec. Bras. 34:2021–2027.

Ploetz, R.C. 2015. Fusarium wilt of banana. Phytopathology 105:1512–1521.

Poerba, Y.S., and F. Ahmad. 2010. Genetic variability among 18 cultivars of cooking bananas and plantains by RAPD and ISSR markers. Biodiversitas. 11(3).

Portal, O., M. Acosta-Suárez, B. Ocaña, W. Schäfer, E. Jiménez, et al. 2012. A green fluorescent protein-transformed *Mycosphaerella fijiensis* strain shows increased aggressiveness on banana. Australasian Plant Pathol. 41:645-647.

Raboin, L.-M., F. Carreel, J.-L. Noyer, F.-C. Baurens, J.-P. Horry, et al. 2005. Diploid ancestors of triploid export banana cultivars: Molecular identification of 2n restitution gamete donors and n gamete donors. Mol. Breed. 16:333–341.

Raemaekers, R.H. 2001. Crop production in tropical Africa. Directorate General for International Cooperation (DGIC), Brussels, Belgium.

Ramirez, J., A. Jarvis, I. Van den Bergh, C. Staver, and D. Turner. 2011. Chapter 20: Changing climates: Effects on growing conditions for banana and plantain (*Musa* spp.) and possible responses, p. 426-438, *In* S. Yadav, B. Redden, J.L. Hattfield, and H. Lotze-Campen, eds. Crop adaptation to climate change. Wiley-Blackwell Publishing Ltd, Chichester, UK.

Ravi, I., and S. Uma. 2011. Phenotyping bananas and plantains for adaptation to drought, *In* M. Philippe, and R. Jean-Marcel, eds. Drought phenotyping in crops: From theory to practice. CGIAR Generation Challenge Programme/CIMMYT, Texcoco, Mexico.

Ravi, I., S. Uma, M.M. Vaganan, and M.M. Mustaffa. 2013. Phenotyping bananas for drought resistance. Front. Physiol. 4: 9.

Ravi, I., and M.M. Vaganan. 2016. Abiotic stress tolerance in banana, p. 207-222, *In* N.K.S. Rao, K.S. Shivashankara, and R.H. Laxman, eds. Abiotic stress physiology of horticultural crops. Springer, India.

Risterucci, A.-M., I. Hippolyte, X. Perrier, L. Xia, V. Caig, et al. 2009. Development and assessment of Diversity Arrays Technology for high-throughput DNA analyses in *Musa*. Theoret. Appl. Genet. 119:1093–1103.

Robinson, J.C. 1996. Banana and plantains. CAB International, Wallingford, UK.

Robinson, J.C. 2000. Banana productivity-the impact of agronomic practices. Acta Hortic. 540:247–258.

Robinson, J.C., and A.J. Alberts. 1986. Growth and yield responses of banana (cultivar 'Williams') to drip irrigation under drought and normal rainfall conditions in the subtropics. Sci. Hortic. 30:187–202.

Robinson, J.C., and V.G. Sauco. 2010. Bananas and Plantains. 2nd ed. CAB International Wallingford, UK.

Rocha, A.D.J., J.M.D.S. Soares, F.D.S. Nascimento, A.S. Santos, V.B.D.O. Amorim, et al. 2021. Improvements in the resistance of the banana species to Fusarium Wilt: A systematic review of methods and perspectives. J. Fungi, 7:249.

Roderick, H., L. Tripathi, A. Babirye, D. Wang, J. Tripathi, et al. 2012. Generation of transgenic plantain (*Musa* spp.) with resistance to plant pathogenic nematodes. Mol. Plant Pathol. 13:842–851.

Sabouri, A., R. Afshari, T. Raiesi, H.B. Raouf, E. Nasiri, et al. 2018. Superior adaptation of aerobic rice under drought stress in Iran and validation test of linked SSR markers to major QTLs by MLM analysis across two years. Mol. Biol. Rep. 45:1037–1053.

Safhi, F.A., S.M. Alshamrani, D.S. Alshaya, M.A. Hussein, and D. Abd El-Moneim. 2023. Genetic diversity analysis of banana cultivars (*Musa* sp.) in Saudi Arabia based on AFLP marker. Current issues. Mol. Biol. 45:1810-1819.

Saha, J., K. Giri, and S. Roy. 2020. Identification and characterization of differentially expressed genes in the rice root following exogenous application of spermidine during salt stress. Genomics. 112:4125-4136.

Sairam, R.K., D.S. Shukla, and D.C. Saxena. 1997. Stress induced injury and antioxidant enzymes in relation to drought tolerance in wheat genotypes. Biol. Plant. 40:357–364.

Sardos, J., C. Breton, X. Perrier, I. Van den Houwe, S. Carpentier, et al. 2022. Hybridization, missing wild ancestors and the domestication of cultivated diploid bananas. Front. Plant Sci. 13:969220.

Satrio, R.D., M.H. Fendiyanto, E.D.J. Supena, Suharsono and Miftahudin, 2019. Identification of drought-responsive regulatory genes by hierarchical selection of expressed sequence tags and their expression under drought stress in rice. Intl J Agric Biol. 22:1524–1532.

Sebuwufu, G., P.R. Rubaihayo, and G. Blomme. 2004. Variability in the root system of East African banana genotypes. Afr. Crop Sci J. 12:85-93.

Shaikh, T.N., J.R. Patel, and B.H. Patel. 2023. Mulching effect on water management in agro-ecological systems: a review. AQUA—Water Infrastruct. Ecosyst. Soc. 72:1664-1680.

Shekhawat, U. K. S., T.R. Ganapathi, and A.B. Hadapad. 2012. Transgenic banana plants expressing small interfering RNAs targeted against viral replication initiation gene display high-level resistance to banana bunchy top virus infection. J. General Virol. 93, 1804‑1813.

Shim, J.S., N. Oh, P.J. Chung, Y.S. Kim, Y.D. Choi, and J.K. Kim. 2018. Overexpression of *OsNAC14* improves drought tolerance in rice. Front. Plant Sci. 9:310.

Shongwe, V.D., R. Tumber, M.D. Masarirambi, and A.N. Mutukumira. 2008. Soil water requirements of tissue-cultured dwarf Cavendish banana (*Musa* spp. L). Phys. Chem. Earth 33:768–774.

Simmonds., N.W. 1962. The evolution of the bananas. Longmans, London, UK.

Simmonds, N.W. 1966. Bananas. 2nd ed. Longmans, London, UK.

Šimoníková, D., A. Němečková, J. Čížková, A. Brown, R. Swennen, et al. 2020. Chromosome painting in cultivated bananas and their wild relatives (*Musa* spp.) reveals differences in chromosome structure. Int. J. Mol Sci. 21:7915.

Singh, H.P., and S. Uma. 2000. Genetic diversity of banana in India, p.136-156, *In* H.P. Singh, and K.L. Chadha, eds. Banana improvement, production and utilization, *In* Proceedings of the conference on challenges for banana production and utilization in 21st century. Trichy, India.

Singh, W.A., N.S. Singh, E.J. Devi, P.J. Handique, and H.S. Devi. 2021. Collection and characterization of banana gene pools (*Musa* spp.) in Manipur (NE India) using PCR–RFLP and RAPD and ISSR markers. Braz J. Bot. 44:671-684.

Skutch, A.F. 1932. Anatomy of the axis of the banana. The Botanical Gazette 43(3).

Song, S., Y.I. Xu, D. Huang, H. Miao, J. Liu, et al. 2018. Identification of a novel promoter from banana aquaporin family gene (*MaTIP1;2*) which responses to drought and salt-stress in transgenic *Arabidopsis thaliana*. Plant Physiol. Biochem. 128:163–169.

Speijer, P.R. 1999. East African Highland banana production as influenced by nematodes and crop management in Uganda. Int.J. Pest Manag. 45:41–49.

Sreedharan, S., U.K.S. Shekhawat, and T.R. Ganapathi. 2013. Transgenic banana plants overexpressing a native plasma membrane aquaporin *MusaPIP1;2* display high tolerance levels to different abiotic stresses. Plant Biotechnol. J. 11:942–952.

Ssali, R.T. 2016. The identification and characterization of resistance *Musa* to *Fusarium Oxysporum* f. sp *cubense* race 1. PhD thesis. Stellenbosch University, South Africa.

Stevens, B., J. Diels, E. Vanuytrecht, A. Brown, S. Bayo, et al. 2020a. Canopy cover evolution, diurnal patterns and leaf area index relationships in a Mchare and Cavendish banana cultivar under different soil moisture regimes. Scientia Hortic. 272:109328.

Stevens, B., J. Diels, A. Brown, S. Bayo, P. A. Ndakidemi, and R. Swennen. 2020b. Banana biomass estimation and yield forecasting from non-destructive measurements for two contrasting cultivars and water regimes. Agronomy. 10:1435.

Subrahmanyeswari, T., and S. Gantait. 2022. Biotechnology of banana (*Musa* spp.): multi-dimensional progress and prospect of in vitro–mediated system. Appl Microbiol. Biotechnol. 106:3923-3947.

Sunisha, C., H.D. Sowmya, T.R. Usharani, M. Umesha, H.R. Gopalkrishna, et al. 2020. Deployment of stacked antimicrobial genes in banana for stable tolerance against *Fusarium oxysporum* f.sp. *cubense* through genetic transformation. Mol. Biotechnol. 62:8–17.

Surendar, K.K., D.D. Devi, I. Ravi, S. Krishnakumar, S.R. Kumar, and K. Velayudham. 2013a. Water stress in banana – A Review. Bull. Environ. Pharmacol. Life Sci. 2:1–18.

Surendar, K.K., D.D. Devi, I. Ravi, P. Jeyakumar, and K. Velayudham. 2013b. Physiological and biochemical behavior in banana cultivars and hybrids under water deficit. Afr J. Agric Res. 8:4198–4208.

Surendar, K.K., D.D. Devi, I. Ravi, P. Jeyakumar, S.R. Kumar, and K. Velayudham. 2013c. Effect of water deficit on yield and epicuticular wax, proline and free amino acid content of banana cultivars and hybrids. World J. Agric. Sci. 9:201–209.

Swennen, R. 1990. Plantain cultivation under West African conditions- A reference manual, p. 24. International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

Swennen, R., and D. Vuylsteke. 2001. Banana (*Musa* L.), p. 530-552, *In* R.H. Raemaekers ed) Crop production in Tropical Africa. DGIC, Brussels, Belgium.

Swennen, R., S.C. Carpentier, I.M. Henry, A. Vertommen, I. Van den houwe, et al. 2011. From fundamental research discoveries to applications for banana improvement. Acta Hortic. 897:47–53.

Tabkhkar, N., B. Rabiei, H.S. Lahiji, and M. Hosseini Chaleshtori. 2018. Genetic variation and association analysis of the SSR Markers linked to the major drought-yield QTLs of rice. Biochem. Genet. 56:356–374.

Taulya, G., P.J.A. van Asten, S. Okech, and C. Gold. 2006. Effect of drought on the yields of an East African highland banana (cv. 'Kisansa') in Uganda. The proceedings of the 23rd Soil Science Society of East Africa conference. Masaka, Uganda.

Tenkouano, A. 2006. Recent advances and challenges in banana and plantain breeding, p. 141–159, *In* H.P. Singh, and S. Uma, eds. Banana technological advances. Westville Publishing House, Trichy, India.

Thingnam, S.S., D.S. Lourembam, P.S. Tongbram, V. Lokya, S. Tiwari, et al. 2023. A perspective review on understanding drought stress tolerance in wild banana genetic resources of northeast India. Genes. 14:370.

Thomas, D.S., and D. Turner. 2001. Banana (*Musa* spp.) leaf gas exchange and chlorophyll fluorescence in response to soil drought, shading and lamina folding. Sci. Hortic. 90:93–108.

Thomas, D.S, D. Turner, and D. Eamus. 1998. Independent effects of the environment on the leaf gas exchange of three banana (*Musa* spp.) cultivars of different genomic constitution. Sci. Hortic. 75:41–57.

Tossi, V.E., L.J. Martinez Tosar, L.E. Laino, J. Iannicelli, J.J. Regalado, et al. 2022. Impact of polyploidy on plant tolerance to abiotic and biotic stresses. Front. Plant Sci. 13:869423.

Tripathi, L., H. Mwaka, J.N. Tripathi, and W.K. Tushemereirwe. 2010. Expression of sweet pepper *Hrap* gene in banana enhances resistance to *Xanthomonas campestris pv. musacearum*. Mol. Plant Pathol. 11:721–731.

Tripathi, L., J.N. Tripathi, W.K. Tushemereirwe, G. Arinaitwe, and A. Kiggundu. 2013. Transgenic bananas with enhanced resistance against Xanthomonas wilt disease. Acta Hortic. 974:81–90.

Tripathi, L., J.N. Tripathi, H. Roderick, and H.J. Atkinson. 2013. Engineering nematode resistant plantains for Sub-Saharan Africa. Acta Hortic. 974:99–107.

Tripathi, L., H. Atkinson, H. Roderick, J. Kubiriba, and J.N. Tripathi. 2017. Genetically engineered bananas resistant to Xanthomonas wilt disease and nematodes. Food Energy Secur. 6:37-47.

Tripathi, L., H. Atkinson, H. Roderick, J. Kubiriba, and J.N. Tripathi. 2017. Genetically engineered bananas resistant to Xanthomonas wilt disease and nematodes. Food. Energy Secur. 6:37–47.

Tripathi, J.N., V.O. Ntui, M. Ron, S.K. Muiruri, A. Britt, et al. 2019. CRISPR/Cas9 editing of endogenous banana streak virus in the B genome of *Musa* spp. overcomes a major challenge in banana breeding. Commun. Biol. 2:46.

Tripathi, L., V.O. Ntui, and J.N. Tripathi. 2019. Application of genetic modification and genome editing for developing climate-smart banana. Food Energy Secur. 8:e00168.

Tripathi, J.N., V.O. Ntui, T. Shah, and L. Tripathi. 2021. CRISPR/Cas9-mediated editing of *DMR6* orthologue in banana (*Musa* spp.) confers enhanced resistance to bacterial diseases. Plant Biotechnol. J. 19:1291–1293.

Tripathi, L., V.O. Ntui, and J.N. Tripathi. 2022. Control of bacterial diseases of banana using CRISPR/Cas-based gene editing. Int J Molecul Sci. 23:3619.

Turner, D.W. 2005. Factors affecting the physiology of the banana root system, p. 107–113, *In* D.W. Turner, and F.E. Rosales, eds. Towards a better understanding of for its productive management. International Network for the Improvement of Banana and Plantain (INIBAP), Montpellier, France.

Ude, G., M. Pillay, E. Ogundiwin, and A. Tenkouano. 2003. Genetic diversity in an African plantain core collection using AFLP and RAPD markers. Theor. Appl. Genet. 107:248–255.

Uma, S., and S. Sathiamoorthy. 2002. Names and synonyms of bananas and plantains in India. National Research Centre for Banana (ICAR), Trichy, India.

Uma, S., S. Sathiamoorthy, H.P. Singh, and M. Dayarani. 2002. Crop improvement in *Musa*-evaluation of germplasm for male and female fertility. Indian J. Plant Genet. Res. 15:137–139.

Uma, S., S. Lakshmi, M.S. Saraswathi, A. Akbar, and M.M. Mustaffa. 2011. Embryo rescue and plant regeneration in banana (*Musa* spp.). Plant Cell, Tissue Organ Cult. 105:105–111.

Uma, S., R. Karthic, S. Kalpana, S. Backiyarani, M. Kumaravel, et al. 2023. An efficient embryogenic cell suspension culture system through secondary somatic embryogenesis and regeneration of true-to-type plants in banana cv. Sabri (silk subgroup AAB). Plant Cell, Tissue Organ Cult. 155:313-322.

Van Asten, P.J.A., A.M. Fermont, and G. Taulya. 2011. Drought is a major yield loss factor for rainfed East African highland banana. Agric. Water. Manag. 98:541–552.

Vanhove, A.C., W. Vermaelen, B. Panis, R. Swennen, and S.C. Carpentier. 2012. Screening the banana biodiversity for drought tolerance: Can an *in vitro* growth model and proteomics be used as a tool to discover tolerant varieties and understand homeostasis. Front. Plant Sci. 3:176.

Van Wesemael, J., E. Kissel, D. Eyland, T. Lawson, R. Swennen, et al. 2019. Using growth and transpiration phenotyping under controlled conditions to select water efficient banana genotypes. Front. Plant Sci. 10:352.

Vásquez, N.M. 2003. Anatomy and morphology of monocotyledonous and dicotyledonous roots, p. 37-42, *In* D.W. Turner, and F.E. Rosales, eds. Banana root system: towards a better understanding for its productive management. Proceedings of an international symposium held in San José, Costa Rica, 3-5 November 2003. International Network for the Improvement of Banana and Plantain (INIBAP), Montpellier, France.

Vishnevetsky, J., T.L. White, A.J. Palmateer, M. Flaishman, Y. Cohen, et al. 2011. Improved tolerance toward fungal diseases in transgenic Cavendish banana (*Musa* spp. AAA group) cv. Grand Nain. Transgenic Res. 20:61-72.

Wairegi, L.W.I., P.J.A. van Asten, M.M. Tenywa, and M.A. Bekunda. 2010. Abiotic constraints override biotic constraints in East African highland banana systems. Field Crops Res. 117:146–153.

Wang, X., H. Wang, S. Liu, A. Ferjani, J. Li, et al., 2016. Genetic variation in *ZmVPP1* contributes to drought tolerance in maize seedlings. Nat. Genet. 48:1233–1241.

Wang, X., R. Yu, and J. Li. 2021. Using genetic engineering techniques to develop banana cultivars with fusarium wilt resistance and ideal plant architecture. Front. Plant Sci. 11:617528.

Wong, W.C., R.Y. Othman, and N. Khalid. 2008. Improvements in the efficiency of Agrobacterium-mediated transformation of embryogenic cell suspensions of banana cv. ‘Mas’ using a low-antibiotic liquid washing-assisted approach. Transgenic Plant J. 2:75–85.

Wonga, G.R., P. Mazumdar, S.E. Lau, and J.A. Harikrishna. 2018. Ectopic expression of a *Musa acuminata* root hair defective 3 (*MaRHD3*) in Arabidopsis enhances drought tolerance. J. Plant Physiol. 231:219–233.

Wu, C., W. Deng, W. Shan, X. Liu, L. Zhu, et al. 2023. Banana MKK1 modulates fruit ripening via the MKK1-MPK6-3/11-4-bZIP21 module. Cell Rep. 42(8).

Xu, Y.I., W. Hu, J. Liu, J. Zhang, C. Jia, et al. 2014. A banana aquaporin gene, *MaPIP1; 1*, is involved in tolerance to drought and salt stresses. BMC Plant Biol. 14:59.

Yaqoob, A., A.A. Shahid, T.R. Samiullah, A.O. Rao, M.A.U. Khan, et al. 2016. Risk assessment of *Bt* crops on the non-target plant-associated insects and soil organisms. J. Sci. Food Agric. 96:2613–2619.

Yue, B., W. Xue, L. Xiong, X. Yu, L. Luo, et al. 2006. Genetic basis of drought resistance at reproductive stage in rice: Separation of drought tolerance from drought avoidance. Genetics 172:1213–1228.

Zhao, D., X. Ni, Z. Zhang, H. Niu, R. Qiu, et al. 2022. Bt protein hasten entomopathogenic fungi-induced death of nontarget pest whitefly by suppressing protective symbionts. Sci. Total Environ. 853:158588.

Zribi, W., R. Aragüés, E. Medina, and J.M. Faci. 2015. Efficiency of inorganic and organic mulching materials for soil evaporation control. Soil Tillage Res. 148:40–45.

Chapter 3[2]: Assessing drought effects on banana production and on-farm coping strategies by farmers – a study in the cattle corridor of Uganda

Abstract

Drought is a major threat to banana production in Uganda, leading to large yield losses. This study documented drought effects on banana production and identified farmers' coping strategies to mitigate the impact of droughts. Interviews were conducted in eight districts, randomly selected from banana-growing districts in Uganda's cattle corridor, characterised by frequent droughts. Data were collected from 120 respondents/farms. Banana production in the study area was dominated by small-scale farmers, growing mostly a combination of cooking and dessert banana types. Among the 15 identified effects of drought stress on banana growth, reduced bunch weight, wilting and drying of leaves, reduced leaf production and reduced number of fingers and clusters were the most reported. 'Mpologoma' and 'FHIA 17' cultivars were reported as the most and least affected by drought stress, respectively. Although the cattle corridor is prone to recurrent droughts, the deployment of drought coping strategies was mostly low, with farmers using one to three strategies. A total of 12 drought mitigation practices were used across the cattle corridor, with mulching being the most common option. Irrigation was perceived as the most effective mitigation option though its deployment was limited by water scarcity and the high cost of water pumps. This study suggests the need to strengthen data collection and climate information systems and development of drought-tolerant cultivars by breeders. Additionally, farmers need to prioritise preventive coping strategies like planting drought-tolerant cultivars, irrigation, mulching, and manure application and ensure timely deployment of mitigation practices.

Keywords: banana production, cattle corridor, coping strategies, drought stress, Uganda

[2] This chapter was published in the journal, Climatic Change: Nansamba, M., J. Sibiya, R. Tumuhimbise, W. Ocimati, E. Kikulwe, et al. 2022. Assessing drought effects on banana production and on-farm coping strategies by farmers — a study in the cattle corridor of Uganda. Clim. Change 173:21. https://doi.org/10.1007/s10584-022-03408-w

3.1 Introduction

Banana (*Musa* spp.) is a major food and cash crop worldwide, particularly in the tropical and subtropical regions. The crop has a global production of 116.8 million tons harvested from an area of 5.16 million hectares (FAOSTAT, 2019). The East and Central African subregion, including Uganda, Tanzania, Kenya, Burundi, Rwanda, and the eastern part of the Democratic Republic of Congo is home to a unique group of bananas well-known as the East African Highland Bananas (EAHBs, *Musa* AAA genome). Moreover, this region is also considered to be a secondary centre of diversity for the EAHBs and plantains (AAB genome) (Simmonds, 1966), with an annual production of 17.31 million tons cultivated on 1.7 million hectares (FAOSTAT, 2019). In Uganda, EAHBs are the main staple food for over 13 million people, with cultivation mostly concentrated in the central and southwestern regions (BMGF, 2014). The crop is grown either as a sole crop or intercrop with other perennial or annual crops, offering additional benefits to the ecosystem in addition to animal feed, fibre, and food (van Asten et al., 2015; Ocimati et al., 2018). Although EAHBs are an important staple and commercial crop to many Ugandans (Bagamba, 2007), actual production remains low (< 30 t ha^{-1} $year^{-1}$) compared with the potential yield (> 70 t ha^{-1} $year^{-1}$) (van Asten et al., 2005) due to a combination of biotic and abiotic stresses.

Several studies in Uganda have reported on biotic stresses such as banana bacterial wilt (*Xanthomonas campestris* pv. *musacearum*) (Ocimati et al., 2014a), banana weevil (*Cosmopolites sordidus*) (Gold et al., 2004; Ocan et al., 2008) parasitic nematodes (*Pratylenchus coffeae and Radopholus similis*) (Speijer, 1999), fusarium wilt (*Fusarium oxysporum* f.sp. *cubense* [FOC]) (Kangire et al., 2000) and black leaf streak disease (*Mycosphaerella fijiensis*) (Barekye et al., 2009). Prominent abiotic stresses include low soil fertility (Wairegi et al., 2010) and drought (inadequate soil moisture) (van Asten et al., 2011a).

Drought is a serious constraint to banana production in Uganda, particularly in the cattle corridor where the country's largest banana production is based. With most of the farmers depending on seasonal rainfall patterns, there is an immediate effect of climate variability (manifesting as drought and unreliable rainfall) on crop production, food security, income, and livelihoods in general (NAPA, 2007; Anwar et al., 2013). The National Environment Management Authority (NEMA) (2016) reported that Uganda's cattle corridor is vulnerable to climate change impacts such as floods, erratic rains, high temperatures and prolonged droughts.

Climate models predicted that temperature and rainfall changes would most likely result in wet regions becoming wetter and dry areas becoming drier (Christensen et al., 2007; MWE 2007), as well as an increase in drought incidences (Hepworth and Goulden 2008). General circulation models projected that mean temperature increases ranging from 0.3°C to 0.5°C would occur every decade, especially in the semi-arid areas (Republic of Uganda, 2010). Moreover, McSweeney et al. (2008) reported a 1.4°C mean temperature increase in Uganda since the 1960s. Although contrasting annual rainfall trends have been reported in Uganda (Hepworth and Goulden 2008; McSweeney et al., 2008), significant seasonal rainfall distribution changes are anticipated. For instance, IGAD (2010) reported less, unreliable, and unevenly distributed rainfall, which was linked to more severe and frequent droughts. According to CRED (2022), Uganda has experienced nine drought events since 1961 to date, affecting almost 5 million people.

Drought effects on several sectors (including agriculture) within Uganda's cattle corridor have been reported (Nimusiima et al., 2013; Kilimani et al., 2015; Owoyesigire et al., 2016; Branch, 2018). Twongyirwe et al. (2019) evaluated farmers' perceptions of how drought affects household food security, existing coping responses and their determinants. They found that households were most vulnerable to drought-induced food insecurity and hence access to financial services and other sources of livelihood may offer resilience to such drought effects. In another study by Mfitumukiza et al. (2017), large crop losses and damages per household due to drought were reported.

However, the above studies did not consider the impact of drought on banana production in isolation but rather on crop production in general. There are gaps in literature regarding the daunting effects of drought stress on banana production and yield. Numerous studies focussing on phenotypic and physiological changes of selected banana genotypes to drought stress (also termed as moisture stress) have been conducted, but most of these have been carried out on-station under controlled conditions (Bananuka et al., 1999), with few studies conducted in the field (Wairegi et al., 2010; Taulya et al., 2014; Uwimana et al., 2021). One of the field studies found that drought results in crop failure and reduced bunch yield (up to 65% losses) when the rainfall falls below 1,100 mm per year (van Asten et al., 2011a).

While several efforts have been made or are being undertaken to increase the resilience of bananas to drought conditions through research and agronomic or crop management (van Asten

et al., 2011a; Nansamba et al., 2020), knowledge of how specific farmer-preferred cultivars respond to drought stress and holistic on-farm adaptive strategies by banana farmers is still limited. Drought effects, particularly on the production of locally grown banana cultivars, have not been systematically documented as a basis for use in banana improvement research. Understanding the impact of drought on different banana varieties and associated management measures by farmers is critical in managing drought effects on production across a scale and supporting the improvement of farmer coping practices. The purpose of this study was to assess the effects of drought on banana production and identify management strategies deployed by farmers. Specifically, the study aimed to (i) document drought effects on all locally grown varieties in terms of impact on plant growth characteristics, yield and yield-related parameters (ii) establish the variability within the set of cultivars maintained by farmers i.e. identify which cultivars are highly sensitive, moderately tolerant, very tolerant or not affected by drought (iii) identify on-farm coping practices employed by farmers for mitigating such adverse effects and (iv) determine the specific purpose(s) for each coping strategy in mitigating drought effects on bananas.

3.2 Methodology

3.2.1 Study area and site selection

This study was conducted in eight districts lying within the cattle corridor of Uganda (Figure 3.1). The cattle corridor dominated mostly by pastoral rangelands covers an area of 84,000 km^2 stretching from North-east to South-west Uganda. In this predominantly semi-arid region, local communities rely mainly on rain-fed crop and livestock production for their livelihood (McGahey and Visser, 2015; FAO, 2019). The annual daily average minimum and maximum temperatures in the region are 21.5°C and 30°C, respectively (Nimusiima et al., 2018). The area receives poorly distributed annual rainfall of 1350 mm (Mfitumukiza, 2015), with two rainy seasons (March to June and late August to November-December) and two dry seasons (June-August and December-February) (Nimusiima et al., 2013; Ogwang et al., 2016). It is characterized by unpredictable weather conditions such as unpredictable rainfall onsets and cessations, flooding, and recurrent and prolonged droughts (USAID, 2011; Nimusiima et al., 2013). Climatic data from the Uganda National Meteorological Authority indicates a decline in annual rainfall (Appendix 3.1) and an increasing trend in maximum and minimum

temperatures (Appendix 3.2) and in the cattle corridor districts of Mbarara, Mubende and Luwero over the past 60 years (1961-2020).

According to Kajobe et al. (2016), Uganda has ten agroecological zones, five of which fall within the cattle corridor, including Southern drylands, Lake Victoria Crescent, Karamoja drylands, part of Mid Northern and part of Eastern agroecological zone. Among these five agroecological zones, the Southern drylands and Lake Victoria Crescent were purposively selected because they host the largest banana-based cropping systems. From the Southern dryland agroecological zone, four districts including Ibanda, Sembabule, Ntungamo and Isingiro were randomly selected. In the Lake Victoria Crescent agroecological zone, the randomly selected districts included Mubende, Luweero, Nakaseke and Kiboga. At district level, two sub-counties were randomly selected from a list of banana-growing sub-counties (Table 3.1).

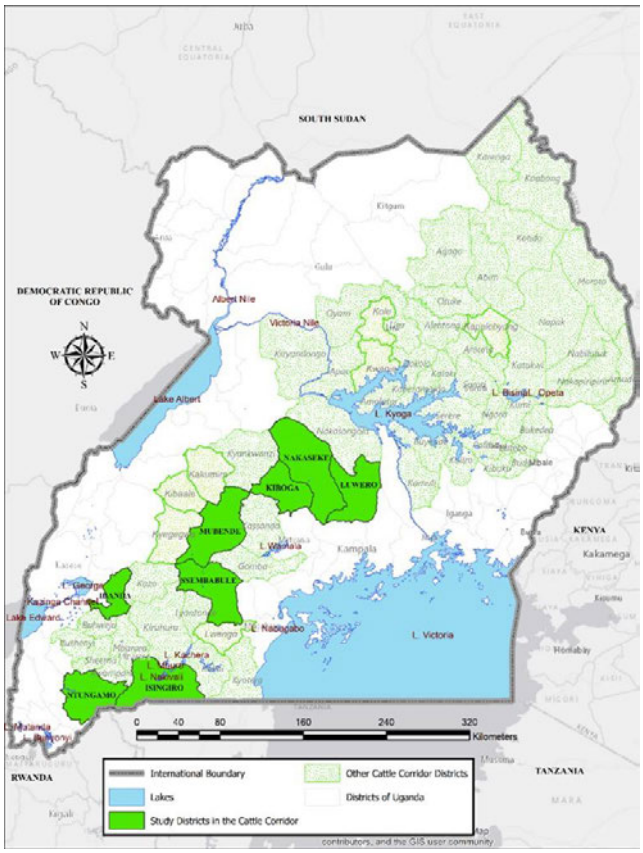


Figure 3.1 Map showing the location of study districts within the cattle corridor of Uganda

Table 3.1 The location of sampled banana farms in the cattle corridor of Uganda

Agroecological zone	District selected	Sub-county
Southern Drylands	Ibanda	Igorora town council, Kijongo
	Sembabule	Mateete, Rwebitakuri
	Ntungamo	Rubaare, Ruhaama
	Isingiro	Masha, Birere
Lake Victoria Crescent	Mubende	Bukuya, Makokoto
	Luweero	Bamunanika, Makulibita
	Nakaseke	Kasagga, Nakaseke
	Kiboga	Lwamata, Kibigga

3.2.2 Sampling, data collection and analysis

A total of 120 banana farms (i.e., 15 farms per district) were randomly selected with the assistance of District Agricultural and Extension Officers in the respective districts. Farms were only considered for selection if the plantation was at least 0.25 acres (with at least 100 banana plants) large and banana was grown as the main crop on that farm. It was assumed that this group of farmers had a better understanding and experience with the effects of drought on banana production, particularly drought effects on plant growth characteristics and yield, the response of different varieties as well as their coping strategies to mitigate adverse drought effects on-farm. Respondents participated in one-on-one interviews guided using a semi-structured questionnaire from which both quantitative and qualitative data were collected. The study was conducted towards the end of the long dry season of December to March 2018. Farm-level data were collected including, the banana plantation status, nature of the cropping system, drought stress effects on plant growth characteristics and farmers' on-farm coping strategies for mitigating drought effects on banana production.

Regarding plantation status, the interviewer recorded the plantation size, plantation management (poorly- or well-managed), plantation age, and banana cultivars grown. Plantations were considered 'well-managed' if the farmer carried out basic agronomic management practices like mulching, water conservation, weeding, and pruning, while 'poorly-managed' plantations were neither mulched nor weeded at the time of visiting. The plantation age was captured and later grouped into six categories for further analysis. A list of banana

varieties grown at each farm was recorded and coded for analysis. Cultivar names varied depending on the local language in that region but were reconciled using banana variety lists developed by Karamura et al. (2012), Marimo et al. (2019) and an agronomy extension training guide prepared by the National Banana Research Program of the National Agricultural Research Organization (NARO) Uganda (NARO, 2019). Following the classification of Karamura (1998), all grown cultivars were then categorized into four groups based on the use of their end products i.e., cooking, dessert, beer/juice, and roasting bananas. Lastly, farmers provided the key reasons for cultivar selection.

In the case of the nature of the cropping system, two options including monocropping (only bananas) and intercropping (banana [main crop] + other crops) systems were considered. Reasons for practicing these two cropping systems were then captured and in case of intercropping, the crops grown with bananas were identified.

The respondents were then probed to describe the effects and symptoms (in terms of changes in plant morphological growth characteristics) of drought stress manifested by sensitive cultivars and the scores for the different symptoms were later coded for analysis. Based on their experience and careful observation, farmers were then required to report the reaction of each cultivar (only those cultivars they cultivated) to drought stress using a four-point rating scale i.e. 3= highly sensitive, 2= moderately tolerant, 1= very tolerant and 0= no effect at all. Interviewers confirmed these effects by observing the plants since these interviews were conducted in farmers' fields. The most popular cultivars (i.e. grown by at least twenty farmers) were then ranked from most to least affected by drought using a weighted average index (WAI) adopted from Ndamani et al. (2016).

$$\mathrm{WAI} = \frac{\mathrm{F_S} \times 3 + \mathrm{F_m} \times 2 + \mathrm{F_t} \times 1 + \mathrm{F_n} \times 0}{\mathrm{N}} \tag{3.1}$$

Where F_s = frequency of responses with highly sensitive, F_m = frequency of responses with moderately tolerant, F_t = frequency of responses with very tolerant, F_n = frequency of responses with no drought stress effect response and N = total number of respondents growing a given cultivar (out of the total 120).

To determine on-farm coping strategies for mitigating adverse drought stress effects on bananas, farmers were asked about their deployment of different agronomic and crop management practices. These practices included mulching, irrigation, intercropping with trees/ shrubs, weeding, reduced leaf harvesting, construction of trenches or contour bands trenches within the plantations, manure application and others, if any. Depending on the number of interventions deployed, each respondent's extent of deployment of such on-farm drought coping practices was determined against a four-point rating scale as no practice (0 coping strategies), low (1-3 strategies), medium (4-6 strategies) and high practice (more than 6 strategies). Respondents were also deliberately probed to specify the purpose of each intervention in mitigating drought effects on banana production and the time of deployment of each coping strategy.

The study data were analysed using STATA software version 17 (StataCorp, 2021) to derive inferential and descriptive statistics. Graphs were then generated in Microsoft Excel 2016 software using the data analysis from STATA 17. Pearson's product moment coefficient of correlation was used to determine the association between the focus and explanatory variables.

3.3 Results and Discussion

3.3.1 Characteristics of selected banana plantations

The banana plantations assessed varied in size, age, management, cultivars, cropping system, among others (Table 3.2). Most of the respondents were small- (57.5%) and medium-scale farmers (21.7%), with an average plantation size of 4.36 acres. All selected fields were more than one year old, with those ranging between 1 to 5 (33.3%) and above 20 years (29.2%) forming the majority. Banana fields that are 30 to 50 years old have been reported to be common in the East and Central African region (Bekunda, 1999; Gold et al., 1999). Over 85% of respondents had well-managed plantations while 14.2% had poorly managed farms. Farmers cultivated a total of 54 banana varieties (mean= 8.4, minimum=2 and maximum=19 cultivars), mostly comprising of a combination of cooking and dessert use types (29.2%), followed by cooking, dessert and roasting types combined (21.7%). Only 13.3% of the farmers cultivated one use type bananas, specifically the cooking type, while 13.3% cultivated all the four use groups. Growing mixed banana varieties is regarded as important because each variety has a

unique set of production and consumption attributes, which vary in terms of composition and levels (Edmeades et al., 2008; Akankwasa et al., 2013; Akankwasa et al., 2020). For instance, the grown banana varieties have different levels of tolerance to adverse weather conditions such as prolonged drought and thus provide farmers with an opportunity to diversify against risks.

Table 3.2 Characteristics of banana plantations owned by respondents in the eight study districts located in the cattle corridor of Uganda.

Category	Proportion of respondents (%)
Plantation size (acres)	
Small (0.25 – 2.5)	57.5
Medium (2.6 – 5.0)	21.7
Large (greater than 5.0)	20.8
Plantation age (scale score: 1-6)	
Less than 1 year	-
1-5 years	33.3
6-10 years	19.2
11-15 years	10.0
16-20 years	8.3
Above 20 years	29.2
Management level	
Well managed	85.8
Poorly managed	14.2
Banana types & combinations grown	
Cooking bananas only	13.3
Dessert bananas only	-
Beer/Juice bananas only	-
Roasting bananas only	-
Cooking & Dessert bananas	29.2
Cooking & Beer/Juice bananas	2.5
Cooking & Roasting bananas	3.3
Dessert & Beer/Juice bananas	-
Dessert & Roasting bananas	-
Beer/Juice & Roasting bananas	-
Cooking, Dessert & Beer/Juice bananas	15.8
Cooking, Dessert & Roasting bananas	21.7
Cooking, Beer/Juice & Roasting bananas	0.8
Dessert, Beer/Juice & Roasting bananas	-
All four banana types	13.3

Category	Proportion of respondents (%)
Main reason for cultivar selection	
Big bunch size	55.0
Tolerance to drought stress	15.0
Pest and disease resistance	8.3
Availability of planting materials	5.0
Fast maturity	5.0
Desirable taste	5.0
Others	6.7
Cropping system	
Monocropping (only bananas)	37.5
Intercropping (bananas + other crops)	62.5

Among the four banana types grown, the cooking banana cultivars comprised the majority (Figure 3.2), with each respondent growing at least one variety. Across the two agroecological zones, the top five cooking type cultivars were all EAHB types (*Musa* AAA genome) and included 'Mbwazirume', 'Kibuzi', 'Nakitembe', 'Mpologoma' and 'Nakabululu'. 'Sukali Ndiizi' (AAB genome) and 'Bogoya' (AAA genome) were the most prominent dessert banana varieties (Table 3.3). However, the proportions of farmers in the southern drylands districts growing 'Mbwazirume', 'Kibuzi' and 'Bogoya' were significantly higher than those in Lake Victoria crescent districts (Table 3.4). 'Mbwazirume' was the most grown local cultivar because of its desirable consumer attributes, including good taste, soft food, and good flavour (Akankwasa et al., 2013; Akankwasa et al., 2020). 'Mbwazirume' is also often used as a reference cultivar in the banana breeding programme at NARO-Uganda and IITA (Nowankunda et al., 2015; Batte et al., 2019; Tumuhimbise et al., 2018; 2019). Farmers attributed their possession of fewer dessert and beer/juice types to destruction by fusarium wilt (caused by fungus *Fusarium oxysporum* f. sp. *cubense*), a soilborne disease. The dessert variety 'Bogoya' (syn. 'Gros Michel') and beer type 'Kayinja' (syn. Pisang Awak) are generally susceptible to *Foc* (Pegg et al., 2019). Consequently, the few stands of dessert, beer and roasting bananas are planted mainly for home consumption with surplus sold in the local market.

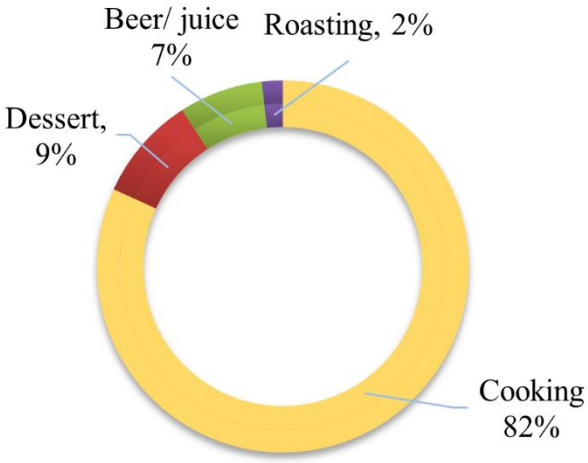


Figure 3.2 Grouping of banana cultivars grown by respondents based on the use of their end products.

Table 3.3 Local names and synonyms of banana cultivars grown by farmers in the eight study districts located in the cattle corridor of Uganda.

Local cultivar name	Synonym(s)	Banana type	Genome group	% of respondents growing cultivar
Mbwazirume	-	Cooking	AAA	65.8
Kibuzi	-	Cooking	AAA	61.7
Nakitembe	Entaragaza	Cooking	AAA	57.5
Mpologoma	Bukadefu	Cooking	AAA	52.5
Nakabululu	Embururu	Cooking	AAA	52.5
Musakala	Enshakara, Rwamugongo	Cooking	AAA	40.0
Enyeru	Nabusa, Enyanshenyi, Enshenyi	Cooking	AAA	35.0
Muvubo	Mujuba, Mayovu	Cooking	AAA	32.5
Ndyabalangira	Enzirabushera	Cooking	AAA	27.5
Kisansa	-	Cooking	AAA	25.8
Enzirabahima	Entukura, Muziranyama	Cooking	AAA	23.3
Kivuuvu	-	Cooking	ABB	21.7
Nakinyika	Enjuuma	Cooking	AAA	20.8
Enjagata	Nandigobe	Cooking	AAA	18.3
Nfuuka	Ntiika, Enfuuka	Cooking	AAA	16.7

Local cultivar name	Synonym(s)	Banana type	Genome group	% of respondents growing cultivar
Butobe	Butoobe	Cooking	AAA	16.7
Nakyetengu	Ekitetengwa, Kitiika	Cooking	AAA	10.8
Atwalira	-	Cooking	AAA	10.0
FHIA 25	-	Cooking	AAB	9.2
Siira	-	Cooking	AAA	9.2
Namwezi	Kasa	Cooking	AAA	8.3
M9	Kiwangaazi, Kabana 6H	Cooking	AAA	7.5
Katwalo	Njoogabakazi, Kasenga, Kabucuragye, Entanzinduka	Cooking	AAA	7.5
Lwaddungu	-	Cooking	AAA	5.0
Nakamaali	Nakamali	Cooking	AAA	2.5
M2	Kabana 7H	Cooking	AAA	1.7
Namunwe	-	Cooking	AAA	1.7
Mukubakonde	Mukubyakonde	Cooking	AAA	1.7
Lumenyamagaali	-	Cooking	AAA	1.7
Lusumba	-	Cooking	AAA	1.7
Enyaruyonga	-	Cooking	AAA	1.7
M19	NAROBan1	Cooking	AAA	0.8
M20	NAROBan2	Cooking	AAA	0.8
M25	NAROBan3	Cooking	AAA	0.8
M27	NAROBan4	Cooking	AAA	0.8
Nakawere	-	Cooking	AAA	0.8
Nalugolima	-	Cooking	AAA	0.8
Nambi	-	Cooking	AAA	0.8
Mukazimugumba	-	Cooking	AAA	0.8
Lwakizita	-	Cooking	AAA	0.8
Enkunku	Bukunku, Makunku	Cooking	AAA	0.8
Namulondo	-	Cooking	AAA	0.8
Enyamanyamunyo	-	Cooking	AAA	0.8
Lwasa	-	Cooking	AAA	0.8
Gonja	Plantain	Roasting	AAB	40.0
Sukali Ndiizi	Kabalagala, Apple banana	Dessert	AAB	61.7

Local cultivar name	Synonym(s)	Banana type	Genome group	% of respondents growing cultivar
Bogoya	Mbogoya, Gros Michel	Dessert	AAA	58.3
FHIA 17	Kabana 3H	Dessert	AAAA	24.2
Yangambi-KM5	Mufunyankobe	Dessert	AAB	9.2
FHIA 23	Kabana 4H	Dessert	AAAA	3.3
Kayinja	Musa	Beer /juice	ABB	20.8
Mbidde	Kabula, Enyarukira, Embiire	Beer /juice	AAA	15.8
Kisubi	-	Beer /juice	AB	3.3
Kibiddebidde	Kibidde	Beer /juice	AAA	1.7

Table 3.4 Proportion of farmers growing the five most popular banana cultivars in the two agroecological zones

Cultivar	Agroecological zone (%)		Chi square value
	Lake Victoria Crescent	Southern Drylands	
Mbwazirume	55.0	76.8	6.260** (0.012)
Kibuzi	40.0	83.3	23.831*** (0.000)
Sukali Ndiizi	61.7	60.0	0.035 (0.852)
Bogoya	48.3	68.3	4.931** (0.026)
Nakitembe	60.0	53.3	0.543 (0.461)

***$p < 0.01$; **$p < 0.05$; *$p < 0.1$; values in parenthesis are p values

Big bunch size was the main reason (55% of the respondents) for selecting specific banana cultivars, distantly followed by tolerance to drought stress (15%). Large bunch size has been reported as a key factor for selecting a specific variety because big bunches or fingers fetch a higher price in the market than their smaller-sized counterparts (Akankwasa et al., 2013; Marimo et al., 2019). However, similar to Bagamba et al. (2010), farmers also reported banana market prices to depend on the season. During the rainy season, the farm-gate price for bananas is low due to the high supply compared to the higher price offered during the dry season when the supply is low. In addition, farmers reported the availability of planting materials (from their old plantations, neighbours, or improved varieties from banana breeders), resistance to pests and diseases, desirable taste, and the cultivar's ability to thrive and yield even in low fertility soils as reasons for selecting cultivars. Similar, observations have been reported in banana systems of Democratic Republic of Congo, Rwanda, Tanzania, and Burundi (Ocimati et al., 2013, 2014b, 2016; Madalla et al., 2023).

3.3.2 Nature of cropping system

More than half of the respondents (62.5%) had an intercropping system, whereas 37.5% reported a monocropping system. Farmers with an intercropping system had plantations with banana as the main crop and at least two other crop or tree species (Table 3.5). 86.1% of those respondents with an intercropping system grew trees/shrubs within or at the edges of their plantations, purposefully to provide stakes (for supporting big banana bunches from toppling or snapping), wood and food (from the edible fruits) and shade to their banana plants during very hot and sunny days (Table 3.5). Legume intercrops (43%), mainly with beans and peas accounted for the next dominant intercrop species. Banana intercropping with crops such as coffee (van Asten et al., 2011b) and beans (Bagamba et al., 1998) has been a common practice among Ugandan farmers over the years as a strategy to maximize crop production, increase family incomes, reduce pest and disease prevalence, improve soil fertility (leaf droppings act as organic mulch) and structure and increase resilience to adverse weather conditions such as drought (van Asten et al., 2015; Gambart et al., 2020). Farmers with an intercropping system also pointed out the need to diversify their diets to boost their intake of required nutrients which are low or lacking in bananas (Ekesa et al., 2013). On the other hand, banana growers with a monocropping system stated avoidance of competition for resources (light, soil nutrients, water) among intercrops and reducing banana shade effects on shorter crops as reasons for their

choice. Moreover, intercropping is time-consuming and labour intensive, thereby adding to costs of operations such as weeding. Similar observations have been reported within the study region by Gambart et al. (2020).

Table 3.5 Other crops grown in association with bananas by study respondents.

Intercrop category	Specific crops grown	% of respondents growing intercrop
Trees /shrubs	Tree crops	86.1
	Mango, avocado, jackfruit, orange, guava, coffee, pawpaw, java plum	
	Wood or fodder trees	
	*Ficus natalens*is, *Albizia coriaria*, *Maesopsis eminii*, *Swietenia mahagoni* (mahogany)	
Legumes	Beans, cowpeas	43.1
Tubers	Cassava, sweet potatoes, yams	35.3
Vegetables	Eggplants, bitter berries, tomatoes, cabbage	19.6
Cucurbits	Pumpkins, bottle brush, calabash	5.9
Cereals	Maize	11.8
Others		
Fruits	Pineapples, passion fruit	
Spices	Mint, lemon grass, rosemary, ginger	
Medicinal plants	*Aloe vera*	
Peanuts	Groundnut	17.6
Grasses	Sugarcane, elephant grass	

3.3.3 Perceived and observed drought stress effects on banana growth characteristics

Figure 3.3 shows the observed effects of drought stress on the morphological characteristics of field-grown bananas. Drought effects observed and reported by farmers included wilting and drying of leaves (Figure 3.3A; 73%), reduced bunch weight (Figure 3.3B; 90% of farmers), and reduced leaf formation (63%) as the most prominent effects of drought stress across all grown banana cultivars (Figure 3.4). Similarly, a reduction in bunch weight was reported by van Asten et al. (2011a) which may partly be attributed to a decline in the plant's photosynthetic capacity and rate (Flexas and Medrano, 2002). However, farmers reported late exposure to drought (after bunch formation) not to affect bunch yield despite causing complete wilting and drying of leaves (Figure 3.3A).

On drought-sensitive cultivars, additional drought effects reported included stunted growth (Figure 3.3C), reduced size and number of fingers and/or clusters (Figure 3.3D), drying and snapping of the pseudostem (Figure 3.3E), halted or reduced sucker production, delayed flowering and bunch formation, longer bunch filling duration, reduced plant size (including the pseudostem and leaves), and rapid leaf senescence. Interestingly, study respondents also reported some cultivar-specific drought stress symptoms. For instance, choking was manifested by only two cultivars: 'Mpologoma' and 'Nakyetengu' (Figure 3.3F). 'Mpologoma' was the only cultivar that displayed leaf petiole rosette formation (Figure 3.3G) and abortion of the inflorescence, which occurs particularly during prolonged dry periods (Figure 3.3H). The disintegration of the pseudostem was only observed in cultivars 'Kibuzi' and 'Entaragaza'.

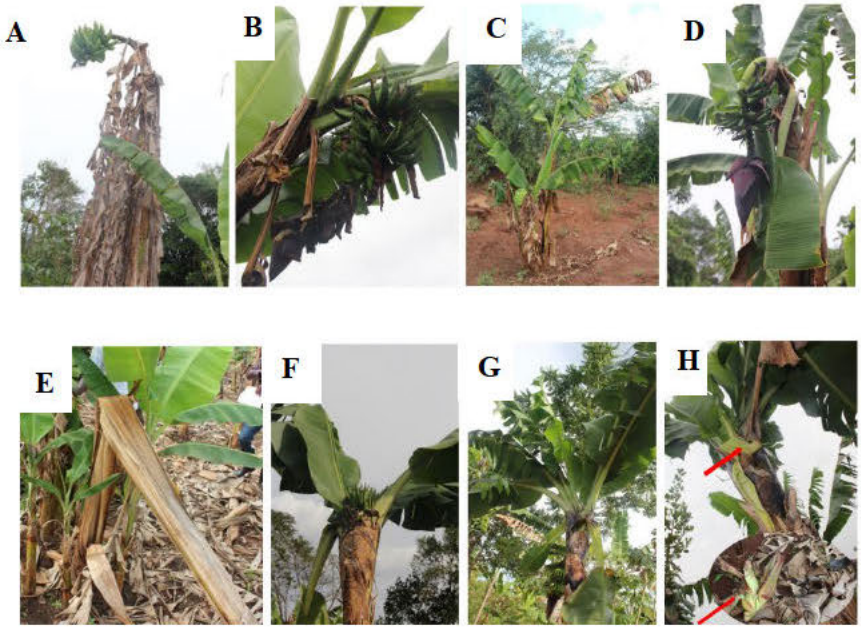


Figure 3.3 Drought stress symptoms manifested by drought-sensitive banana cultivars. (A) wilting and drying of leaves (B) reduced bunch size (C) stunted growth (D) reduced number of fingers and clusters (E) drying and snapping of pseudostem (F) choking (G) formation of petiole rosette (H) abortion of the inflorescence (upper arrow – point of attachment of inflorescence while on the plant, lower arrow - aborted inflorescence on the ground)

These observations confirm some of the adverse effects of drought on banana growth that have been reported in previous field studies. For instance, Ravi and Uma (2011) reported drought-susceptible *Musa acuminata* diploids to produce fewer hands, ill-filled fruits and to experience bunch choking. Similarly, delays in phenological processes like flower development and increased fruit filling duration due to limiting soil water have been reported (Turner et al., 2007; Taulya et al., 2014).

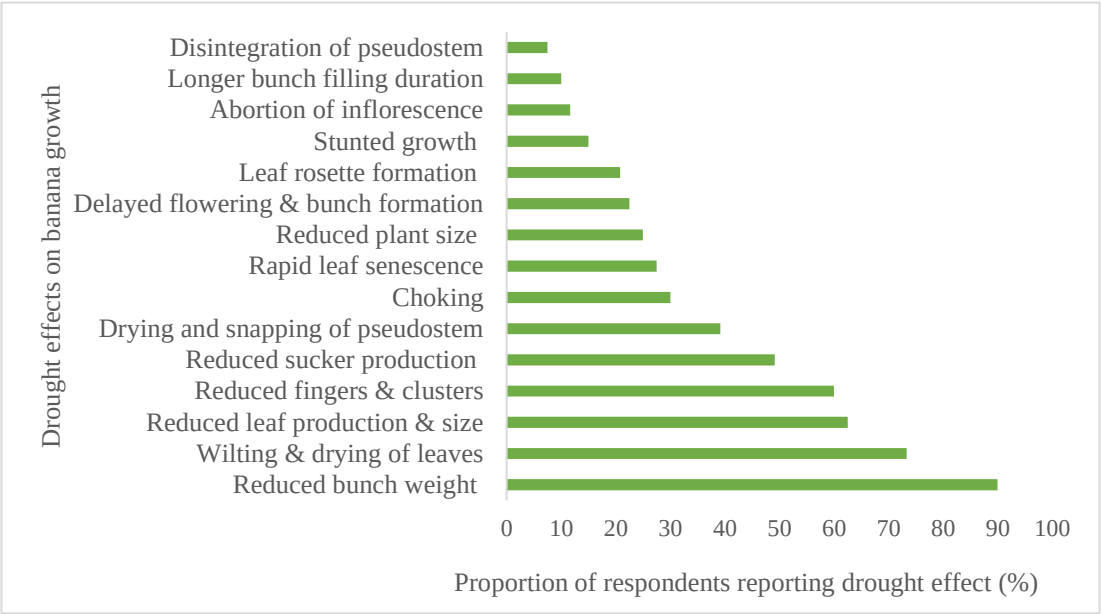


Figure 3.4 Proportion of farmers reporting different drought effects on the growth of field-grown banana cultivars in the study sites.

Table 3.6 shows the most popularly grown banana cultivars (grown by at least 20 farmers) and their respective responses to drought stress as perceived by the study respondents based on the estimates of the WAI estimates. The larger the WAI value, the higher the impact of drought stress on a given cultivar. The cooking type cultivar 'Mpologoma' was reported as the most adversely impacted by drought stress (WAI = 2.94), whereas 'FHIA 17', an exotic improved dessert hybrid, was ranked as the least affected (WAI = 0.90). Farmers partly attributed the high sensitivity of 'Mpologoma' to drought stress to a high transpiration rate through the leaves and pseudostems (formed by tightly overlapping leaf sheaths). Although water is mainly lost from the banana plant through the leaf stomata by transpiration (Liu et al., 2008), part of it is also lost from the fleshy pseudostems. Compared to moderately tolerant cultivars like 'Nakabululu', 'Mpologoma' has very thin leaf sheaths (Figure 3.5) through which water is easily lost, thereby resulting in quicker desiccation, weakening, and snapping of the pseudostem during drought conditions. On the other hand, cultivars like 'Nakabululu' have thicker leaf sheaths which minimise the loss of water, hence their moderate tolerance to drought stress. However, it was interesting to note from farmers that 'Mpologoma' recovers much faster than other cultivars when drought conditions cease. On the other hand, 'FHIA 17' might have

inherited its high tolerance to drought stress from one of its parent cultivars, 'Gros Michel', locally known as 'Bogoya' (Van den Bergh et al., 2020). According to the WAI value in Table 3.6, 'Bogoya' was reported to be moderately tolerant to drought.

Table 3.6 Ranking of the most popular banana cultivars based on their response to drought stress as perceived by the study respondents (from most to least affected by drought)

		Number of respondents reporting cultivar response to drought stress					
Cultivar local name	Frequency (n=120)	Highly sensitive	Moderately tolerant	Very tolerant	No effect	WAI	Rank
Mpologoma	63	60	2	1	0	2.94	1
Kisansa	31	11	18	2	0	2.29	2
Enjagata	22	6	16	0	0	2.27	3
Musakala	48	13	33	2	0	2.23	4
Gonja	48	20	19	9	0	2.23	4
Ndyabalangira	33	8	22	3	0	2.15	6
Enzirabahima	28	10	12	6	0	2.14	7
Muvubo	39	6	31	2	0	2.10	8
Kibuzi	74	17	45	12	0	2.07	9
Butobe	20	5	9	6	0	1.95	10
Nakitembe	69	7	51	11	0	1.94	11
Mbwazirume	79	9	54	16	0	1.91	12
Nfuuka	20	1	16	3	0	1.90	13
Bogoya	70	13	30	27	0	1.80	14
Nakinyika	25	2	14	9	0	1.72	15
Enyeru	42	3	20	19	0	1.62	16
Nakabululu	63	3	25	35	0	1.49	17
Sukali Ndiizi	74	1	29	44	0	1.42	18
Kayinja	25	0	9	16	0	1.36	19
Kivuuvu	26	0	10	15	1	1.35	20
FHIA 17	29	1	4	15	11	0.90	21

WAI denotes Weighted Average Index

Figure 3.5 Thickness of leaf sheaths of highly drought-sensitive and moderately tolerant banana cultivars. (A) cultivar 'Mpologoma' (drought-sensitive) - thin leaf sheath layers (B) cultivar 'Nakabululu' (moderately drought-tolerant)- thicker leaf sheath layers.

Considering the top five most grown cultivars, 'Kibuzi' was reported as the most affected by drought (WAI=2.07), followed by 'Nakitembe' (WAI=1.94). However, a significantly higher number of respondents in the Lake Victoria Crescent region reported both 'Kibuzi' and 'Nakitembe' to be moderately tolerant to drought stress than those in the Southern Drylands zone (Table 3.7).

3.3.4 On-farm coping strategies to mitigate drought stress effects on banana

A total of 12 on-farm coping strategies were reported for minimising the daunting effects of drought on banana production (Figure 3.6). Each practice was deployed for a specific purpose and time of deployment (Table 3.8). The five most deployed practices were mulching (56%), planting of mixed cultivars (35%), construction of water retention trenches or contour bands (23%), manure application (11%) and reduced leaf harvesting (18%). Some of these strategies such as mulching, application of manure and construction of water retention trenches form basic plantation management and soil-water conservation practices by Ugandan banana farmers (NARO, 2019). Common mulch types used by farmers included residues from banana plants such as fresh or dry leaves and pseudostems, grass (e.g., spear grass, elephant grass), residues of other crops and dried weeds. Such organic mulch options were also reported by Bekunda (1999). As a precautionary measure, farmers apply manure or fertilisers, especially potassium, during the rainy season to ensure a healthy banana plant (Smithson et al. 2001; Zhang et al. 2020) that can withstand moisture stress conditions, thereby masking the water

stress effects on plant growth (Taulya, 2013; Panelo and Diza 2017; Meya et al., 2020; 2023a; 2023b). Although fertiliser application, particularly farm manure, was reported as desirable, its deployment was constrained by the insufficient supply and high cost of organic manure except for farmers who reared livestock, e.g., cows, goats and chickens. Other drought coping options deployed by respondents included irrigation, weeding, staking of weak plants (particularly for late season drought), tying of disintegrated pseudostems with banana fibres, intercropping with trees and cover crops, heaping of soil around exposed plant roots and sucker removal (they leave three to four plants per mat) (Figure 3.7). Even though mulching was the most used coping practice (55.8%), many of the farmers (81.7%) pointed out irrigation as the most effective measure to mitigate drought effects on banana production.

Table 3.7 Respondents' perceptions of the response of the five most grown banana cultivars to drought stress across the two selected agroecological zones.

Cultivar	Drought sensitivity level	Agroecological zone (%)		Chi-square value
		Lake Victoria Crescent	Southern Drylands	
Mbwazirume	1	18.2	21.7	4.50 (0.105)
	2	78.8	60.9	
	3	3.0	17.4	
Kibuzi	1	23.1	16.0	7.80** (0.02)
	2	73.1	52.0	
	3	3.8	32.0	
Sukali Ndiizi	1	51.3	66.7	2.43 (0.297)
	2	46.0	33.3	
	3	2.7	0.0	
Bogoya	1	27.6	46.3	3.348 (0.187)
	2	55.2	34.2	
	3	17.2	19.5	
Nakitembe	1	16.7	12.4	4.71* (0.095)
	2	80.5	68.8	
	3	2.8	18.8	

***$p < 0.01$; **$p < 0.05$; *$p < 0.1$; values in parenthesis are p values. Cultivar drought sensitivity levels: 1- very tolerant; 2-moderately tolerant; 3-highly sensitive

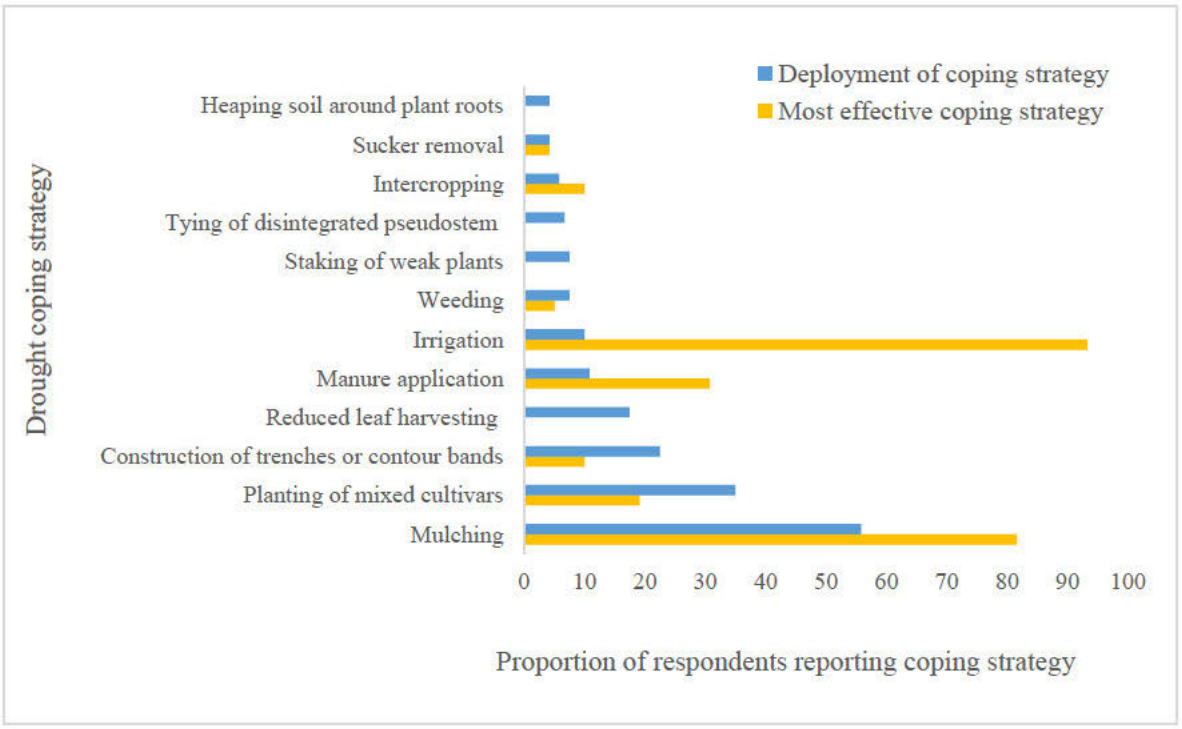


Figure 3.6 On-farm drought stress coping strategies by banana farmers in the study districts located in the cattle corridor of Uganda.

Farmers reported several drought coping strategies, some of which are already documented in literature and others not (Figure 3.6). However, farm-level use of drought coping strategies was predominantly low (72%), with most of the farmers deploying one to three drought coping strategies. Only 4% of the farmers used four to six practices (medium practice) while none of the farmers used more than six practices (high practice) to mitigate drought effects on their bananas. 24% of farmers did not deploy any of the drought coping strategies.

Table 3.8 Purpose and time of deployment of on-farm drought coping strategies by study respondents.

Drought coping practice	Purpose of intervention	Time of deployment
Mulching	Preserving soil moisture by reducing evaporation	Throughout the growing season or during the drought period
Planting mixed cultivars	Diversify risk (due to drought effects) from a single cultivar	At planting
Irrigation	Replenish lost or used soil moisture	During the drought period or
Intercropping	Tall trees or shrubs provide shade to bananas, especially on very hot days Short trees, shrubs and cover crops serve as soil cover	Any time during the growing season
Reduced leaf harvesting	Ensure plants have adequate leaves for photosynthesis Leaves shade the pseudostem and ground, thereby reducing desiccation	During the drought period
Construction of ditches or contour bands	For soil and water retention	At planting or At onset of rainfall
Weeding	Reduces competition for resources e.g. soil water and nutrients	Throughout the growing season
Staking of plants	Support the weight of heavy bunches on weak or snapped plants	During the drought period
Manure application	Increased organic matter improves water retention. Fertilizers ensure a healthy and strong plant that can withstand drought	At the onset of rains or At planting
Tying of disintegrated pseudostem	To keep the disintegrated pseudostem intact	During the drought period
Sucker removal	Reduce competition among plants on the same mat for the scarce resources including water	Throughout the growing season, but more purposely at onset and during the drought period
Heaping soil around plant roots	Protect exposed roots from desiccation and improve water and nutrient uptake	During the drought period

Farmers' management of climate change-related conditions such as drought is often influenced by prevailing climatic, economic, and social factors (Shrestha et al., 2017). Rural communities are often resource limited and hence lack the means to combat the adverse effects of climate change (Mardy et al., 2018). In this study, farmers who did not practice any or a few on-farm drought coping strategies attributed their failure to the high cost of inputs e.g. water and mulch, water scarcity (there is not even enough water for home consumption), limited time and old age. Drought coping practices such as weeding, sourcing of mulch and mulching, collecting water from long distances for irrigation require a considerable amount of physical labour, which farmers lack and are hence limited in their capability to partake in such drought management activities. Moreover, their lack of monetary resources hinders them from sourcing for external labour. Limited access and high cost of irrigation equipment were also mentioned as hindrances for irrigation during dry periods. This confirms previous findings by Kabunga et al. (2012). Therefore, there is a need for intervention by development practitioners, particularly the government e.g., through subsidising agricultural equipment such as irrigation pumps.

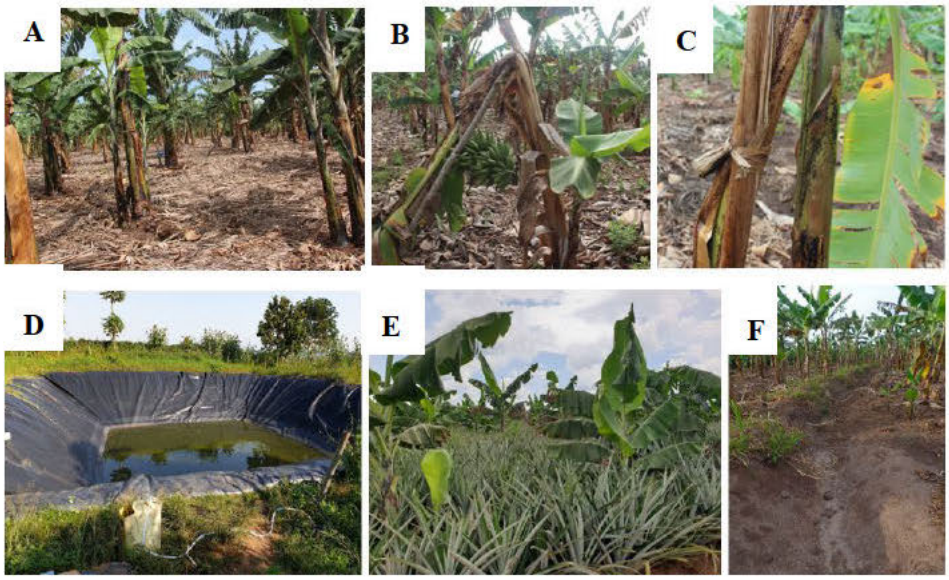


Figure 3.7 Farmers' drought stress mitigation practices in the study districts. (A) mulching with grass and banana residues (B) staking of snapped plants (C) tying of disintegrated pseudostem with banana fibres (D) water catchment from which irrigation water is drawn (E) banana intercropped with pineapple, which serves as a cover crop (F) trench for trapping soil and water.

3.3.5 Correlations of farm characteristics and the extent of deployment of coping strategies

The association between the extent of deployment of drought coping practices (focus variable) and two farm characteristics, plantation size and age, as explanatory variables were determined. Pearson's product-moment correlation coefficient (*r*) indicated significant negative associations between the extent of deployment of drought strategies and the two explanatory variables, i.e., *r* values -0.126 ($p = 9.55 \times 10^{-23}$) and -0.008 ($p = 1.22 \times 10^{-6}$) for plantation age and size, respectively. The negative association between the extent of deploying coping strategies and plantation size could reflect resource limitation to deploy drought mitigation practices that are mostly costly on larger farms. The bigger the banana plantation, the more demanding and expensive it is to access drought management requirements such as hired labour, mulch, manure, irrigation water and equipment.

3.4 Conclusions and future perspectives

This study has shown that the banana production systems in the cattle corridor of Uganda are threatened by drought. Farmers reported drought effects on the plant's vegetative growth and, later, on the floral, fruit and yield-related traits. Six of the 15 drought stress indicators or traits reported (including reduced bunch size, reduced size and number of fingers and clusters, delayed flowering and bunch formation, longer bunch filling duration, choking and abortion of inflorescence) occurred at flower and fruit formation and development stages. Given that, in the end, the most relevant indicator is the impact of drought on the yield of cultivars, researchers should prioritise the above floral and yield-related traits (including flowering time, bunch weight, number and size of hands and fingers) when conducting field assessment drought tolerance experiments. Ideally, a tolerant cultivar should be able to yield during the drought season. Also, considering that the amount of leaf area left after the drought stress contributes significantly to the yield, scientists should consider vegetative traits related to leaf production (number of functional leaves and leaf cigars formed) and size (total functional leaf area). Plant biomass and pseudostem height (reported by farmers as reduced plant size and stunted growth, respectively) should also be monitored. The advantage of vegetative growth traits is that they can be measured at any stage of plant growth. Future drought evaluation experiments should be based on the above traits used by farmers to ensure correct translation

of measured traits back to the farmers' measures or indicators of drought tolerance or sensitivity of cultivars. Giving priority to farmers' traits will also ease farmer acceptance of new drought-tolerant hybrids.

In successive chapters (i.e. Chapters 4 and 7), drought assessment experiments will be carried out under screen house conditions. Field experiments were not possible due to time restrictions and limited financial resources. Therefore, from the traits reported by farmers, only plant traits pertaining to stunted growth, leaf death (including wilting and drying of leaves and rapid leaf senescence), and leaf production and size will be considered. For instance, in Chapter 4, stunted growth will be estimated by measuring the plant height while wilting and senescing of leaves will be determined from the leaf relative water content and the chlorophyll content, respectively. The number of leaf cigars formed during the drought period, number of functional leaves, and total leaf area will be recorded to ascertain the impact of drought on leaf production and size. The total plant dry matter will also be recorded to reflect the significant negative impact of drought on plant size as reported by farmers. For Chapter 7, which primarily focuses on the variability in water usage, growth and transpiration response of *Musa* progenies and their parental genotypes to declining soil water content in relation to fluctuating environmental factors that influence the evaporative demand (vapour pressure deficit and light intensity), the total functional leaf area, leaf damage and transpiration rates will be given priority. Knowledge of the impact of drought on early growth stages is crucial as drought tends to prolong the vegetative stage, consequently leading to fewer bunches per mat over time (Uwimana et al., 2020). Nonetheless, the above recommended traits should be considered in future banana drought tolerance breeding and evaluation experiments.

To mitigate adverse drought effects, banana farmers in the Ugandan cattle corridor deployed several coping practices such as mulching, planting mixed cultivars, construction of water retention trenches or contour bands, reduced leaf harvesting, manure application, irrigation, weeding and staking of weak plants. Additional practices included tying disintegrated pseudostems with banana fibres, intercropping with trees and cover crops, heaping soil around exposed plant roots, and removal of excess suckers. Mulching was the most practiced, while irrigation though among the least applied practices, was considered by farmers to be the most effective in reducing drought effects on banana production. Therefore, control plants in subsequent phenotyping chapters (Chapter 4 and Chapter 7) will be watered regularly to ensure

optimal growth conditions, which in turn will result in contrasting phenotypes between well-watered plants and their water stressed counterparts. Despite the importance of the above on-farm practices in mitigating drought effects on banana, the extent of their deployment was still limited by a shortage of resources (monetary, and human resources). Deployment of some of the mitigation measures (e.g. irrigation) will require government and policy interventions in terms of providing logistical support to farmers and subsidies.

Given the nature of climate change-related phenomena like unpredictable withdrawal of rains and prolonged droughts, farmers need to prioritise preventive coping initiatives such as planting drought-tolerant cultivars, mulching (all year round) and manure application which protect plants against potential adverse drought effects rather than curative strategies. Moreover, such preventive strategies should be deployed at planting or before the onset of drought instead of adopting these practices during drought conditions or after the damage has already been caused.

Despite limited modelling work on climate trends in Uganda, past climate data shows that drought occurrences may exacerbate, especially in the semi-arid regions (including the cattle corridor). Therefore, there is need to generate more reliable weather data, improve climate projections and strengthen current information systems to better prepare for future drought incidences. Additionally, development practitioners and agricultural extension programs need to better inform farmers on mitigation options to avert drought effects based on reliable climate forecasts. The development of drought-tolerant cultivars that require much less water for optimal growth should be explored by banana breeding programmes. Moreover, some of the popular cultivars that were reported in this study as tolerant (e.g. ‘Sukali Ndiizi’ and ‘Kayinja’) and most affected (‘Mpologoma’) by drought stress are recommended as reference cultivars when screening banana germplasm for drought tolerance. In this thesis, ‘Mpologoma’ and ‘Sukali Ndiizi’ will be included as drought-sensitive and drought-tolerant reference varieties in the germplasm screening experiment of the next chapter, Chapter 4. ‘Mpologoma’ and ‘Sukali Ndiizi’ will also be two of the 55 accessions genotyped in Chapter 6. ‘Enzirabahima’, one of the EAHB cultivars grown by farmers in this study, is the female parent of the tetraploid hybrid ‘917K-2’, which is the female parent of the *Musa* progenies that will later be developed, genotyped and phenotyped in chapters 5, 6 and 7, respectively.

References

Akankwasa, K., G.F. Ortmann, E. Wale, and W.K. Tushemereirwe. 2013. Farmers' choice among recently developed hybrid banana varieties in Uganda: A multinomial logit analysis. Agrekon: Agric. Econ. Res. Policy. Practice. Southern Africa. 52:25-51.

Akankwasa, K., P. Marimo, R. Tumuhimbise, M. Asasira, E. Khakasa, et al. 2020. The East African highland cooking bananas 'Matooke' preferences of farmers and traders: Implications for variety development. Int. J. Food Sci. Technol. 56:1124–1134.

Anwar, R.M., D.L. Liu, I. Macadam, and G. Kelly. 2013. Adapting agriculture to climate change: a review. Theor. Appl. Climatol. 113:225-245.

Bagamba, F., J. Ssennyonga, W.K. Tushemereirwe, and C. Gold. 1998. Performance and profitability of the banana subsector in Uganda farming systems, p. 729-740, *In* C. Picq, E. Fouré, and E.A. Frison, eds. Bananas and Food Security. International Network for the Improvement of Banana and Plantain (INIBAP), Montpellier, France.

Bagamba, F. 2007. Market access and agricultural production: The case of banana production in Uganda. PhD thesis. Wageningen University, The Netherlands.

Bagamba, F., K. Burger, and W.K. Tushemereirwe. 2010. Banana (*Musa* spp.) production characteristics and performance in Uganda. Acta Hortic. 879:187-198.

Bananuka, J.A., P.R. Rubaihayo, and M.M. Tenywa. 1999. Reactions of *Musa* genotypes to drought stress. Afr. Crop Sci. J. 7:333–339.

Barekye, A., P. Tongoona, J. Derera, and W.K. Tushemereirwe. 2009. Variation in diploid (AA) and tetraploid (AAAA) banana populations for black Sigatoka resistance and agronomic traits. Afr. Crop Sci. J. 9:509–516.

Batte, M., R. Swennen, B. Uwimana, V. Akech, A. Brown, et al. 2019. Crossbreeding East African highland bananas: lessons learnt relevant to the botany of the crop after 21 years of genetic enhancement. Front. Plant Sci. 10:81.

Bekunda, M. 1999. Farmers' responses to soil fertility decline in banana-based cropping systems of Uganda. Managing Africa's soils No. 4. Russell Press, Nottingham, UK.

BMGF. 2014. Multi crop value chain phase II Tanzania/Uganda cooking banana. Bill and Melinda Gates Foundation (BMGF) technical report. Seattle, Washington. https://gatesopenresearch.org/documents/3-716 . Accessed 22 June 2021.

Branch, A. 2018. From disaster to devastation: Drought as war in northern Uganda. Disasters 42:S306−S327.

Christensen, J.H., B. Hewitso, A. Busuioc, A. Chen, X. Gao, et al. 2007. Regional climate projections, p. 847-940, *In* S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, et al., eds. Climate Change 2007: The physical science basis. Contribution of working group I to the fourth assessment report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK.

CRED. 2012. Centre for Research on the Epidemiology of Disasters (CRED). EM-DAT: The International Disasters Database – Uganda. https://public.emdat.be/mapping . Accessed 5 June 2022.

Edmeades, S., D.J. Phaneuf, M. Smale, and M. Renkow. 2008. Modelling the crop variety demand of semi-subsistence households: Bananas in Uganda. J. Agric Econ. 59:329-349.

Ekesa, B.N., J. Kimiywe, I. Van den Berg, G. Blomme, C. Dhuique-Mayer, M. Davey. 2013. Content and retention of provitamin A carotenoids following ripening and local processing of four popular *Musa* cultivars from Eastern Democratic Republic of Congo. Sustain. Agric. Res. 2 2.

FAO. 2019. FAO, EU and Government of Uganda launch new initiative to promote adaptation to climate change in vulnerable districts in Uganda. Food and Agricultural Organisation (FAO). Available at: http://www.fao.org/uganda/news/detail-events/zh/c/1201051/ [Accessed 18 March 2021].

FAOSTAT. 2019. Food and Agricultural Organization (FAO). http://www.fao.org/faostat/en/#data/QC . Accessed 22 February 2021.

Flexas, J., and H. Medrano. 2002. Drought-inhibition of photosynthesis in C_3 plants: Stomatal and non-stomatal limitations revisited. Ann. Bot. 89:183–189.

Gambart, C., R. Swennen, G. Blomme, J.C. Groot, R. Remans, and W. Ocimati. 2020. Impact and opportunities of agro-ecological intensification strategies on farm performance: a case study of banana-based systems in central and south-western Uganda. Front. Sustain. Food Syst. 4: 87.

Gold, C.S., G.H. Kagezi, G. Night, and P.E. Ragama. 2004. The effects of banana weevil, *Cosmopolites sordidus* (Germar), damage on highland banana growth, yield and stand duration in Uganda. Ann. Appl. Biol. 145:263–269.

Hepworth, N., and M. Goulden. 2008 Climate change in Uganda: Understanding the implications and appraising the response. LTS International, Edinburgh, UK.

IGAD. 2010. Intergovernmental Authority on Development (IGAD). IGAD Environment Outlook.

http://igad.int/index.php?option=com_docman&task=cat_view&gid=58&Itemid=144 Accessed 5 June 2022.

Kabunga, N.S., T. Dubois, and M. Qaim. 2012. Yield effects of tissue culture bananas in Kenya: Accounting for selection bias and the role of complementary inputs. J. Agric. Econ. 63:444–464.

Kajobe, R., E.K. Kato, S.A. Otim, P. Kasangaki, and P.P. Abila. 2016. The status of honeybee pests in Uganda. Bull. Anim Health. Prod. Afr. 64:105–117.

Kangire, A., E.B. Karamura, C. Gold, and M.A. Rutherford. 2000. Fusarium wilt of banana in Uganda, with special emphasis on wilt-like symptoms observed on East African Highland cooking cultivars (*Musa* spp., AAA). Acta Hortic. 540:343-353.

Karamura, D.A. 1998. Numerical Taxonomic Studies of the East African Highland Bananas (*Musa* AAA-East Africa) in Uganda. PhD thesis, University of Reading, England.

Karamura, D.A., E.B. Karamura, and W. Tinzaara. 2012. Banana cultivar names, synonyms and their usage in Eastern Africa. Bioversity International, Kampala, Uganda.

Kilimani, N., J. van Heerden, H. Bohlmann, and L. Roos. 2015. Impact of drought on the Ugandan economy, p.1-29. The proceedings of the Economic Society of South Africa (ESSA) Biennial Conference, held on 2-4 September. University of Cape Town, South Africa.

Liu, H.J., S. Cohen, J. Tanny, J.H. Lemcoff, and G. Huang. 2008. Transpiration estimation of banana (*Musa* spp.) plants with the thermal dissipation method. Plant Soil. 308:227–238.

Madalla, N.A., R. Swennen, A. Brown, S. Carpentier, I. Van den Bergh, et al. 2023. Farmers' preferences for East African highland cooking banana 'Matooke' hybrids and local cultivars. Agric. Food Security. 12:2.

Mardy, T., M.N. Uddin, M.A. Sarker, D. Roy, and E.S. Dunn. 2018. Assessing coping strategies in response to drought: A micro level study in the North-West region of Bangladesh. Climate 6:23.

Marimo, P., D. Karamura, R. Tumuhimbise, M.M. Shimwela, I. Van den Bergh, et al. 2019. Post-harvest use of banana in Uganda and Tanzania: Product characteristics and cultivar preferences of male and female farmers. CGIAR Research Program on Roots, Tubers and Bananas (RTB). RTB Working Paper. No. 2019-3. International Potato Centre, Lima, Peru.

McGahey, D., and Z. Visser. 2015. Climate change vulnerability and adaptation in Uganda's cattle corridor. Adaptation at Scale in Semi-Arid Regions (ASSAR). http://www.assar.uct.ac.za/news/climate-change-vulnerability-and-adaptationuganda%E2%80%99s-cattle-corridor. Accessed 20 March 2021.

McSweeney, C., M. New, and G. Lizcano. 2008. United Nations Development Programme (UNDP) Climate change country profiles: Uganda. UNDP, New York, USA.

Meya, A.L., P. Ndakidemi, K.M. Mtei, R. Swennen, and R. Merckx. 2020. Optimizing soil fertility management strategies to enhance banana production in volcanic soils of the northern highlands, Tanzania. Agronomy. 10:289.

Meya, A.I., R. Swennen, P.A. Ndakidemi, K.M. Mtei and R. Merckx. 2023a. Maize stover transfers from maize fields to banana-based agroforestry homegardens and the corresponding nutrient flows in central-northern Tanzania. Front. Forests. Global Change 6:1131328.

Meya, A.I., R. Swennen, P.A. Ndakidemi, K.M. Mtei, and R. Merckx. 2023b. Better nitrogen fertilizer management improved Mchare banana productivity and profitability in northern highlands, Tanzania. Agronomy. 13:1418.

Mfitumukiza, D. 2015. Climate variability/change in the cattle corridor of Uganda: vulnerability and adaptation perspectives. Climate change lecture series. Makerere University Centre for Climate Change Research and Innovations (MUCCRI), Kampala, Uganda.

Mfitumukiza, D., B. Barasa, and E. Ntale. 2017. Ecosystem-based adaptation to drought among agro-pastoral farmers: opportunities and constraints in Nakasongola district, Central Uganda. Environ. Manag. Sustain. Dev. 6:31-50.

MWE. 2007. Ministry of Water and Environment. Environment and Natural Resources Sector Investment Plan: 2008/09 – 2017/18. Republic of Uganda.

Nansamba, M., J. Sibiya, R. Tumuhimbise, D. Karamura, J. Kubiriba, and E. Karamura. 2020. Breeding banana (*Musa* spp.) for drought tolerance: A review. Plant Breed. 139:685-696.

NAPA. 2007. Climate Change: Uganda National Adaptation Programmes of Action (NAPA). Ministry of Water and Environment, Kampala, Uganda.

NARO. 2019. Growing bananas better – Banana agronomy extension training guide. National Agricultural Research Organisation Uganda (NARO). http://africasoilhealth.cabi.org/wpcms/wp-content/uploads/2019/09/Banana-agronomy-extension-guide-final.pdf . Accessed 2 February 2021.

Ndamani, F., and T. Watanabe. 2016. Determinants of farmers' adaptation to climate change: A micro level analysis in Ghana. Sci. Agric. 73:201–208.

NEMA. 2016. State of the environment report for Uganda 2014. National Environment Management Authority (NEMA), Kampala, Uganda.

Nimusiima, A., C.P.K Basalirwa, M. Mwanjalolo, W. Otim-Nape, J. Okello-Onen, et al. 2013. Nature and dynamics of climate variability in the Uganda cattle corridor. Afr. J. Environ. Sci. Technol. 7:770-782.

Nimusiima, A., C.P.K. Basalirwa, G.M. Jackson, J.G.M. Majaliwa, D. Kirya, and R. Twinomuhangi. 2018. Predicting the impacts of climate change scenarios on maize yield in the cattle corridor of central Uganda. J. Environ. Agric. Sci. 14:3-78.

Nowakunda, K., A. Barekye, R.T. Ssali, J. Namaganda, W.K. Tushemereirwe, G. Nabulya, et al. 2015. 'Kiwangaazi' (syn 'KABANA 6H') Black Sigatoka nematode and banana weevil tolerant 'Matooke' hybrid banana released in Uganda. HortScience 50:621–623.

Ocan, D., H.H. Mukasa, P.R. Rubaihayo, W. Tinzaara, and G. Blomme. 2008. Effects of banana weevil damage on plant growth and yield of East African *Musa* genotypes. J. Appl. Biosci. 9:407 415.

Ocimati, W., G. Blomme, D. Karamura, P. Ragama, P. Lepoint, et al. 2013. On-farm *Musa* germplasm diversity in different agroeecologies of Burundi. Int. J. Biodivers. Conserv. 5:751-760.

Ocimati, W., G.V. Nakato, K.K.M. Fiaboe, F. Beed, and G. Blomme. 2014a. Incomplete systemic movement of *Xanthomonas campestris* pv. *Musacearum* and the occurrence of latent infections in Xanthomonas wilt-infected banana mats. Plant Pathol. 64:81–90.

Ocimati, W., G. Blomme, A. Rutikanga, D. Karamura, P. Ragama, et al. 2014b. *Musa* germplasm diversity status across a wide range of agro-ecological zones in Rwanda. J. Appl. Biosci. 73:5979–5990.

Ocimati, W., D. Karamura, C. Sivirihauma, V. Ndungo, E. De Langhe, et al. 2016. On-farm banana (*Musa*) cultivar diversity status across different altitudes in North and South Kivu provinces of eastern Democratic Republic of Congo. Acta Hortic. 1114:35-44.

Ocimati, W., J.C.J. Groot, P. Tittonell, G. Taulya, and G. Blomme. 2018. Effects of Xanthomonas wilt and other banana diseases on ecosystem services in banana-based agroecosystems. Acta Hortic. 1196:19-32.

Ogwang, B.A., A. Nimusiima, T. Tindamanyire, M.N. Serwanga, G. Ayesiga, et al., 2016. Characteristics and changes in SON rainfall over Uganda (1901-2013). J. Environ. Agric. Sci. 8:45–53.

Owoyesigire, B., D. Mpairwe, P. Ericksen, and D. Peden. 2016. Trends in variability and extremes of rainfall and temperature in the cattle corridor of Uganda. Uganda J. Agric Sci. 17:231–244.

Panelo, B.C., and T.M. Diza. 2017. Growth and yield performance of banana (*Musa acuminate* L.) as affected by different farm manures. Asia Pac. J. Multidiscip. Res. 5:199-203.

Pegg, K.G., L.M. Coates, W.T. O'Neill, and D.W. Turner. 2019. The epidemiology of Fusarium wilt of banana. Front. Plant Sci. 10:1395.

Ravi, I., and S. Uma. 2011. Phenotyping bananas and plantains for adaptation to drought, *In* M. Philippe, and R. Jean-Marcel, eds. Drought phenotyping in crops: From theory to practice. CGIAR Generation Challenge Programme/CIMMYT, Texcoco, Mexico.

Republic of Uganda. 2010. Climate Change vulnerability assessment, adaptation strategy and action plan for the water resources sector in Uganda. https://oneworldgroup.co.za/wp-content/uploads/2019/07/Vulnerability-Assessment-Report-vS3.pdf. Accessed 5 June 2022.

Shrestha, R.P., N. Chaweewan, and S. Arunyawat. 2017. Adaptation to climate change by rural ethnic communities of Northern Thailand. Climate 5:57.

Simmonds, N.W. 1966. Bananas. 2nd ed. Longmans, London, UK.

Smithson, P.C., B.D. McIntyre, C.S. Gold, H. Ssali, and I.N. Kashaija. 2001. Nitrogen and potassium fertilizer vs. nematode and weevil effects on yield and foliar nutrient status of banana in Uganda. Nutr. Cycl. Agroecosystems. 59:239–250.

Speijer, P.R.1999. East African Highland banana production as influenced by nematodes and crop management in Uganda. Int. J. Pest Manag. 45:41–49.

StataCorp. 2021. Stata Statistical Software: Release 17. StataCorp LLC, College Station, Texas, USA.

Taulya, G. 2013. East African highland bananas (*Musa* spp. AAA-EA) 'worry' more about potassium deficiency than drought stress. Field Crops Res. 151:45–55.

Taulya, G., P.J.A. van Asten, P.A. Leffelaar, and K.E. Giller. 2014. Phenological development of East African highland banana involves trade-offs between physiological age and chronological age. Eur. J. Agron. 60:41–53.

Tumuhimbise, R., A. Barekye, J. Kubiriba, K. Akankwasa, I.K. Arinaitwe IK, et al. 2018. New high-yield cooking banana cultivars with multiple resistances to pests and diseases ('NAROBan1', 'NAROBan2', 'NAROBan3', and 'NAROBan4') released in Uganda. HortScience. 53:1387–1389.

Tumuhimbise, R., H. Buregyeya, J. Kubiriba, W.K. Tushemereirwe, A. Barekye A, et al. 2019. 'NABIO808' (Syn. 'NAROBAN5'): A tasty cooking banana cultivar with resistance to pests and diseases. Crop Breed. Appl. Biotechnol. 19:491-495.

Turner, D.W., J.A. Fortescue, and D.S. Thomas. 2007. Environmental physiology of the bananas (*Musa* spp.). Braz. J. Plant Physiol. 19:63–484.

Twongyirwe, R., D. Mfitumukiza, B. Barasa, B.R. Naggayi, H. Odongo, et al. 2019. Perceived effects of drought on household food security in South-western Uganda: Coping responses and determinants. Weather Clim. Extremes 24:100201.

USAID. 2011. Climate change and conflict in Uganda: The cattle corridor and Karamoja. CMM Discussion Paper No. 3. United States Agency for International Development, Uganda (USAID). http://www.fess-global.org/Publications/Other/Climate_Change_and_Conflic_%20in_Uganda.pdf Accessed 2 May 2021.

Uwimana, B., Y. Zorrilla-Fontanesi, J. van Wesemael, H. Mduma, A. Brown, et al. 2021. Effect of seasonal drought on the agronomic performance of four banana genotypes (*Musa* spp.) in the East African Highlands. Agronomy. 11:4.

Van Asten, P.J.A., C.S. Gold, J. Wendt, D. De Waele, S.H.O. Okech, et al. 2005. The contribution of soil quality to yield and its relation with other banana yield loss factors in Uganda, p.100-115. The proceedings of a workshop held on Farmer participatory testing of IPM options for sustainable banana production in Eastern Africa, held on 8-9 December 2003 in Seeta, Uganda. International Plant Genetic Resources Institute, Montpellier, France.

Van Asten, P.J.A., A.M. Fermont, and G. Taulya. 2011a. Drought is a major yield loss factor for rainfed East African highland banana. Agric. Water Manag. 98:541–552.

Van Asten, P.J.A., L.W.I. Waireg, D. Mukasa, and N.O. Uringi. 2011b. Agronomic and economic benefits of coffee–banana intercropping in Uganda's smallholder farming systems. Agric. Syst. 104:326-334.

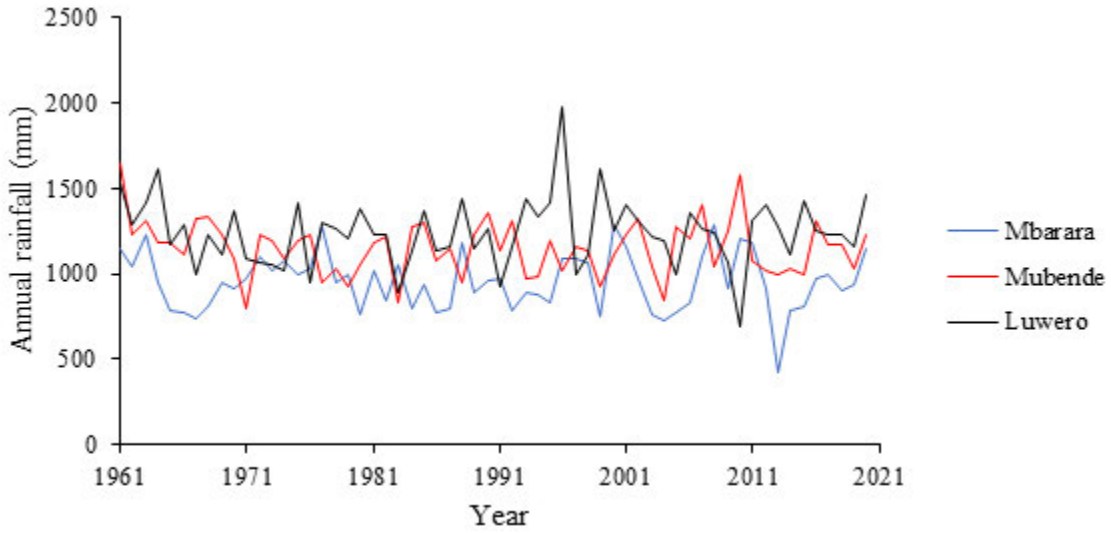
Van Asten, P., D. Ochola, L. Wairegi, A. Nibasumba, L. Jassogne, and D. Mukasa. 2015. Coffee-Banana intercropping: Implementation guidance for policymakers and investors. Climate-smart agriculture practice brief. CGIAR Research Program on Climate Change, Agriculture and Food Security (CCAFS), Copenhagen. https://biblio1.iita.org/bitstream/handle/20.500.12478/723/CCAFSpbCoffee-Banana.pdf?sequence=1 Accessed 14 May 2021.

I. Van den Berg, A. Vézina, and R. Crichton. 2020. FHIA-17. https://www.promusa.org/FHIA-17 Accessed 5 May 2021.

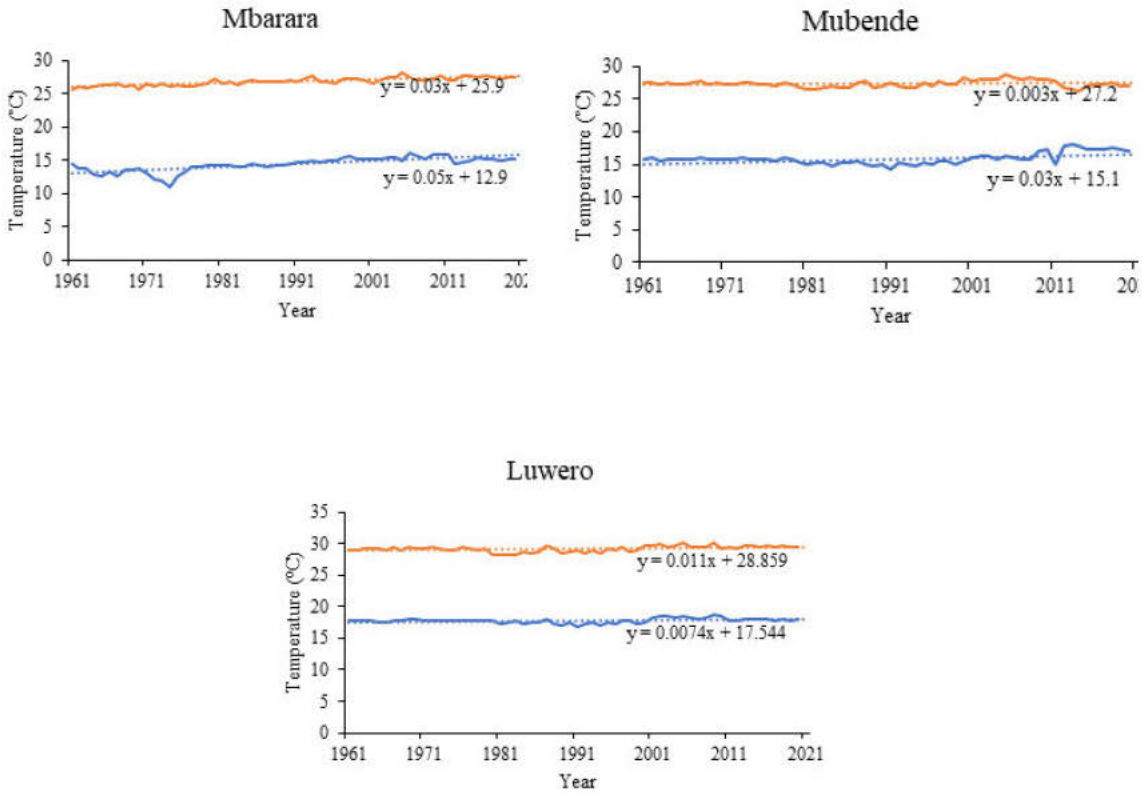
Wairegi, L.W.I., P.J.A. van Asten, M.M. Tenywa, and M.A. Bekunda. 2010. Abiotic constraints override biotic constraints in East African highland banana systems. Field Crops Res. 117:146–153.

Zhang, J., B. Li, J. Zhang, P. Christie, and X. Li. 2020. Organic fertilizer application and Mg fertilizer promote banana yield and quality in an Udic Ferralsol. PLoS One. 15(3): e0230593.

3.5 Appendices



Appendix 3.1 Trends in total annual precipitation recorded at Mbarara, Mubende and Luwero weather stations from 1961 to 2020. Source of data - Uganda National Meteorological Authority (UNMA).



Appendix 3.2 Trends in average maximum (brown) and minimum (blue) temperatures recorded at Mbarara, Mubende and Luwero weather stations from 1961 to 2020. Source of data - Uganda National Meteorological Authority (UNMA).

Chapter 4[3]: Response of banana (*Musa* spp.) to water stress based on phenotypic and physiological traits

Abstract

Banana (*Musa* spp.), an important staple food in the tropical and subtropical regions, is highly susceptible to drought. Developing drought-tolerant bananas using available germplasm offers a long-term solution to mitigate drought effects. The East and Central Africa Banana Germplasm Collection in Uganda contains genetically diverse genotypes whose potential for drought tolerance breeding is yet to be established. This study aimed to determine the response of 16 *Musa* genotypes to water stress based on phenotypic and physiological traits and to select promising genotypes for use in banana drought tolerance breeding. Four genotypes with a known reaction to water stress conditions were included as local checks. Three-month-old tissue culture-derived plantlets were completely deprived of water for four weeks while control plants were regularly irrigated back to field capacity, and both sets maintained under screen-house conditions. Water stress resulted in significant reductions in plant height, total leaf area, number of leaf cigars and functional leaves, total dry matter, chlorophyll, and relative water content. Water use efficiency (WUE) of 12 genotypes increased under stress conditions. Stomatal conductance was affected by the genotype x water treatment interaction. Genotype 'ITC.0987' was the most tolerant, considering that moisture stress had the least effect on its above-ground growth. Among the improved diploids, 'TMB2x9722-1' had the least total dry matter reduction and highest WUE, while 'TMB2x9172' showed the least decrease in relative water content and highest root-shoot ratio increase under stress. Thus, 'ITC.0987', 'TMB2x9722-1' and 'TMB2x9172' are essential drought-tolerant candidates that may be utilized in breeding.

Keywords: chlorophyll content, *Musa* spp., plant growth, relative water content, stomatal conductance, water stress, water use efficiency

[3] This chapter was published in the Journal of Crop Improvement: Nansamba, M., J. Sibiya, R. Tumuhimbise, D. Karamura, J. Ssekandi, et al. 2022. Response of banana (*Musa* spp.) to drought stress based on phenotypic and physiological traits. J. Crop Improv. 37:751-775. https://doi.org/10.1080/15427528.2022.2148313

4.1 Introduction

Bananas (*Musa* spp.) constitute a major source of dietary energy and micronutrients for millions of people in Asia and Africa (Heslop-Harrison and Schwarzacher, 2007). In Uganda, banana ranks first among the top five food crops grown, with a total planted area of 729,000 ha and total production of 11,230,000 tons reported in the financial year 2022/2023 (UBOS [Uganda Bureau of Statistics], 2023). Over 6,223 tons of bananas valued at 3,713,000 USD are exported from Uganda, mainly to Kenya, the Democratic Republic of Congo, and South Sudan (UBOS, 2023). However, due to its high-water requirement, banana is sensitive to changes in climatic conditions such as atmospheric temperatures (Turner and Lahav, 1983; Ramirez et al., 2011) and prolonged droughts (Srikul and Turner, 1995; Nansamba et al., 2020; Uwimana et al., 2020). It is envisaged that by 2050, climate change could cause significant declines in global banana yield gains, from an average of 1.37 to 0.19 tons ha−1 in the major production and export countries (Varma and Bebber, 2019).

Banana requires high volumes of water ranging between 1,200 and 2,600 mm per annum for optimal growth (Carr, 2009; Van Asten et al., 2011; Mustaffa and Kumar, 2012). Since major banana growing sites and breeding programs are in the humid tropics and subtropics, production is often affected by unpredictable and prolonged rainfall withdrawals. The East African highland region, one of the major banana production systems, experiences seasonal droughts which cause substantial yield losses (Wairegi et al., 2010; Kayongo et al., 2015; Uwimana et al., 2020). For instance, for every 100 mm drop in available soil water, 8–10% yield losses occur in East African Highland bananas (EAHBs), which require 1,200– 1,300 mm water per year (Van Asten et al., 2011). Bunch weight reductions of 20–65% are likely to occur in the drier parts (receiving 900–1100 mm yr^{-1}) of the East African Highland cropping system, making breeding for drought-tolerant varieties the topmost intervention to mitigate the drought effects.

Breeding drought-tolerant cultivars requires sources of tolerance genes which can be identified through screening of existing genotypes, from which water-efficient candidates are selected for use as male and/or female parents in subsequent conventional crosses. Many researchers have evaluated different banana cultivars at various growth stages for their response to moisture stress under controlled and field conditions (Vanhove et al., 2012; Xu et al., 2014; Muthusamy et al., 2016; Uwimana et al., 2021). Drought-sensitive banana genotypes display stress

symptoms such as wilting, increased degradation of leaf chlorophyll, reduced net photosynthetic rates, stomatal conductance and total plant biomass production, irrespective of the growth stage (Thomas and Turner, 1998; Mahouachi, 2009; Nansamba et al., 2020). Genotypes that perform better under drought conditions often have enhanced water use efficiency (WUE) and increased biomass allocation to the root system (Kissel et al., 2015; Delfin et al., 2016; Santos et al., 2020). Under field conditions, adverse moisture stress effects on critical growth stages such as flower initiation, fruit initiation and filling, resulting in fewer fingers/ hands and reduced bunch weight, have been reported (Goenaga and Irizarry, 1995; Mahouachi, 2007). Stevens et al. (2020) reported delays in banana phenological processes such as flowering time and harvest date, as well as significant reductions in biomass build-up and yields of two cultivars, 'Mchare Huti-Green Bell' (AA) and 'Grand Naine' (AAA) due to water stress.

Among the *Musa* spp. genotypes currently maintained at the East and Central Africa banana Germplasm Collection (ECAGC) hosted at the Zonal Agricultural Research Development Institute in Mbarara, Uganda, are diploids (including wild, edible, landraces, and improved hybrids) (Karamura et al., 2016). Some of these diploids are important sources of resistance to banana pests (weevils and nematodes) and diseases (black Sigatoka) (Nowakunda et al., 2015; Tumuhimbise et al., 2018), but their levels of tolerance to drought are not known. It is, thus, vital to assess them to identify drought-tolerant germplasm for the mainstream banana genetic improvement programs. Therefore, this study aimed to determine the response of 16 *Musa* genotypes to water stress based on phenotypic and physiological traits and to select promising genotypes for use in banana drought tolerance breeding.

4.2 Materials and methods

4.2.1 Plant materials, soil composition and experimental design

16 *Musa* spp. genotypes consisting of edible, wild, landraces and improved species and selected based on possession of the 'B' genome and /or desirable taste and breeding attributes were evaluated for their response to water stress for four weeks in the screen house (Table 4.1). Some studies have linked the B genome to enhanced tolerance to water stress (Ekanayake et al., 1994; Vanhove et al., 2012). Additionally, diploids were selected for evaluation because of their

current use in banana breeding as male-fertile parents (Ortiz and Swennen, 2014; Batte et al., 2019). Two cultivars, 'Mpologoma' (AAA) and 'Williams' (AAA), were included as drought-sensitive checks while 'Monthan' (ABB) and 'Sukali Ndiizi' (AAB) were included as a drought-tolerant reference varieties (Robinson and Alberts, 1986; Ravi et al., 2013). In Chapter 3, banana farmers in the cattle corridor region of Uganda reported 'Mpologoma' (an EAHB cultivar) to be the most sensitive to drought while 'Sukali Ndiizi' was reported among the top five least affected popular cultivar (Table 3.6; Table 3.7). The plant materials used were sourced from the ECAGC in Mbarara, Uganda and the International Transit Centre (ITC) in Belgium.

Before imposition of water stress, 3-month-old tissue culture-derived plantlets were acclimated for four weeks, after which they were transplanted into 10-litre plastic buckets containing 9 kg of soil. The potting medium comprised black soil, sand, and organic manure in the ratio of 3:1:1, respectively and had moisture retention of 24% at field capacity.

The experiment was set up following a completely randomized design (CRD) with three replications and one plant per pot. Three plants of each genotype were maintained under well-watered conditions and three were water stressed. Water stress was imposed two months after transplanting by completely ceasing irrigation of the stressed plants for four weeks, while control plants continued to be watered back to field capacity every three days. The experiment was conducted in a screen-house at the National Agricultural Research Laboratories (NARL) in Kawanda, Uganda. Screen- house temperatures and relative humidity were monitored with a Tinytag Plus 2 data logger (model TGP-4017, Chichester, UK). During the evaluation period, the weekly relative humidity of the screen-house ranged from 53.3% to 100%, with temperatures ranging from 18 ℃ to 32.3 ℃ (Table 4.2). The soil water content (expressed in terms of pressure) was monitored with soil moisture sensors (model 200SS WATERMARK sensor, Irrometer Company Inc., Riverside, USA). The moisture sensors were vertically buried and maintained in the potted soil for the entire evaluation period. Soil water content measurements were recorded every three days using a hand-held soil moisture meter (WATERMARK Meter, Irrometer) attached to the sensor's wires.

Table 4.1 List of *Musa* spp. genotypes evaluated for drought tolerance at the National Agricultural Research Laboratories, Kawanda in Uganda under screen-house conditions.

Genotype Name/ID	Ploidy	Genome constitution	Origin	Banana type	Desirable traits
TMB2x9172	2x	AA	IITA, Nigeria	Improved diploid	Black Sigatoka resistant, high bunch yield
TMB2x6142-1	2x	AA	IITA, Nigeria	Improved diploid	Black sigatoka resistant, high bunch yield
TMB2x8075-7	2x	AA	IITA, Nigeria	Improved diploid	Black sigatoka & Nematode resistant, high bunch yield
TMB2x9128-3	2x	AA	IITA, Nigeria	Improved diploid	Fusarium wilt resistant, high bunch yield
TMB2x9722-1	2x	Unknown	IITA, Nigeria	Improved diploid	Rich in vitamin A
ITC.1239	2x	AB	Papua New Guinea	Landrace	Edible dessert
ITC.1638	2x	AB	India	Landrace	Edible diploid
ITC.0987	2x	AB	Papua New Guinea	Landrace	Edible dessert
Kunnan	2x	AB	Unknown	Landrace	-
Musa balbisiana	2x	BB	South-East Asia	Wild *Musa* species	Associated with resistance to abiotic stresses
Calcutta-4	2x	AA	Unknown	Wild *Musa* species	High male and female fertility, high pollen production, Resistant to fusarium wilt, black sigatoka, and nematodes
Vunapope	2x	AB	Papua New Guinea	Landrace	Edible cooking type
Sukali Ndiizi	3x	AAB	Kiisi, Kenya	Dessert banana	Edible dessert, drought-tolerant check
Mpologoma	3x	AAA	Kiboga, Uganda	East African Highland Banana	Edible cooking type, drought-sensitive check
Williams	3x	AAA	Unknown	Cavendish	Drought-sensitive check
Monthan	3x	ABB	Unknown	Monthan	Drought-tolerant check

Table 4.2 Weekly weather conditions at the National Agricultural Research Laboratories, Kawanda during the evaluation of *Musa* spp. genotypes for drought tolerance

Week	Tmin (^oC)	Tmax (^oC)	RHmin (%)	RHmax (%)
1	18.8	30.6	61.1	100.0
2	18.7	28.4	71.8	100.0
3	18.2	32.3	67.6	100.0
4	18.0	31.4	65.8	100.0

Tmin, average minimum temperature; Tmax, average maximum temperature; RHmin, average minimum relative humidity; RHmax, average maximum relative humidity

4.2.2 Measurement of plant phenotypic and physiological traits

4.2.2.1 Phenotypic traits

Data was taken on the following phenotypic traits pertaining to plant growth: Plant height (in cm) was measured (at 28 days after stress imposition) from the soil surface to the meeting point of the two youngest unfurled leaves. The total number of functional leaves was recorded weekly. Leaves were only considered functional if they had at least 50% green tissue; otherwise, they were non-functional (Bananuka et al., 1999). The number of new leaf cigars was monitored every three days throughout the evaluation period.

Total leaf area (TLA) of each plant was determined as follows. For every plant, the length (cm) and width (cm) at the broadest part of each fully opened leaf were measured using a tape measure. Individual leaf areas were calculated according to Summerville (1944).

$$\text{Leaf Area} = \text{Leaf length} \times \text{Leaf width} \times 0.83 \quad (4.1)$$

Where 0.83 is a leaf area factor

The TLA of each plant was then obtained by summing up all the individual leaf areas and recorded in cm^2.

Biomass production and allocation: At trial termination, fresh plant samples were partitioned into roots, leaves and stems (pseudostems and corms) and weighed (in grams) separately for each plant. The roots were thoroughly washed and gently patted dry before determining their fresh weights. All fresh samples were then dried in an oven at 70 °C for three days. After oven drying, sample dry weights were determined. Subsequently, root-shoot ratios were computed from the following equation:

$$\mathrm{RSR} = \frac{\mathrm{RDW}}{\mathrm{SDW}} \tag{4.2}$$

Where RSR corresponds to the root-shoot ratio, RDW and SDW are root and shoot dry weights (both in grams).

4.2.2.2 Plant physiological parameters

Physiological traits related to plant function and plant water status were monitored. Stomatal conductance was recorded every three days between 8:30 am and 12 noon using a steady-state leaf porometer (model SC-1, Decagon Devices, Pullman, USA). Readings were taken on the third youngest fully unfurled leaf of each plant. Leaf chlorophyll contents were recorded every seven days on the third youngest fully opened leaf using a chlorophyll meter (model SPAD-502Plus, Konica Minolta Inc., Tokyo, Japan) and readings recorded in SPAD units. Both stomatal conductance and leaf chlorophyll contents were taken on the third youngest fully unfurled leaf of each plant.

Water use efficiency (WUE) was determined at the end of the drought treatment period and calculated following a method by Ehdaie and Waines (1993).

$$\mathrm{WUE} = \frac{\text{Total plant dry weight (g)}}{\text{Total water used (Kg)}} \tag{4.3}$$

The amount of water used by the plant was determined by weighing the pots every three days and the reduction in pot weight ascribed to plant use. Unlike water stressed plants, control plants were regularly re-watered back to field capacity to replace the loss in pot weight. Pots containing the soil mixture but without plants were maintained to account for water loss due to evaporation.

The relative water content (RWC) was determined at the end of the experiment following a procedure by Barrs and Weatherly (1962). Briefly, using a cork borer, twenty 0.5 cm discs were excised from the third youngest fully opened leaf. The weights of the leaf discs were determined immediately with an analytical balance, taking care to ensure minimal water loss from the fresh samples. To determine the turgid weight, the leaf discs were immediately placed in plastic tubes to which 10ml of distilled water was added and stored in a refrigerator for 24 hrs. After 24 hours of saturation under refrigeration, the discs were dried with tissue paper and their turgid weights recorded. After weighing, the leaf discs were oven-dried at 70°C for 24 hrs for dry weight determination. The RWC was computed as follows:

$$\text{RWC} = \frac{(\text{Fresh weight} - \text{Dry weight})}{(\text{Turgid weight} - \text{Dry weight})} \times 100 \quad (4.4)$$

4.2.3 Statistical analysis

The data were subjected to a two-way analysis of variance (ANOVA) using Genstat® 18th Edition software (VSN International, Hemel Hempstead, UK, 2015) to test for significant differences between genotypes. Comparisons were then made between parameter means using Tukey's Honest Significant Difference (HSD) test at a significance level of $\alpha = 0.05$. Correlation coefficients among the phenotypic and /or physiological traits were calculated using Pearson's correlation (*r*) method, with a two-sided test applied ($\alpha = 0.05$).

4.3 Results

The soil moisture content of water stressed plants, expressed in terms of pressure, increased from 2 kPa on the last day of watering to 186 kPa at the termination of the experiment, indicating that the soil was severely dry. On the other hand, well-watered plants were maintained in adequately wet soils with a moisture content range of 2 kPa to 12 kPa.

Table 4.3 summarizes the analysis of variance for phenotypic and physiological traits measured. Significant differences were observed among tested *Musa* spp. genotypes and water treatments for total leaf area, plant height, total dry matter, number of leaf cigars, number of functional leaves, chlorophyll content, WUE and RWC. The effect of the genotype x water treatment interaction was significant for total leaf area, plant height, number of leaf cigars,

number of functional leaves and RWC. In contrast, the total plant dry matter, root-shoot ratio, chlorophyll content and WUE were not affected by the genotype x water treatment interaction.

Table 4.3 Analysis of variance (mean squares) for phenotypic and physiological traits of 16 *Musa* spp. genotypes evaluated across well-watered and water stress conditions

Sources of variation	df	Total leaf area	Plant height	Total dry matter	Root-shoot ratio	Number of leaf cigars	Number of functional leaves	Chlorophyll content	Water use efficiency	Relative water content
Genotype	15	4442861**	696.7**	2654.4**	0.063*	0.688**	6.5248**	248.17**	17.286**	112.02**
Water treatment	1	38259737**	2758.4**	2133.9**	0.002 ns	32.181**	145.628**	1878.38**	8.559**	5202.35**
Genotype*Water treatment	15	1190013**	127.5**	207.5 ns	0.001 ns	0.510*	4.880**	34.48 ns	1.447 ns	44.59**
Residual	62	351171	18.1	199.1	0.01	0.269	0.527	41.77	1.032	10.06

ns, non-significant; *, $p < 0.05$; **, $p < 0.01$ level

4.3.1 Effect of water stress on plant growth

4.3.1.1 Total leaf area

Means comparison of the two water treatments showed that higher TLA (2881.5 cm^2) was obtained from the plants exposed to the well-watered treatment while their water stressed counterparts produced 1605.6 cm^2 TLA, which had a 44.3% decrease compared to well-watered plants (Table 4.4). Water stress caused significant TLA reductions ranging between 19.7 and 84.7%. The highest and third highest TLA reductions were found in the drought-sensitive checks, 'Williams' and 'Mpologoma', respectively, while the improved diploid, 'TMB2x9172', had the lowest reduction. The drought-tolerant checks, 'Monthan', and 'Sukali Ndiizi' experienced less than 50% reductions in TLA due to water stress. While plants of 15 out of 16 tested genotypes had reduced TLA under water stress conditions, the 'ITC.0987' genotype had a greater TLA under the same treatment (Table 4.4). Such an inverse response is contrary to the general trend when well-watered plants had higher TLA than those deprived of water for four weeks. Among the improved diploids, 'TMB2x6142-1' had the highest TLA under well-watered and water stress conditions.

4.3.1.2 Plant height

For all genotypes, well- watered plants were consistently taller than their water stressed counterparts (Table 4.4). Water stress significantly reduced the plant heights of all tested genotypes during the four weeks of water stress imposition (Table 4.4). Water stress-induced reductions in plant height ranged between 6.2 and 62.4%, with the lowest and highest reductions observed in the improved diploid, 'TMB2x9722-1' and drought-sensitive check, 'Williams', respectively. 'Mpologoma' displayed a significantly lower reduction in plant height than 'Williams' when subjected to water stress conditions. Regarding the drought-tolerant checks, water stress caused three times more reduction in plant height in 'Sukali Ndiizi 'than in 'Monthan'. In terms of frequencies, 75% of the genotypes had plant height reductions of less than 50%, whereas 25% showed height reductions greater than 50%. Genotypes including 'ITC.0987', 'Kunnan', '*M. balbisiana*', 'Vunapope' and 'Monthan' (drought-tolerant check) were relatively short species (24.3–27.5 cm) even under well-watered conditions. The observed significant plant height

reductions among water stressed plants clearly indicate the negative impact of water stress on the plant's potential to grow taller.

Table 4.4 Mean total leaf area and plant height of 16 *Musa* spp. genotypes evaluated under water stress and well-watered conditions at four weeks after stress imposition

Total leaf area, cm^2 per plant				Plant height, cm per plant			
Genotype	Water stress	Well-watered	Reduction (%)	Genotype	Water stress	Well-watered	Reduction (%)
ITC.0987	479.4 [a]	253.5 [a]	-89.1	TMB2x9722-1	29.0 [cd]	30.9 [ab]	6.2
TMB2x9172	2308.8 [d-f]	2876.4 [b-d]	19.7	ITC.0987	22.2 [bc]	24.3 [a]	8.8
ITC.1638	3209.9 [f]	4013.2 [cd]	20.0	TMB2x6142-1	47.7 [f]	52.5 [c-e]	10.1
TMB2x9722-1	1175.8 [a-c]	1543.4 [ab]	23.8	TMB2x8075-7	48.0 [f]	53.8 [de]	10.8
ITC.1239	2659.9 [ef]	3603.2 [b-d]	26.2	Monthan	23.1 [bc]	26.1 [a]	11.5
Musa balbisiana	1223.2 [a-c]	1779 [ab]	31.2	Vunapope	22.1 [bc]	25.1 [a]	12.0
Kunnan	1170.4 [a-c]	1748.4 [a-c]	33.1	Mpologoma	41.2 [ef]	46.8 [c-e]	12.1
Monthan	1230.9 [a-c]	1915.7 [a-c]	35.7	TMB2x9128-3	41.7 [ef]	47.5 [c-e]	12.3
TMB2x8075-7	2117.2 [c-e]	3515.8 [b-d]	39.8	ITC.1239	39.0 [ef]	49.5 [c-e]	21.2
TMB2x6142-1	2366.4 [d-f]	4039.1 [cd]	41.4	Calcutta-4	20.2 [a-c]	29.2 [ab]	30.6
Sukali Ndiizi	1985 [c-e]	3545.6 [b-d]	44.0	*Musa balbisiana*	18.7 [ab]	27.5 [a]	31.9
Vunapope	1538.5 [b-d]	3198.3 [b-d]	51.9	Sukali Ndiizi	37.0 [de]	55.7 [e]	33.5
TMB2x9128-3	1312.6 [a-d]	2808.9 [b-d]	53.3	TMB2x9172	20.5 [a-c]	44.0 [cd]	53.4
Mpologoma	1549.3 [b-d]	4620.4 [d]	66.5	Kunnan	11.7 [a]	26.8 [a]	56.4
Calcutta-4	764.4 [ab]	3307.9 [b-d]	76.9	ITC.1638	21.1 [a-c]	52.8 [de]	60.1
Williams	452.5 [a]	2958.3 [b-d]	84.7	Williams	15.2 [ab]	40.5 [bc]	62.4
Mean[1]	1605.6 [a]	2881.5 [b]			29.0 [a]	39.8 [b]	
SED	376.5	376.5			4.2	4.2	
CV (%)	26.5	14.4			17.4	9.3	

[1]Means in a column or row followed by the same letter(s) are not significantly different at 5% level Tukey HSD

4.3.1.3 Biomass production and allocation

4.3.1.3.1 Total dry matter

This study revealed significant variations in total dry matter due to the water treatment ($p < 0.05$) and the genotype ($p < 0.05$) (Table 4.5). For most of the genotypes (13 out of 16), water stress resulted in total dry matter weight reductions ranging from 6 to 52%. 'Mpologoma' (a drought-sensitive check) and 'Calcutta-4' (a wild *Musa* species), displayed the highest reductions, while the remaining 11 genotypes showed total dry matter reductions not exceeding 50%. Among the genotypes whose total dry matter was reduced by water stress, 'Sukali Ndiizi', a drought-tolerant check, was the fourth least affected genotype with a reduction of 23.3% while Monthan had a reduction of up to 35%. In contrast, three improved diploids including, 'TMB2x6142-1', 'TMB2x8075-7' and 'TMB2x9722-1' presented greater total dry matter weights under water stress treatment in comparison to well-watered conditions.

4.3.1.3.2 Root-shoot ratio

The root-shoot ratios did not differ significantly across the two water treatments, while significant genotypic differences were observed under each water treatment (Table 4.5). The two drought-sensitive checks, 'Mpologoma' and 'Williams', and five other genotypes showed a general root-shoot ratio reduction of less than 50%. In this category, the lowest and highest reductions in root-shoot ratios were recorded in 'Sukali Ndiizi' and 'TMB2x9128-3', respectively. On the other hand, nine (9) out of 16 tested genotypes showed a general increase in the root-shoot ratios, ranging from 2 to 61%. 'TMB2x9172' had a significantly higher percent increase in root-shoot ratio compared to the rest of the entries. 'Kunnan' closely followed by 'Monthan' (a drought-tolerant check) also had root-shoot ratio increases (> 50%) under the water stress treatment, while 'ITC.1239', 'ITC.1638' and '*M. balbisiana*' showed relatively low percent increases (< 10%).

Table 4.5 Total dry matter and root-shoot ratios of 16 *Musa* spp. genotypes grown under well-watered and water stress conditions after four weeks of stress imposition

	Total dry matter, g per plant				Root-shoot ratio		
Genotype	Water stress	Well-watered	Reduction (%)	Genotype	Water stress	Well-watered	Reduction (%)
TMB2x9722-1	22.2 $^{a-d}$	19.7 ^{ab}	-13.0	TMB2x9172	0.233 ^{ab}	0.144 ^{a}	-61.0
TMB2x8075-7	53.6 ^{ef}	48.6 $^{a-d}$	-10.3	Kunnan	0.583 ^{b}	0.375 ^{ab}	-55.6
TMB2x6142-1	51.7 ^{ef}	48.3 $^{a-d}$	-6.9	Monthan	0.331 ^{ab}	0.215 ^{ab}	-53.9
Musa balbisiana	29.8 $^{a-e}$	31.7 $^{a-c}$	6.1	TMB2x8075-7	0.232 ^{ab}	0.168 ^{ab}	-38.3
TMB2x9172	60.0 ^{f}	70.0 ^{cd}	14.3	TMB2x9722-1	0.353 ^{ab}	0.291 ^{ab}	-21.5
ITC.1638	51.7 ^{ef}	61.7 $^{b-d}$	16.2	ITC.0987	0.077 ^{a}	0.066 ^{a}	-17.4
Sukali Ndiizi	55.0 ^{ef}	71.7 ^{cd}	23.3	*Musa balbisiana*	0.525 ^{b}	0.489 ^{b}	-7.3
TMB2x9128-3	46.7 $^{d-f}$	63.3 $^{b-d}$	26.3	ITC.1239	0.216 ^{ab}	0.207 ^{ab}	-4.0
Kunnan	20.0 $^{a-d}$	27.5 $^{a-c}$	27.3	ITC.1638	0.236 ^{ab}	0.231 ^{ab}	-2.4
ITC.1239	40.0 $^{b-f}$	58.3 $^{b-d}$	31.4	Sukali Ndiizi	0.224 ^{ab}	0.229 ^{ab}	2.1
Vunapope	14.7 $^{a-c}$	22.0 ^{ab}	33.2	Mpologoma	0.29 ^{ab}	0.311 ^{ab}	6.9
Monthan	8.2 ^{ab}	12.6 ^{a}	35.4	TMB2x6142-1	0.233 ^{ab}	0.258 ^{ab}	9.6
Williams	13.4 $^{a-c}$	23.4 ^{ab}	42.8	Vunapope	0.166 ^{ab}	0.192 ^{ab}	13.1
ITC.0987	5.1 ^{a}	9.9 ^{a}	48.4	Williams	0.206 ^{ab}	0.286 ^{ab}	28.0
Mpologoma	41.7 $^{c-f}$	86.7 ^{d}	51.9	Calcutta-4	0.257 ^{ab}	0.361 ^{ab}	28.8
Calcutta-4	9.3 ^{ab}	19.4 ^{ab}	52.0	TMB2x9128-3	0.203 ^{ab}	0.341 ^{ab}	40.4
Mean[1]	32.9 ^{a}	42.5 ^{b}			0.264 ^{a}	0.258 ^{a}	
SED	8.4	8.4			0.05	0.05	
CV (%)	23.1	17.3			28.0	21.3	

[1]Means in a column or row followed by the same letter(s) are not significantly different at 5% probability level Tukey HSD

4.3.1.4 Leaf cigar formation

Significant differences ($p < 0.05$) were observed for the number of leaf cigars formed across the two water treatments (Table 4.6). On average, water stressed plants produced only one leaf cigar during the four-week stress imposition period while well-watered plants formed two new leaf cigars, hence an equivalent reduction of about 50%. Interestingly, the drought-sensitive check, 'Mpologoma', had a lower reduction in leaf cigars formed than 'Monthan', a drought-tolerant check. The lowest reduction in the number of leaf cigars formed was observed in the drought-tolerant check, 'Sukali Ndiizi', while the highest reduction was recorded in the improved diploid, 'TMB2x9128-3', as no new leaves were formed in this genotype during the entire experimental period.

Significant differences due to the genotype were only observed for the water stress treatment but not for well-watered conditions (Table 4.6). The genotype 'ITC.0987' formed the same number of leaf cigars (2) under both water stress and well-watered treatments, which indicates that water stress did not affect its leaf cigar production.

4.3.1.5 Number of functional leaves

The number of functional leaves of the well-watered plants was consistently superior to that of water stressed plants except for the 'ITC.0987' genotype (Table 4.6). In this context, water stressed plants of 'ITC.0987' had six functional leaves, while the well-watered plants had five. For the rest of the genotypes, significant reductions caused by water stress (ranging between 20% and 81.1%) were recorded. The five genotypes most affected by water stress (with greater than 50% reduction) included the two drought-sensitive checks ('Mpologoma' and 'Williams'), 'Calcutta-4', 'Vunapope', and 'Sukali Ndiizi'. Considering the drought-tolerant checks, the reduction in the number of functional leaves was two times higher in 'Sukali Ndiizi' than in 'Monthan'. Among the five improved diploids tested, 'TMB2x8075-7' showed the highest reduction in the number of functional leaves. The remaining four improved diploids had less than 50% reduction in the average number of functional leaves under water stress conditions.

Table 4.6 Number of leaf cigars and functional leaves produced by the 16 *Musa* spp. genotypes grown under and water stress and well-watered conditions during the four weeks of stress imposition

	Number of leaf cigars				Number of functional leaves		
Genotype	Water stress	Well-watered	Reduction (%)	Genotype	Water stress	Well-watered	Reduction (%)
ITC.0987	2.0 ^{c}	2.0 ^{a}	0.0	ITC.0987	6.3 ^{d}	5.0 ^{ab}	-26.7
Sukali Ndiizi	1.3 ^{b}	2.0 ^{a}	33.3	TMB2x9172	4.0 $^{b-d}$	5.0 ^{ab}	20.0
ITC.1638	1.0 ^{ab}	1.7 ^{a}	40.0	ITC.1638	5.0 ^{cd}	6.7 $^{c-e}$	25.0
Mpologoma	1.3 ^{b}	2.3 ^{a}	42.9	Monthan	3.0 $^{a-c}$	4.0 ^{a}	25.0
ITC.1239	1.7 ^{bc}	3.0 ^{a}	44.4	ITC.1239	3.0 $^{a-c}$	4.0 ^{a}	25.0
TMB2x9172	0.7 ^{ab}	1.3 ^{a}	50.0	Kunnan	5.0 ^{cd}	7.0 $^{c-f}$	28.6
TMB2x6142-1	1.0 ^{ab}	2.0 ^{a}	50.0	TMB2x6142-1	5.0 ^{cd}	7.7 $^{d-f}$	34.8
Kunnan	1.0 ^{ab}	2.0 ^{a}	50.0	*Musa balbisiana*	4.0 $^{b-d}$	6.3 $^{b-d}$	36.8
Musa balbisiana	1.0 ^{ab}	2.0 ^{a}	50.0	TMB2x9128-3	3.3 $^{a-c}$	5.3 $^{a-c}$	37.5
Monthan	1.0 ^{ab}	2.0 ^{a}	50.0	TMB2x9722-1	3.0 $^{a-c}$	5.3 $^{a-c}$	43.7
TMB2x8075-7	1.0 ^{ab}	2.3 ^{a}	57.1	TMB2x8075-7	2.7 $^{a-c}$	5.0 ^{ab}	46.0
TMB2x9722-1	1.0 ^{ab}	2.3 ^{a}	57.1	Mpologoma	4.0 $^{b-d}$	8.3 ^{f}	52.0
Williams	0.7 ^{ab}	2.0 ^{a}	66.7	Sukali Ndiizi	2.0 ^{ab}	4.3 ^{a}	53.8
Vunapope	0.7 ^{ab}	3.0 ^{a}	77.8	Vunapope	3.3 $^{a-c}$	8.0 ^{ef}	58.3
Calcutta-4	0.7 ^{ab}	2.7 ^{a}	75.0	Calcutta-4	2.0 ^{ab}	7.3 $^{d-f}$	72.7
TMB2x9128-3	0.0 ^{ab}	2.0 ^{a}	100.0	Williams	1.3 ^{a}	7.0 $^{c-f}$	81.0
Mean[1]	1.0 ^{a}	2.2 ^{b}			3.5 ^{a}	6.0 ^{b}	
SED	0.2	0.2			0.5	0.5	
CV (%)	17.7	33.2			19.1	24.8	

[1]Means in a column or row followed by the same letter(s) are not significantly different at 5% probability level based on Tukey HSD

4.3.4 Effect of drought on plant physiological processes

4.3.4.1 Stomatal conductance

The stomatal conductance of well-watered and water stressed plants did not differ significantly until nine days after stress imposition (Table 4.7). After that, the stomatal conductances of water stressed plants were consistently lower than those of well-watered plants. However, at every time porometer readings were taken, significant genotypic variations in stomatal conductance were observed under both water treatments. While well-watered plants maintained relatively high stomatal conductance throughout the experiment, the stomatal conductance of water stressed plants continued to reduce with declining soil moisture (Figure 4.1). On the last day of taking porometer readings (i.e. the 27th day), 'Mpologoma' and 'Vunapope' had the highest and lowest stomatal conductances, respectively, under optimal conditions. On the same day, under water stress conditions, all the genotypes, except 'TMB2x9172' and 'ITC0987', had stomatal conductances of less than 50 mmol/m^{2}s. 'Kunnan' had the lowest stomatal conductance, followed by the drought-sensitive check, 'Williams'. Among the 14 genotypes with stomatal conductance of less than 50 mmol/m^{2}s under water stress conditions, 'Monthan' (drought-tolerant check) had the highest (44.5 mmol/m^{2}s) on the final day porometer readings were taken. On the last day, the stomatal conductances of the drought-tolerant checks did not differ significantly from those of the drought-sensitive check genotypes under water stress conditions. However, 'TMB2x9172' and 'ITC0987' had significantly higher stomatal conductances than the rest of the genotypes.

Genotypes showed significantly different stomatal conductance with declining soil moisture (Figure 4.1). While the stomatal conductance of ten genotypes decreased as early as three days after the last day of watering (Figure 4.1; Group 1), the remaining six genotypes showed an increase on the same day (Figure 4.1; Group 2).

Group 1 consisted of genotypes whose stomatal conductance were high on the first day of stress imposition but fluctuated (often dropped and increased) mainly between the third and 15th day (with their maximum porometer readings in that interval) and declined after that with minor fluctuations. However, on the 21st day of water stress, the 'ITC.0987' genotype showed an increase in stomatal conductance, which continued to rise even after

that, suggesting continued stomatal opening and transpiration despite declining soil moisture.

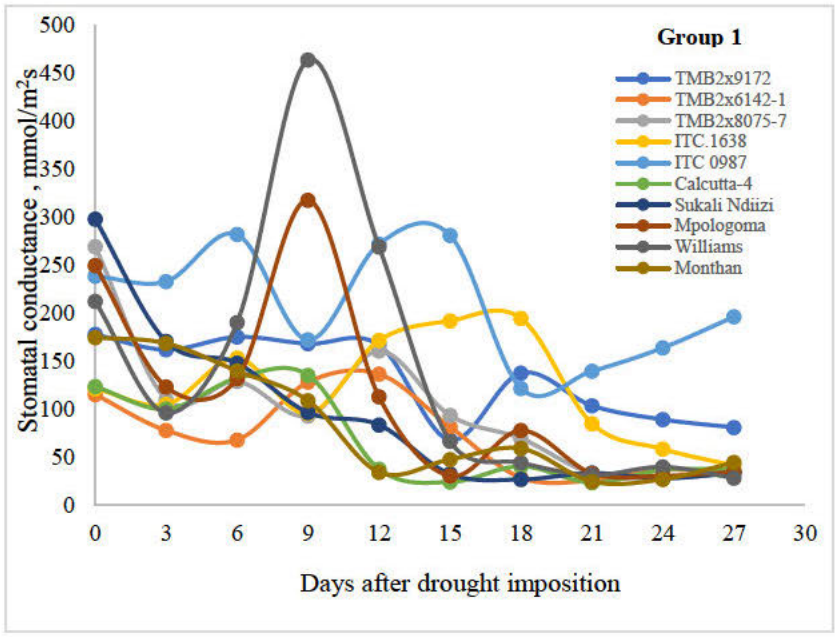
Group 2 genotypes attained maximum stomatal conductance values between the third and twelfth day after ceasing irrigation, but readings continued to drop gradually to less than 50 mmol/m^2s in subsequent days of declining soil moisture. "*M. balbisiana*" showed maximum stomatal conductance on the 6^{th} day after last irrigation, after which it decreased tremendously and almost instantly.

In addition to the main effects of the genotypes and water treatments, the stomatal conductance was also significantly affected by the interaction of the genotype and water treatment ($p < 0.05$).

Table 4.7 Stomatal conductance of 16 *Musa* spp. genotypes grown under well-watered and water stress conditions during the 28 days of stress imposition

	Days after water stress imposition																			
	0		3		6		9		12		15		18		21		24		27	
Genotype	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2
TMB2x9172	200.9b-d	178.0cd	190.7cd	162.1a-c	171.6ab	175.3ab	221.6c-e	168.3a-c	188.3a-c	166.9bc	349.3b-d	67.9 a	118.6 a	137.7 c	217.9a-d	103.8 c	173.9b	89.3ab	192.1ab	111.1b
TMB2x6142-1	111.0ab	115.4 b	94.5a	78.0 a	85.0 a	68.1 a	125.9a-c	128.3ab	134.8 a	136.6ab	143.2 a	81.7 a	167.8 ab	28.7 a	104.3 a	25.7 a	96.3a	36.7a	217.5ab	33.5a
TMB2x8075-7	265.3d	269.4ef	127.9ab	110.4ab	126.6ab	129.1ab	95.7 a	93.1 ab	163.9 ab	160.8 c	223.7a-d	93.6 ab	241.8c-e	69.6 bc	271.0b-e	33.1 ab	189.4bc	28.3a	203.5ab	33.7a
TMB2x9128-3	156.2a-d	150.1b-d	139.4a-c	180.0a-c	111.2a	103.8ab	92.4 a	61.3 a	165.2 ab	138.6ab	185.6 ab	22.9 a	261.5 de	36.4 ab	323.7 c-f	27.7 a	223.1b-d	29.1a	208.3ab	31.4a
TMB2x9722-1	133.2a-c	117.9b	113.5 ab	343.8bc	167.5ab	138.7ab	211.0c-e	188.2a-c	347.2 ef	39.8 a	277.8a-d	22.1 a	276.2d-f	29.5 a	314.4 c-f	27.9 a	320.9fg	48.8ab	3678.0f	28.8a
ITC.1239	153.5a-d	128.3bc	154.1a-c	131.9ab	146.4ab	166.4ab	210.5c-e	210.5 bc	249.1b-e	228.1 c	226.9a-d	121.1ab	165.0 ab	70.1 bc	228.7a-e	31.2 ab	174.7b	29.7a	250.3a-c	38.5a
ITC.1638	131.5a-c	121.4b	115.9ab	105.1ab	153.6ab	153.2ab	198.5b-e	95.6 ab	168.8 ab	171.7bc	201.8a-c	192.1ab	192.0 bc	194.7 c	218.3a-d	85.1 bc	176.8b	58.5ab	225.2ab	41.3a
ITC.0987	165.4a-d	238.8de	217.1d	233.2a-d	235.4b	282.1a-c	287.4 ef	172.3a-c	172.1 ab	272.2 c	273.0a-d	281.2 b	281.9 ef	121.8 c	195.3a-c	139.7 c	269.8d-f	164.1b	263.8a-d	196.3b
Kunnan	171.4a-d	196.6c-e	112.2ab	388.5c	172.5ab	344.3 c	265.1 ef	265.1bc	329.2 d-f	110.6ab	378.4c-d	35.4 a	443.3 g	41.0 ab	383.6 ef	40.9a-c	343.0g	47.8ab	362.8d-f	24.2a
M. balbisiana	53.5a	38.9a	92.2 a	338.1bc	144.7ab	358.0 c	298.1 ef	98.2 ab	350.0 ef	44.3 a	189.8 ab	23.8 a	265.4 de	32.4 a	330.1 c-f	33.4 ab	345.2g	40.4a	360.9ef	36.1a
Calcutta-4	106.8ab	123.6bc	113.6ab	100.2ab	156.4ab	132.3ab	151.6a-d	135.5a-c	213.0a-d	37.7 a	241.0a-d	24.1 a	233.8b-e	40.5 ab	188.7a-c	23.5 a	286.9e-g	36.7a	221.0ab	37.4a
Vunapope	120.5ab	130.9bc	116.8ab	123.6ab	141.5ab	35.6 a	150.0a-d	136.3a-c	214.9a-d	53.1 a	179.7 a	31.1 a	170.6 ab	57.3a-c	151.8 ab	25.7 a	167.2b	38.8a	180.1a	37.3a
Sukali Ndiizi	206.5b-d	298.2f	144.1a-c	170.7a-c	116.6a	148.0ab	338.0 f	96.6 ab	293.3c-e	83.7 ab	171.9 a	32.5 a	282.8 ef	26.8 a	264.0b-e	33.5 ab	187.1bc	28.1a	321.6c-f	33.5a
Mpologoma	250.9cd	249.9d-f	118.9ab	123.4ab	137.7ab	132.1ab	330.7 f	317.9 bc	421.5 f	112.9ab	382.9	30.5 a	342.5 f	77.8 bc	445.6 f	33.2 ab	422.7h	30.6a	404.7f	34.6a
Williams	96.3ab	212.3c-e	198.0cd	96.0a	186.0ab	190.3ab	240.7d-f	463.9 c	438.7 f	269.2 c	279.7a-d	66.6 a	281.6 ef	44.3 ab	352.1d-f	31.5 ab	307.8fg	39.9a	258.7a-c	28.3a
Monthan	141.5a-d	174.8cd	163.7b-d	168.7a-c	146.7ab	139.9ab	102.9ab	108.9ab	210.5a-d	34.4 a	250.4a-d	47.7 a	208.4b-d	59.1a-c	258.4b-e	24.6 a	243.4c-e	27.0a	276.6b-e	44.5a
Mean[1]	153.5 a	172.20 a	167.4 a	170.2 a	124.5 a	140.1 a	206.3 b	162.3 a	252. 2 b	131.1 a	244.4 b	75.3 a	241.6 b	68.1 a	263.0 b	45.5 a	243.3b	48.4a	267.7b	50.4a
SED	30.1	30.1	27.1	27.1	30.6	30.6	37.3	37.3	37.1	37.1	30.2	30.2	25.5	25.5	26.5	26.5	24.3	24.3	23.5	23.5
CV (%)	21.9	33.7	29.8	23.1	30.3	18.7	21.4	25.5	19.6	23.8	33.8	25.3	30.8	17.7	26.8	19.3	35.4	22.2	27.5	11.6

[1]Means in a column or row followed by the same letter(s) are not significantly different at 5% probability level based on Tukey HSD. T1 – Well-watered treatment, T2- Water stress treatment



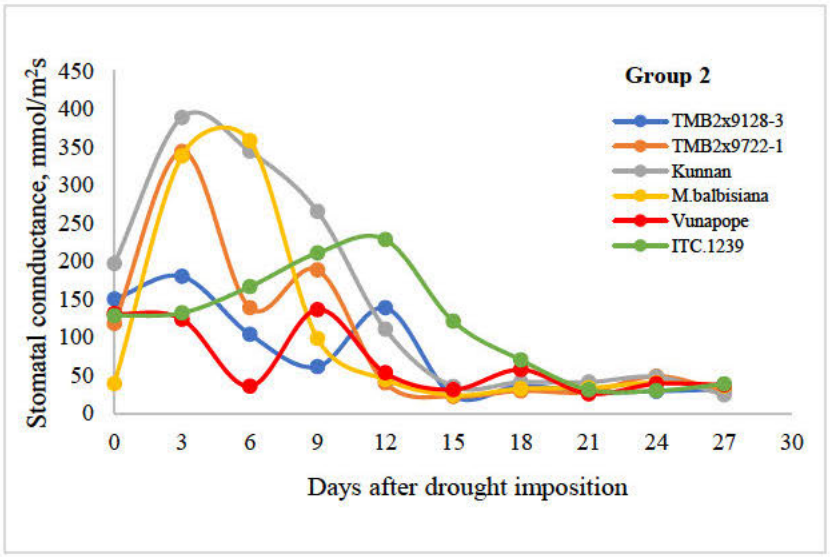


Figure 4.1 Stomatal conductance of 16 *Musa* spp. genotypes evaluated under water stress conditions for 28 days in a screen-house at the National Agricultural Research Laboratories, Uganda. Group 1 - Genotypes with high stomatal conductance on the first day of stress imposition but fluctuated mainly between the third and 15th day and declined after that with minor fluctuations. Group 2 - Genotypes with maximum stomatal conductance between the third and 12th day, but porometer readings continued to drop gradually with declining soil moisture.

4.3.4.2 Chlorophyll content

This study indicated gradual chlorophyll degradation among water stressed plants, with the maximum and minimum mean contents recorded on the first and last days of water stress imposition, respectively (Table 4.8). However, in the first three weeks of stress imposition, the chlorophyll content did not differ significantly between the well-watered and water stress plants. Significant differences in chlorophyll content were only found at four weeks after watering was ceased. Nonetheless, significant genotypic differences in chlorophyll content were observed under each water treatment at every week. At four weeks, water stress induced chlorophyll content reductions of up to 40%, with the highest reduction exhibited by 'ITC.1239', followed by 'Calcutta-4', 'Williams' and 'TMB2x8075-7' in close range (Table 4.8, Table 4.9). On the contrary, 'Mpologoma', had significantly less chlorophyll content reduction than the above genotypes. 'Sukali Ndiizi' had a slightly higher chlorophyll content reduction (i.e. 4%) than 'Mpologoma'. Genotypes including the drought-tolerant check 'Monthan', 'Kunnan' and 'ITC.0987' maintained at least 90% of their chlorophyll content despite the imposed water stress.

Table 4.8 Chlorophyll content of 16 *Musa* spp. genotypes grown under well-watered and water stress conditions during the 28 days of stress imposition

	Days after water stress imposition									
	0		7		14		21		28	
Genotype	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2
TMB2x9172	40.7 ^{a}	58.2 ^{ab}	39.0 $^{a-d}$	46.5 $^{b-f}$	35.2 $^{a-c}$	45.2 $^{b-e}$	35.7 $^{a-c}$	43.2 $^{b-f}$	44.6 $^{a-d}$	36.5 $^{a-c}$
TMB2x6142-1	36.8 ^{a}	44.4 ^{ab}	36.6 $^{a-c}$	36.0 ^{a}	33.5 $^{a-c}$	32.7 ^{a}	32.9 ^{ab}	29.8 ^{a}	36.9 ^{ab}	30.9 ^{ab}
TMB2x8075-7	45.1 ^{ab}	41.0 ^{a}	36.8 $^{a-c}$	41.1 $^{a-d}$	35.5 $^{a-c}$	37.4 $^{a-c}$	35.7 $^{a-c}$	31.5 ^{ab}	39.2 $^{a-c}$	27.2 ^{ab}
TMB2x9128-3	40.1 ^{a}	56.4 ^{ab}	47.0 $^{c-e}$	43.7 $^{a-e}$	37.4 $^{a-d}$	40.5 $^{a-d}$	36.8 $^{a-d}$	38.1 $^{a-d}$	36.9 ^{ab}	28.3 ^{ab}
TMB2x9722-1	42.5 ^{ab}	38.7 ^{a}	49.4 $^{c-e}$	40.7 $^{a-d}$	50.0 $^{e-g}$	37.8 $^{a-c}$	50.0 ^{de}	37.5 $^{a-d}$	49.0 $^{b-d}$	35.7 $^{a-c}$
ITC.1239	37.6 ^{a}	39.3 ^{a}	33.9 ^{ab}	39.2 $^{a-c}$	30.5 ^{ab}	39.1 $^{a-d}$	31.1 ^{ab}	39.2 $^{a-d}$	39.1 $^{a-c}$	23.3 ^{a}
ITC.1638	41.3 ^{ab}	42.3 ^{ab}	40.4 $^{a-d}$	37.6 ^{ab}	37.8 $^{a-d}$	36.4 ^{ab}	36.2 $^{a-d}$	36.2 $^{a-d}$	37.4 ^{ab}	31.8 $^{a-c}$
ITC.0987	48.3 ^{ab}	49.3 ^{ab}	47.8 $^{c-e}$	49.6 $^{d-f}$	49.8 $^{e-g}$	55.5 ^{e}	47.2 $^{c-e}$	47.4 $^{d-f}$	43.2 $^{a-d}$	39.1 $^{a-c}$
Kunnan	42.3 ^{ab}	37.1 ^{a}	39.6 $^{a-d}$	39.0 $^{a-d}$	41.6 $^{b-f}$	38.8 $^{a-d}$	40.6 $^{a-e}$	39.3 $^{a-e}$	42.1 $^{a-d}$	39.2 $^{a-c}$
Musa balbisiana	37.5 ^{a}	34.9 ^{a}	36.8 $^{a-c}$	34.7 ^{a}	39.1 $^{b-e}$	33.5 ^{a}	36.5 $^{a-d}$	33.6 $^{a-c}$	37.0 ^{ab}	32.8 $^{a-c}$
Calcutta-4	52.7 ^{ab}	48.9 ^{ab}	50.5 ^{de}	48.0 $^{c-f}$	51.3 ^{fg}	47.5 $^{c-e}$	52.0 ^{e}	45.4 $^{c-f}$	50.8 ^{cd}	32.5 $^{a-c}$
Vunapope	53.4 ^{ab}	50.9 ^{ab}	54.9 ^{e}	52.6 ^{ef}	54.6 ^{g}	53.7 ^{e}	54.1 ^{e}	52.1 ^{ef}	52.8 ^{d}	45.4 ^{bc}
Sukali Ndiizi	40.8 ^{a}	46.6 ^{ab}	31.0 ^{a}	35.0 ^{a}	27.2 ^{a}	33.7 ^{a}	29.9 ^{a}	31.2 ^{ab}	34.7 ^{a}	27.5 ^{ab}
Mpologoma	60.4 ^{b}	67.7 ^{b}	44.9 $^{b-e}$	47.6 $^{b-f}$	44.5 $^{c-g}$	48.9 ^{de}	45.0 $^{b-e}$	47.9 $^{d-f}$	44.6 $^{a-d}$	37.1 $^{a-c}$
Williams	49.7 ^{ab}	53.7 ^{ab}	48.2 $^{c-e}$	52.8 ^{ef}	48.3 $^{d-g}$	49.0 ^{de}	49.0 $^{c-e}$	37.0 $^{a-d}$	48.3 $^{b-d}$	31.8 $^{a-c}$
Monthan	54.4 ^{ab}	51.0 ^{ab}	55.2 ^{e}	54.3 ^{f}	55.5 ^{g}	54.1 ^{e}	56.3 ^{e}	54.1 ^{f}	55.9 ^{d}	52.2 ^{c}
Mean	45.2 ^{a}	47.5 ^{a}	43.3 ^{a}	43.6 ^{a}	42.0 ^{a}	42.7 ^{a}	41.8 ^{a}	40.2 ^{a}	43.3 ^{b}	34.4 ^{a}
SED	3.5	3.5	2.7	2.7	3.1	3.1	3.1	3.1	3.3	3.3
CV (%)	19.4	23.1	18.9	16.3	21.9	19.2	22.4	20.1	16.9	26.7

T1 – Well-watered treatment, T2- Water stress treatment

Table 4.9 Chlorophyll content of 16 *Musa* spp. genotypes grown under well-watered and water stress conditions during the four weeks of drought stress imposition

	Chlorophyll content, SPAD units		
Genotype	Water stress	Well-watered	Reduction (%)
Monthan	52.2 [c]	55.9 [d]	6.6
Kunnan	39.2 [a-c]	42.1 [a-d]	6.9
ITC.0987	39.1 [a-c]	43.2 [a-d]	9.3
Musa balbisiana	32.8 [a-c]	37.0 [ab]	11.5
Vunapope	45.4 [bc]	52.8 [d]	14.1
ITC.1638	31.8 [a-c]	37.4 [ab]	14.9
TMB2x6142-1	30.9 [ab]	36.9 [ab]	16.3
Mpologoma	37.1 [a-c]	44.6 [a-d]	16.8
TMB2x9172	36.5 [a-c]	44.8 [a-d]	18.0
Sukali Ndiizi	27.5 [ab]	34.7 [a]	20.7
TMB2x9128-3	28.3 [ab]	36.9 [ab]	23.2
TMB2x9722-1	35.7 [a-c]	49.0 [b-d]	27.0
TMB2x8075-7	27.2 [ab]	39.2 [a-c]	30.6
Williams	31.8 [a-c]	48.3 [b-d]	34.2
Calcutta-4	32.5 [a-c]	50.8 [cd]	36.0
ITC.1239	23.3 [a]	39.1 [a-c]	40.3
Mean[1]	34.4 [a]	43.3 [b]	
SED	2.9	2.9	
CV (%)	26.7	16.9	

[1]Means in a column or row followed by the same letter(s) are not significantly different at 5% level Tukey HSD

4.3.4.3 Water use efficiency

WUE varied significantly across the two water treatments and among the banana genotypes ($p < 0.05$) (Table 4.10). The mean WUE of plants subjected to water stress conditions was significantly higher than that of well-watered plants. The WUE of the two drought-sensitive checks, 'Mpologoma' and 'Williams' and two other genotypes including, 'ITC.0987', and 'Calcutta-4', reduced significantly under water stress conditions while the remaining 12 *Musa* genotypes exhibited WUE increases ranging from 6.8% to 79.4%. 'Mpologoma' and 'TMB2x9172' had the highest WUE, 6.08 and 6.15 g per kg of water used, under well-watered and water stress conditions, respectively, while 'Monthan' and 'ITC.0987' showed the least WUE, 0.78 and 0.57 g per kg of water used, under well-watered and water stress conditions, respectively. Between the two drought-tolerant checks, the increase in WUE under water stress conditions was almost two times higher in

'Sukali Ndiizi' than in 'Monthan'. Of the five improved diploids, three, including 'TMB2x6142-1', 'TMB2x8075-7' and 'TMB2x9722-1', showed WUE increases greater than 50%.

4.3.4.4 Relative water content

The RWC of plants subjected to water stress treatment was significantly lower than that of plants grown under well-watered conditions ($p < 0.05$) (Table 4.10). All the tested *Musa* spp. genotypes exhibited RWC values higher than 60%. Contrary to the above-mentioned parameters (except for leaf cigars formed), water stress caused a lower RWC reduction in 'Mpologoma' than in 'Monthan'. 'Sukali Ndiizi', on the other hand, had the third least reduction in RWC, closely following 'TMB2x9172' (second) and 'ITC 0987'(first). The lowest RWC (63.2%) was observed in the water stressed plants of 'ITC.1638' while the highest mean RWC (93.4%) was exhibited in the well-watered plants of 'TMB2x8075-7' (Table 4.10). Water stress caused the least (5.8%) and highest (27.3%) mean RWC reductions in the genotypes 'ITC.0987' and 'ITC.1239', respectively.

4.3.3 Correlations between measured traits

Table 4.11 shows the correlation coefficients (r) describing the strength of relationships among measured phenotypic and physiological traits. Under optimal conditions (well-watered), chlorophyll content had a significant positive or negative correlation ($p < 0.05$), with all the measured traits except for the root-shoot ratio. Under water stress, the chlorophyll content was negatively correlated with plant height, total dry matter and WUE, but positively associated with the number of functional leaves. The total leaf area showed strong positive correlations ($r \geq 0.7$) with the total dry matter and WUE under well-watered and water stressed conditions. Plant height also had significant positive associations with the total dry matter, total leaf area and WUE in both water treatments, although stronger correlations were recorded under optimum conditions. Total dry matter showed a moderate negative correlation with the number of functional leaves under well-watered conditions but a weak and non-significant correlation under water stress conditions. Notably, WUE was strongly and positively correlated with the total dry matter under well-watered (r = 0.981) and stress (r = 0.998) conditions. The root-shoot ratio had weak non-significant correlations ($r < 0.3$, $p > 0.05$) with all the other traits under both water stress and well-

watered treatments. Under well-watered conditions, RWC significantly correlated with chlorophyll content, number of functional leaves and plant height. However, RWC significantly correlated with only plant height under stress conditions.

Table 4.10 Water use efficiency and relative water contents of 16 *Musa* spp. genotypes evaluated under well-watered and water stress conditions for four weeks

	Water use efficiency, g per kg of water used				Relative water content, %		
Genotype	Well-watered	Water stress	Decrease (%)	Genotype	Water stress	Well-watered	Reduction (%)
ITC.0987	0.90 [a]	0.57 [a]	36.7	ITC.0987	82.7 [e]	87.8 [a-c]	5.8
Mpologoma	6.08 [f]	4.24 [ef]	30.3	TMB2x9172	79.6 [cd]	86.3 [ab]	7.8
Calcutta-4	1.17 [ab]	0.97 [ab]	17.1	Sukali Ndiizi	80.2 [cd]	87.7 [a-c]	8.7
Williams	1.47 [ab]	1.42 [bc]	3.4	TMB2x8075-7	81.6 [c-e]	91.0 [cd]	10.3
Vunapope	1.49 [ab]	1.57 [bc]	-6.8	Mpologoma	78.9 [cd]	91.2 [cd]	13.5
ITC.1239	3.58 [cd]	4.01[ef]	-12.2	Calcutta-4	68.2 [a-c]	80.3 [a]	15.1
Kunnan	1.96 [a-c]	2.21 [c-e]	-12.8	TMB2x9128-3	75.3 [b-d]	88.8 [bc]	15.2
Monthan	0.78 [a]	0.88 [a]	-12.8	TMB2x6142-1	74.0 [bc]	90.7 [cd]	18.4
Sukali Ndiizi	4.57 [e]	5.57 [f-i]	-22.4	Monthan	72.2 [bc]	88.6 [bc]	18.5
TMB2x9128-3	3.95 [c-e]	4.91 [e-g]	-24.1	Vunapope	66.3 [ab]	83.3 [a]	20.4
TMB2x9172	4.55 [e]	6.15 [i]	-35.4	TMB2x9722-1	69.1 [a-c]	87.2 [a-c]	20.7
ITC.1638	3.73 [c-e]	5.25 [f-h]	-40.7	*Musa balbisiana*	73.2 [bc]	93.4 [d]	21.6
Musa balbisiana	2.21 [bc]	3.29 [c-f]	-48.9	ITC.1638	63.2 [a]	82.5 [ab]	23.4
TMB2x8075-7	3.64 [cd]	5.5 [f-i]	-51.3	Williams	68.6 [a-c]	90.1 [cd]	23.9
TMB2x6142-1	3.31 [cd]	5.25 [f-h]	-58.4	Kunnan	64.9 [ab]	86.2 [ab]	24.8
TMB2x9722-1	1.36 [ab]	2.45 [c-e]	-79.4	ITC.1239	65.1 [ab]	89.5 [b-d]	27.3
Mean[1]	2.81 [a]	3.42 [b]			72.86 [a]	87.83 [b]	
SED	0.63	0.63			1.8	1.8	
CV (%)	10.42	7.61			2.29	1.85	

[1]Means in a column or row followed by the same letter(s) are not significantly different at 5% level Tukey HSD

Table 4.11 Pearson's correlation coefficients (*r*) showing the association of eight phenotypic and physiological traits of 16 *Musa* spp. genotypes evaluated under water stress (upper diagonal) and well-watered (lower diagonal) conditions

		Water stress conditions								
		CC	FL	LC	PH	RS	TDM	TLA	WUE	RWC
Well-watered conditions	CC	1	0.326*	0.097 ns	-0.318*	0.086 ns	-0.399**	-0.235 ns	-0.399**	0.106 ns
	FL	0.405**	1	0.292*	-0.091 ns	0.029 ns	-0.089 ns	0.183 ns	-0.078 ns	0.067 ns
	LC	0.352*	-0.044 ns	1	0.068 ns	-0.057 ns	-0.170 ns	0.015 ns	-0.184 ns	0.162 ns
	PH	-0.600**	-0.405**	-0.238 ns	1	-0.240 ns	0.536**	0.401**	0.534**	0.368*
	RS	-0.063 ns	0.281 ns	0.032 ns	-0.172 ns	1	0.038 ns	-0.039 ns	0.051 ns	-0.093 ns
	TDM	-0.508**	-0.492**	-0.246 ns	0.750**	-0.153 ns	1	0.745**	0.998**	0.250 ns
	TLA	-0.304*	-0.056 ns	-0.063 ns	0.713**	-0.097 ns	0.717**	1	0.734**	0.093 ns
	WUE	-0.479**	-0.516**	-0.213 ns	0.732**	-0.144 ns	0.981**	0.682**	1	0.255 ns
	RWC	0.3533*	-0.315*	-0.150 ns	0.331*	-0.071 ns	0.193 ns	0.087 ns	0.235 ns	1

CC, chlorophyll content; FL, Number of functional leaves; LC, number of leaf cigars formed; PH, plant height; RS, root-shoot ratio; TDM, total dry matter; TLA, total leaf area; WUE, water use efficiency; RWC, Relative water content; ns, non-significant; *, $p < 0.05$ (2-tailed); **, $p < 0.01$ level (2-tailed)

4.4 Discussion

Screening germplasm for drought tolerance is essential for effective identification and selection of tolerant candidates for advanced crop improvement programs. In this study, a set of 16 edible, wild and improved banana genotypes were evaluated in a screen-house facility at NARL-Kawanda in Uganda. Similar to Eyland et al. (2021a), the genotypes were evaluated for four weeks, unlike other studies with shorter assessment periods (Delfin et al., 2016; Mattos-Moreira et al., 2018; Santos et al., 2020). The highly significant variations due to the water treatment, genotype and their interaction observed for the traits measured in this study indicate the genetic diversity among the genotypes evaluated across the two water treatments (Table 4.3).

The negative impacts of water stress on bananas have been reported in several studies (Bananuka et al., 1999; Ravi et al., 2013; Kissel et al., 2015; Eyland et al., 2020; 2022). The significant reductions in plant height, total leaf area, number of leaf cigars, number of functional leaves, total dry matter, leaf chlorophyll content and relative water content observed in this study confirm the vulnerability of bananas to water stress, particularly at the seedling stage. 'Mpologoma', the EAHB susceptible check, manifested high reductions in the total leaf area, number of functional leaves, number of leaf cigars, total dry matter as well as reduction in the WUE and root-shoot ratio under water stress conditions. This observation confirms the drought-sensitive status of 'Mpologoma' which was reported by farmers in the earlier participatory study carried out in the cattle corridor of Uganda (Chapter 3). Therefore, 'Mpologoma' can be used as a drought-sensitive check in future evaluation of banana germplasm for drought tolerance.

Although farmers in the cattle corridor reported 'Sukali Ndiizi' to be the fourth least drought-sensitive cultivar among the top 20 popularly grown varieties (Chapter 3; Table 3.6), in this study, it experienced moderately high reductions in the number of functional leaves (53.8%) and total leaf area (44%) due to water stress. This contradiction may be due to the difference in the size of the plants, given that the intensity of the effects of water stress depends on the plant growth stage. Considering that field plants are usually bigger in size and have a more developed root system (wider and deeper) than potted plants, they are more likely to withstand

water stress conditions better as they can explore deeper and wider soil layers for additional water. On the contrary, the root system of potted plants is only limited to the space and water available in the pot. Nonetheless, water stress led to lower reductions of less than 35 % in other traits of 'Sukali Ndiizi', suggesting its moderate tolerance to drought.

The results obtained in this study reflect some of the negative drought effects on the vegetative growth of banana that were reported by farmers in Chapter 3. For instance, stunted growth, reduced leaf production, reduced leaf size, leaf senescence, reduced plant size, and wilting of leaves were reflected in this study as significant reductions in plant height, number of leaf cigars produced, total leaf area, chlorophyll content, total dry matter and RWC, respectively. Water stress has been shown to slow down early vegetative growth by significantly reducing plant height, total leaf area, number of functional leaves, total dry matter and the production of new leaf cigars (Delfin et al., 2016; Uwimana et al., 2020; Eyland et al., 2022).

The chlorophyll content reduction observed in water stressed plants could be due to photooxidation of chlorophyll which is often caused by uncoupling of photosystems I and II during water stress conditions (Ravi et al., 2013). Such drought-induced chlorophyll degradation results in accelerated leaf senescence, an adaptive mechanism plants deploy to reduce their water requirement while remobilizing nutrients from older leaves to young growing tissues (Munné-Bosch and Alegre, 2004). Because chlorophyll is a requirement of photosynthesis, its reduction ultimately results in reduced plant biomass production (Göksoy et al., 2004). However, the significant negative association between leaf chlorophyll content and total plant dry matter observed under well-watered and water stress conditions corroborates that biomass production depends not only on the chlorophyll amount but also on other factors such as water and carbon dioxide.

Similar to Thomas and Turner (1998) and Delfin et al. (2016), this study showed a general reduction in stomatal conductance over the water stress treatment period. Previously, banana plants subjected to water stress treatment had higher stomatal resistance than well-watered plants (Santos et al., 2020). The decline in stomatal conductance indicates increased stomata closure, a major drought avoidance strategy bananas use to reduce water loss by transpiration (Thomas and Turner, 1998; van Asten et al., 2011; Eyland et al., 2020). Once the roots detect

a decline in soil moisture, they relay a series of signals to the leaves, which cause stomatal closure hence reduced stomatal conductance (Schroeder et al., 2001). Leaf stomatal closure can also be stimulated by high atmospheric vapour pressure deficits and low light intensities (Gosa et al., 2019; Eyland et al., 2021). However, this closure of stomata prevents the entry of carbon dioxide, which is needed for photosynthesis, consequently affecting biomass production and accumulation (Cornic, 2000). The occasional small increments in stomatal conductance observed in this study could have been due to increased solar radiation and higher temperature on the day porometer readings were taken, both of which resulted in the increased opening of leaf stomata. Nonetheless, the genotype differences in the stomata movement can be used to characterize bananas as sensitive or tolerant to drought.

The sharp reduction of stomatal conductance of the improved diploid 'TMB2x9722-1' and '*M. balbisiana*' after the third and sixth day of drought imposition (Figure 4.1; Group 2), respectively, suggests a drastic and quick response to declining soil water content. Moreover, 'TMB2x9722-1' and '*M. balbisiana*' showed maximum stomatal conductance on the third and sixth day of stress treatment. Such early and rapid drought avoidance behaviour is desirable, particularly in environments with high evaporative demands. Early stomatal driven drought avoidance in banana has been shown in a previous high-throughput phenotyping characterization (Eyland et al., 2021). The transpiration rates of all the genotypes tested in that study were reduced at relatively high soil water contents. On the other hand, 'ITC.1239' and 'ITC.1638' had gradual stomatal conductance increases until the 12^{th} and 18^{th} day of progressive soil water reduction. Such delayed stomata closure suggests a lower critical soil water content threshold than 'TMB2x9722-1' and '*M. balbisiana*'. Although maintained stomatal opening allows continuous carbon uptake, it also results in higher transpiration rates (Eyland et al., 2021). We, therefore, presume that those genotypes do not thrive well in environments with a long dry season.

The increase in WUE observed in this study in most genotypes under stress conditions is similar to that reported in other crops (Blankenagel et al., 2018; Zhang et al., 2018). In wheat, WUE varied from 10.0 to 13.1 mg g^{-1} in the dry treatment compared to 9.7 – 11.7 mg g^{-1} in the control treatment (Van Den Boogaard et al., 1997). The higher WUE exhibited by *Musa* spp. genotypes under drought imply efficient use of limited water, which is essential to sustain

plant growth and ensure survival in dry environments. Hatfield and Dold (2019) suggested that the increase in WUE under water deficit conditions results from the reduction in the stressed plant's net photosynthetic rate being less than the reduction in stomatal conductance or transpiration rate. Additionally, the strong positive correlation observed between WUE and total dry matter under well-watered and water stress conditions (Table 4.11) suggests that an increase in WUE can improve biomass production. On the other hand, the reductions in WUE shown by the genotypes, 'ITC.0987', 'Calcutta-4', 'Mpologoma' and 'Williams' under stress treatment are similar to the decreases reported in cowpea (Anyia and Herzog, 2004) and wheat (Ehdaie, 1995; Sikder et al., 2016). Under water stress, photosynthesis is limited by gradual reductions of carbon dioxide assimilation rates due to stomatal closure, as observed for higher plants (Reddy et al., 2004).

Delfin et al. (2016) reported that *Musa* spp. triploids were superior to the diploids in terms of WUE under stress treatment. Our findings, however, showed that diploids generally performed relatively the same or even much better than the triploids tested. For instance, the top seven genotypes with the highest increase in WUE under water stress conditions were all diploids (Table 4.10). Moreover, the WUE of 'Mpologoma' and 'Williams' (both triploids) decreased under moisture stress conditions. Therefore, this study draws no substantial conclusion regarding the effect of ploidy number on the WUE.

Nine out of 16 genotypes were characterized by higher root-shoot ratios under water stress than well-watered conditions (Table 4.5). Such higher root-shoot ratios exhibited by water stressed plants suggest that those genotypes allocate more biomass to their root system than their well-watered counterparts during limiting soil moisture conditions. Santos et al. (2020) showed that under mild stress conditions, some genotypes increase root growth (in terms of number and/ or length of the main root) to reach deeper or wider soil levels in search of water. Van Wesemael et al. (2019) also showed that *Musa* spp. genotypes invested more in root than shoot growth when subjected to osmotic stress, which suggests sustained water uptake and productivity in environments with short dry seasons.

Despite the significant reductions in RWC under stress conditions, all genotypes maintained a relatively high RWC, supporting the behaviour shown for a genetically diverse set of

genotypes (Ravi et al., 2013; Sreedharan et al., 2013). Results of this study support reports that the stomata close with declining soil moisture, allowing banana leaves to remain highly hydrated, perhaps through root pressure (Turner et al., 2007). Regarding stress effects on RWC, 'ITC.1239' was the most sensitive to declining soil water content followed by the 'Kunnan' genotype, which even with this proneness to water stress had the second-highest root-shoot ratio increase.

While water stress significantly affected the growth of most of the genotypes, it had little to no effect on the performance of 'ITC.0987' for most measured traits. For instance, water stressed plants had significantly more functional leaves, total leaf area, root-shoot ratio and relatively small reductions in plant height and chlorophyll content. Leaf cigar production was not affected by declining soil moisture (Table 4.6). Although leaf cigar formation was not significantly affected by water stress, it is related to leaf emergence rate which is a very sensitive and useful indicator of the vegetative growth rate of a banana plant (Ravi et al., 2013). 'ITC.0987' was relatively small in size compared to other genotypes evaluated. It was the shortest genotype even under well-watered conditions with the least total leaf area (due to small leaf size) and total dry matter. Because of its small size, we assume that it has a significantly less water requirement than the rest of the genotypes. Consequently, soil moisture depletion by plant use was much slower; therefore, it continued growing optimally for a longer time, even after watering ceased. Moreover, it was the only genotype in which increasing stomatal conductance values were recorded even after 18 days of water stress imposition (Figure 4.1; Group 1), suggesting continued stomatal opening and transpiration. Further, water stressed plants had higher total dry matter than well-watered plants.

The positive correlation between total leaf area and total dry matter observed under well-watered and water stress conditions (Table 4.11) suggests that it is essential for banana genotypes to retain as much functional photosynthetic tissue as possible to ensure higher production of biomass, which is required for continuous plant growth and survival, even more so during water stress conditions. We also found significant positive correlations between total leaf area and WUE under both water treatments as well as between total dry matter and WUE (Table 4.11). High WUE whether under well-watered or water stress treatment, results in increased total dry matter, which a plant can then allocate or invest in plant growth. Plant

height is often used as a measure of plant growth in banana drought assessment studies (Uwimana et al., 2020). Moreover, in this study, plant height positively and significantly correlated to total dry matter under both water treatments. Plant height also positively correlated with total leaf area and WUE. Based on the above correlations (all above 0.4), plant height, total leaf area, total dry matter and WUE are promising traits for detecting the impact of water stress on the early growth stage of bananas. Therefore, we suggest using of these traits in future pre-breeding and breeding initiatives.

4.5 Conclusions

This study investigated the water stress tolerance levels of 16 *Musa* spp. genotypes using phenotypic and physiological traits. All tested genotypes showed some level of sensitivity to water stress, irrespective of whether they contain the B genome. The study also showed significant genotypic variations for all the traits under well-watered and water stress conditions, thereby suggesting genetic diversity for drought tolerance within the germplasm used. While water stress caused significant reductions in most of the traits, significant increments in WUE and root-shoot ratios were observed for most genotypes. This study also confirms that 'Mpologoma' is very sensitive to water stress, as previously reported by farmers in the cattle corridor of Uganda (Chapter 3). Therefore, we recommend the use of 'Mpologoma' as a drought-sensitive check variety in routine screening of banana germplasm for drought. Plant height, total leaf area, total dry matter and WUE were promising traits to detect the impact of water stress on the early growth stage of banana plants. Therefore, researchers should consider these traits in future screening and breeding initiatives. Although this study shows that the soil moisture content declined progressively in stressed plants over the evaluation period, further studies are required to determine the critical soil water content thresholds below which water stress is experienced for each genotype. Knowledge of threshold soil water content is crucial as it informs at what point drought avoidance mechanisms such as stomatal closure start. Lastly, field screening of the three promising genotypes (including 'ITC.0987', 'TMB2x9722-1' and 'TMB2x9172') should be done to test whether the increased WUE under water stress treatment observed in this screen-house study will be translated into high yield. Moreover, the floral, fruit and yield-related traits that were reported by farmers

(Chapter 3) should be prioritised in these field assessments to ensure that the traits used by scientists reflect farmers' measures and/ or indicators of drought tolerance or sensitivity on-farm.

References

Anyia, A.O., and H. Herzog. 2004. Water-use efficiency, leaf area and leaf gas exchange of cowpeas under mid-season drought. Eur. J. Agron. 20:327-339.

Bananuka, J.A., P.R. Rubaihayo, and M.M. Tenywa. 1999. Reactions of *Musa* genotypes to drought stress. Afr. Crop Sci. J. 7:333–339.

Barrs, H.D., and P.E. Weatherley. 1962. A re-examination of the relative turgidity techniques for estimating water deficits in leaves. Aust. J. Biol. Sci. 15:413–428.

Batte, M., R. Swennen, B. Uwimana, V. Akech, A. Brown, et al. 2019. Crossbreeding East African Highland bananas: Lessons learnt relevant to the botany of the crop after 21 years of genetic enhancement. Front. Plant Sci. 10:81.

Blankenagel, S., Z. Yang, V. Avramova, C.C. Schön, and E. Grill. 2018. Generating plants with improved water use efficiency. Agronomy. 8:194.

Carr, M.K.V. 2009. The water relations and irrigation requirements of banana (*Musa* spp.). Exp. Agric. 45:333–371.

Cornic, G. 2000. Drought stress inhibits photosynthesis by decreasing stomatal aperture – not by affecting ATP synthesis. Trends Plant Sci. 5:187-188.

Delfin, E.F., E.T.M. Ocampo, F.M. Cueva, O.P. Damasco, F. de la Cruz, et al. 2016. Response of wild and edible *Musa* spp. seedlings to limiting moisture stress. Philipp J. Crop Sci. 41:1–12.

Ehdaie, B., and J.G. Waines. 1993. Variation in water-use efficiency and its components in wheat: I. well-watered pot experiment. Crop Sci. 33:294-299.

Ehdaie, B. 1995. Variation in water use efficiency and its components in wheat: II. pot and field experiments. Crop Sci. 35:1617-1626.

Ekanayake, I.J., R. Ortiz, and D.R. Vuylsteke. 1994. Influence of leaf age, soil moisture, VPD and time of day on leaf conductance of various *Musa* genotypes in a humid forest-moist savanna transition site. Ann. Bot. 74:173–178.

Eyland, D., C. Breton, J. Sardos, S. Kallow, B. Panis, et al. 2020. Filling the gaps in gene banks: Collecting, characterizing, and phenotyping wild banana relatives of Papua New Guinea. Crop Sci. 61:137–149.

Eyland, D., J. van Wesemael, T. Lawson, and S. Carpentier. 2021. The impact of slow stomatal kinetics on photosynthesis and water use efficiency under fluctuating light. Plant Physiol. 186:998–1012.

Eyland, D., N. Luchaire, L. Cabrera-Bosquet, B. Parent, S.B. Janssens, et al. 2022. High-throughput phenotyping reveals differential transpiration behaviour within the banana wild relatives highlighting diversity in drought tolerance. Plant, Cell Environ. 45:1647-1663.

Goenaga, R., and H. Irizarry. 1995. Yield performance of banana irrigated with fractions of class A pan evaporation in a semi-arid environment. Agron. J. 82:172-176.

Göksoy, A.T., A.O. Demir, Z.M. Turana, and N. Dağüstü. 2004. Responses of sunflower (*Helianthus annuus* L.) to full and limited irrigation at different growth stages. Field Crops Res. 87:167–178.

Gosa, S.C., Y. Lupo, and M. Moshelion. 2019. Quantitative and comparative analysis of whole-plant performance for functional physiological traits phenotyping: new tools to support pre-breeding and plant stress physiology studies. Plant Sci. 282:49-59.

Hatfield, J.L., and C. Dold. 2019. Water use efficiency: Advances and challenges in a changing climate. Front. Plant Sci. 10:103.

Heslop-Harrison, J.S., and T. Schwarzacher. 2007. Domestication, genomics and the future for banana. Ann. Bot. 100:1073-1084.

Karamura, D., R. Tumuhimbise, S. Muhangi, M. Nyine, M. Pillay, et al. 2016. Ploidy level of the banana (*Musa* spp.) accessions at the germplasm collection centre for the East and Central Africa. Afr J. Biotechnol. 15:1692-1698.

Kayongo, S.N., J.M. Sebuliba, and K. Nyombi. 2015. Responses of East African highland banana (EAHB-AAA) cultivars to drought stress. Uganda J. Agric Sci. 16:55–81.

Kissel, E., P. van Asten, R. Swennen, J. Lorenzen, and S.C. Carpentier. 2015. Transpiration efficiency versus growth: Exploring the banana biodiversity for drought tolerance. Sci. Hortic. 185:175–182.

Mahouachi, J. 2007. Growth and mineral nutrient content of developing fruit on banana plants (*Musa acuminata* AAA, 'Grand Nain') subjected to water stress and recovery. J. Hortic. Sci. Biotechnol. 82:839-844.

Mahouachi, J. 2009. Changes in nutrient concentrations and leaf gas exchange parameters in banana plantlets under gradual soil moisture depletion. Sci. Hortic. 120:460–466.

Mattos-Moreira, L.A., C.F. Ferreira, E.P. Amorim, C.P. Pirovani, E.M. de Andrade, et al. 2018. Differentially expressed proteins associated with drought tolerance in bananas (*Musa* spp.). Acta Physiol. Plant. 40:60.

Munné-Bosch, S., and L. Alegre. 2004. Die and let live: Leaf senescence contributes to plant survival under drought stress. Funct. Plant Biol. 31:203-216.

Mustaffa, M.M., and V. Kumar. 2012. Banana production and productivity enhancement through spatial, water and nutrient management. J. Hortic. Sci. 7:1-28.

Muthusamy, M., S. Uma, S. Backiyarani, M.S. Saraswathi, and A. Chandrasekar. 2016. Transcriptomic changes of drought-tolerant and sensitive cultivars exposed to drought stress. Front. Plant Sci. 7: 1609.

Nansamba, M., J. Sibiya, R. Tumuhimbise, D. Karamura, J. Kubiriba, and E. Karamura. 2020. Breeding banana (*Musa* spp.) for drought tolerance: A review. Plant Breed. 139:685-696.

Nowakunda, K., A. Barekye, T.R. Ssali, J. Namaganda, W.K. Tushemereirwe, et al. 2015. 'Kiwangaazi' (syn 'KABANA 6H') Black Sigatoka nematode and banana weevil tolerant 'Matooke' hybrid banana released in Uganda. HortScience 50:621–623.

Ortiz, R., and R. Swennen. 2014. From crossbreeding to biotechnology-facilitated improvement of banana and plantain. Biotechnol. Adv. 32:158–169.

Ramirez, J., A. Jarvis, I. Van den Bergh, C. Staver, and D. Turner. 2011. Chapter 20: Changing climates: Effects on growing conditions for banana and plantain (*Musa* spp.) and possible responses, p. 426-438, *In* S. Yadav, B. Redden, J.L. Hattfield, and H. Lotze-Campen, eds. Crop adaptation to climate change. Wiley-Blackwell, Chichester, England.

Ravi, I., S. Uma, M.M. Vaganan, and M.M. Mustaffa. 2013. Phenotyping bananas for drought resistance. Front. Physiol. 4:9.

Reddy, A.R., K.V. Chaitanya, and M. Vivekananda. 2004. Drought induced responses of photosynthesis and antioxidant metabolism in higher plant. J. Plant Physiol. 161:1189–1202.

Robinson, J.C., and A.J. Alberts. 1986. Growth and yield responses of banana (cultivar 'Williams') to drip irrigation under drought and normal rainfall conditions in the subtropics. Sci. Hortic. 30:187-202.

Robinson, J.C., and V.G. Sauco. 2010. Bananas and Plantains. CAB International, Wallingford, England.

Santos, A.S., E.P. Amorim, C.F. Ferreira, and C.P. Pirovani. 2018. Water stress in *Musa* spp.: A systematic review. PLOS One. 13: e0208052.

Santos, A.S., E.P. Amorim, A-A.F. Almeida, C.F. Ferreira, M.A.C. Filho, and C.P. Pirovani. 2020. Diversity of banana diploid hybrids: An assessment based on a hydroponic system. Plant Breed. 139:1251–1265.

Schroeder, J.I., G.H. Allen, V. Hugouvieux, J.M. Kwak, and D. Waner. 2001. Guard cell signal transduction. Ann. Rev. Plant Physiol. Plant Mol. Biol. 52:627-658.

Sikder, S., Y. Qiao, B. Dong, C. Shi, and M. Liu. 2016. Effect of water stress on leaf level gas exchange capacity and water-use efficiency of wheat cultivars. Indian J. Plant Physiol. 21:300–305.

Sreedharan, S., U.K.S. Shekhawat, and T.R. Ganapathi. 2013. Transgenic banana plants overexpressing a native plasma membrane aquaporin *MusaPIP1;2* display high tolerance levels to different abiotic stresses. Plant Biotechnol. J. 11:942–952.

Srikul, S., and D.W. Turner. 1995. High N supply and soil water deficits change the rate of fruit growth of bananas (cv. 'Williams') and promote tendency to ripen. Sci. Hortic. 62: 165–174.

Stevens, B., J. Diels, A. Brown, S. Bayo, P.A. Ndakidemi, and R. Swennen. 2020. Banana biomass estimation and yield forecasting from non-destructive measurements for two contrasting cultivars and water regimes. Agronomy. 10:1435.

Summerville, W.A.T. 1944. Studies on nutrition as qualified by development in *Musa cavendishii* Lamb. J. Agric Meteorol. 12:361–389.

Thomas, D.S., and D.W. Turner. 1998. Leaf gas exchange of droughted and irrigated banana cv. Williams (*Musa* spp.) growing in hot, arid conditions. J. Hortic. Sci. Biotechnol. 73:419-429.

Tumuhimbise, R., A. Barekye, J. Kubiriba, K. Akankwasa, I.K. Arinaitwe, et al. 2018. New high-yield cooking banana cultivars with multiple resistances to pests and diseases ('NAROBan1', 'NAROBan2', 'NAROBan3', and 'NAROBan4') released in Uganda. HortScience. 53:1387–1389.

Turner, D.W., and E. Lahav. 1983. The growth of banana plants in relation to temperature. Aust. J. Plant Physiol. 10:43–53.

Turner, D.W., J. Fortescue, and D.S. Thomas. 2007. Environmental physiology of the bananas (*Musa* spp.). Braz. J. Plant Physiol. 19:463–484.

UBOS. 2023. Statistical Abstract on the Environmental, Demographic, socio-economic, Production and Macroeconomic Sectors. Uganda Bureau of Statistics (UBOS), Kampala, Uganda.

Uwimana, B., Y. Zorrilla-Fontanesi, J. van Wesemael, H. Mduma, A. Brown, et al. 2020. Effect of seasonal drought on the agronomic performance of four banana genotypes (*Musa* spp.) in the East African Highlands. Agronomy. 11:4.

Van Asten, P.J.A., A.M. Fermont, and G. Taulya. 2011. Drought is a major yield loss factor for rainfed East African highland banana. Agric. Water Manage. 98:541-552.

Van den Boogaard, R., D. Alewijnse, E.J. Veneklaas, and H. Lamber. 1997. Growth and water use efficiency of 10 *Triticum aestivum* cultivars at different water availability in relation to allocation of biomass. Plant. Cell. Environ. 20:200–210.

Vanhove, A.C., W. Vermaelen, B. Panis, R. Swennen, and S.C. Carpentier. 2012. Screening the banana biodiversity for drought tolerance: can an invitro growth model and proteomics be used as a tool to discover tolerant varieties and understand homeostasis. Front. Plant Sci. 3:176.

Van Wesemael, J., E. Kissel, D. Eyland, T. Lawson, R. Swennen, and S. Carpentier. 2019. Using growth and transpiration phenotyping under controlled conditions to select water efficient banana genotypes. Front. Plant Sci. 10:354.

Varma, V., and D.P. Bebber. 2019. Climate change impacts on banana yields around the world. Nat. Clim Change 9:752–757.

VSN International. 2015. Genstat for Windows. 18th ed. VSN International, Hemel Hempstead, UK.

Wairegi, L.W.I., P.J.A. van Asten, M.M. Tenywa, and M.A. Bekunda. 2010. Abiotic constraints override biotic constraints in East African highland banana systems. Field Crops Res. 117:146–153.

Xu, Y., W. Hu, J. Liu, J. Zhang, C. Jia, et al. 2014. A banana aquaporin gene, *MaPIP1;1*, is involved in tolerance to drought and salt stresses. BMC Plant Biol. 14:59.

Zhang, J., H. Jiang, X. Song, J. Jin, and X. Zhang. 2018. The responses of plant leaf CO_2/H_2O exchange and water use efficiency to drought: A meta-analysis. Sustainability. 10:551.

Chapter 5: Evaluation of hybridisation success of two improved drought-tolerant candidate diploid bananas (*Musa* spp.)

Abstract

Banana (*Musa* spp.) improvement through crossbreeding is hindered by poor seed set and low embryo germination rates. This study aimed to analyse the hybridisation success of two selected improved *Musa* diploid drought-tolerant candidate male parents and to generate genotypes for use in subsequent genetic drought tolerance analyses. Four improved tetraploid genotypes ('660K-1', '917K-2', '1201K-1' and '222K-1') were each pollinated with pollen from two male diploid parents, 'TMB2x9722-1' and 'TMB2x9172'. Pollination success, seed set rate, embryo recovery and germination success were recorded and analysed in Genstat software. Significant effects of the female parent, male parent and their interactions were observed for seed set, embryo recovery and embryo germination. Among the female parents, '222K-1' and '917K-2' were the best performers with the highest pollination successes of 73% and 70%, respectively. '917K-2' had the highest mean seed set (95.9 seeds), followed by '222K-1' (79.4 seeds). The male diploid, 'TMB2x9172' had the highest pollination success of 83.3% while only 35% bunches of 'TMB2x9772-1' contained seeds. Crosses with 'TMB2x9172' produced significantly more seeds (110.5 seeds per bunch) than those with 'TMB2x9722-1' (3.7 seeds per bunch). The '917K-2' x 'TMB2x9172' cross produced the highest number of seeds per bunch (184.3 seeds per bunch), while the '1201K-1' x 'TMB2x9722-1' cross had the least seed set (1.8 seeds per bunch). Embryo germination rate varied among crosses with 'TMB2x9172': 12.3% for '917K-2', 8.5% for '660K-1', 3.2% for '222K-1' and 0.7% for '1201K-1'. Conversely, none of the embryos of seeds obtained from bunches pollinated with pollen from 'TMB2x9722-1' germinated. The study revealed 'TMB2x9172' with high pollination success, seed set and germination success as the more suitable male parent to generate hybrid progenies for subsequent genetic drought tolerance studies. Nonetheless, the usefulness of 'TMB2x9172' in banana drought tolerance breeding will depend on its ability to transfer favourable alleles to the offspring and the ability to produce a considerable number of tolerant progenies.

Keywords: banana, embryo germination, embryo rescue, improved *Musa* diploids, improved tetraploids, pollination success

5.1 Introduction

Breeding for desirable agronomic traits such as host plant resistance or tolerance, by far, offers the most effective and long-term solution to biotic and abiotic stresses affecting crop production. In banana, such crossbreeding involves intra- and interspecific hybridization of two parental lines through controlled hand pollinations (Ortiz and Vuylsteke, 1995; Batte et al., 2020; Brown et al., 2020; Waniale et al., 2021a). However, the development of banana hybrids is often challenged by male or female sterility and low seed set, which are attributed to a combination of inherent and environmental factors (Pillay and Tripathi, 2007; Amah et al., 2020). For instance, banana improvement is often complicated by physiological and reproductive barriers such as irregular meiotic behaviour, poor seed viability, male sterility in some cultivars, parthenocarpy, diverse genome constitutions and long crop cycles (Ssebuliba et al., 2006a; Ortiz and Swennen 2014; Brown et al., 2017). Female sterility, particularly in the East African highland cooking bananas (EAHB, AAA), has been associated with abnormalities in pistil morphological traits and individual genotype influences (Ssebuliba et al., 2005). In general, triploid bananas are less fertile than diploids due to the unequal distribution of chromosomes among daughter nuclei during gamete production. According to Shepherd (1960), only nuclei containing balanced and complete chromosome sets form viable gametes. Seed set is also influenced by environmental conditions such as solar radiation, relative humidity, temperature, and rainfall (Ortiz and Vuylsteke, 1995; Ssebuliba et al., 2009). Waniale et al. (2021b) recently reported that maximum temperature at the time of pollination enhances seed set in EAHBs. In an earlier study, Vuylsteke et al. (1990) reported higher germination rates in embryos obtained from seeds of bunches pollinated during the wet season.

Despite the abovementioned complications, improvement of *Musa* species has been achieved through the classical crossbreeding approach (Tenkouano et al., 2003; Aguilar-Morán, 2013; Tumuhimbise et al., 2018). For instance, the banana breeding programs of the National Agricultural Research Organization (NARO-Uganda) and the International Institute of Tropical Agriculture (IITA) in Uganda have developed and released superior hybrids with multiple pest and disease resistances and higher yields compared to the existing local varieties

(Batte et al., 2019; Tumuhimbise et al., 2019; Nowankunda et al., 2015). Ultimately, the success of banana crossbreeding depends on identifying compatible parent lines, which, when cross-pollinated, result in high pollination success, seed set and embryo germination. Besides, among the EAHB cultivars and tetraploid bred hybrids, hybridization success is highly variable between genotypes (Ssebuliba et al., 2005; Batte et al., 2019). Artificial hybridization of a given female fertile genotype with different male parents results in variable mean seed sets due to differences in male fertility (Ssebuliba et al., 2009).

At NARO, Calcutta-4 (*Musa acuminata* spp. *burmannicoides*, AA), a seeded wild, pollen fertile *Musa* species, has been used in several crosses as an important source of resistance to multiple banana pests and diseases (Tushemereirwe et al., 2015). Although wild crop relatives naturally possess beneficial breeding traits, they also often contain undesirable agronomic traits which are inherited by the offspring (Iskra-Caruana et al., 2014; Prohens et al., 2017). Fixation of desirable agronomic and fruit characteristics through crossbreeding would require several generations of backcrossing, a demanding task given the highly heterozygous loci, making it challenging to predict progeny phenotypes based on parental performance (Ortiz, 2000). Consequently, banana improvement programs utilise improved diploids that have resistance to multiple production stresses to minimise the co-inheritance of undesirable traits while overcoming the crossbreeding complications previously described (Brown et al., 2017).

Drought stress is a major threat to banana production systems in Uganda (van Asten et al., 2011; Nansamba et al., 2022), hence a need to develop drought-tolerant cultivars. In Chapter 4 of this thesis, a diverse collection of banana genotypes acquired from the International Transit Centre (ITC) and the East and Central germplasm collection maintained in Mbarara, Uganda, were assessed under well-watered and water stress conditions to select at least two drought-tolerant candidate male parental lines. The two parental lines would then be used in subsequent crosses with improved tetraploids to generate secondary progenies. Because banana improvement is often hindered by several factors that result in male and female sterility, it is important for selected parental lines to not only possess breeding attributes of interest but also generate hybrid seeds when utilised in crosses. For instance, although EAHBs are characterised by a set of unique, desirable food qualities (Akankwasa et al., 2021), majority of cultivars have not been extensively utilised in banana improvement due to poor hybrid seed production caused by low female fertility (Ssebuliba et al., 2005). Thus, selecting parental genotypes that generate

a considerable number of hybrid seeds when inter-crossed is pertinent. Among the improved diploids assessed for drought tolerance in Chapter 4, 'TMB2x9172' and 'TMB2x9722-1' had the highest sustained aboveground growth under water stressed conditions and hence recommended as essential drought-tolerant candidates that may be utilized in breeding. However, the usability and efficiency of 'TMB2x9172' and 'TMB2x9722-1' genotypes in producing progenies through crossbreeding is not known. Therefore, the objectives of this study were to analyse the hybridisation success of the two selected drought-tolerant candidate diploid male parents based on pollination success, embryo recovery and embryo germination rates and to generate genotypes for use in subsequent genetic drought tolerance analyses.

5.2 Materials and methods

5.2.1 Parental genotypes used to generate progenies

Four improved *Musa* tetraploids '660K-1', '917K-2', '1201K-1' and '222K-1' were selected as female parents based on their superior agronomic attributes (Table 5.1) and higher seed production compared to triploids (Batte et al., 2019). The tetraploids were each pollinated with pollen from two improved diploid parents: 'TMB2x9172' and 'TMB2x9722-1'. The two improved male parents (pollen sources) were screened for drought-tolerance under screen-house conditions (Chapter 4) and selected as drought-tolerant candidates. These two hybrid diploids also possess desirable breeding attributes (Table 5.1), some of which will presumably be inherited by their progenies. In this study, pollinations were purposely made between hybrids (improved tetraploids x diploids) to minimise co-inheritance of poor agronomic traits, usually contained in wild *Musa* relatives or unimproved cultivars (Iskra-Caruana et al., 2014; Prohens et al., 2017).

Table 5.1 Description of the four improved *Musa* spp. tetraploids (4x) and two improved diploid (2x) parents used in the study

Parent name	Ploidy (genome)	Pedigree	Special breeding attributes
660K-1	Tetraploid (AAAA)	Enzirabahima × Calcutta-4	Female fertility, large bunch size, *matooke qualities, resistance to black sigatoka
917K-2	Tetraploid (AAAA)	Enzirabahima × Calcutta-4	Female fertility, large bunch size, matooke qualities, resistance to black sigatoka
1201K-1	Tetraploid (AAAA)	Nakawere × Calcutta-4	Female fertility, large bunch size, matooke qualities, resistance to black sigatoka
222K-1	Tetraploid (AAAA)	Nfuuka × Calcutta-4	Female fertility, large bunch size, matooke qualities, resistance to black sigatoka
TMB2x9172	Diploid (AA)	Obino L'Ewai x Calcutta-4	Drought-tolerant candidate, resistance to black sigatoka, improved bunch size
TMB2x9722-1	Diploid (unknown)	Bobby Tannap x Calcutta-4	Drought-tolerant candidate, rich in vitamin A

*Desirable matooke food qualities include soft or tender texture, deep yellow colour, good aroma and taste.

5.2.2 Pollination, seed extraction, and embryo germination

Pollinations were carried out at the East and Central Africa *Musa* germplasm collection centre in Mbarara district, Uganda. This gene bank is located at an elevation of 1433 m above sea level at latitude 00º 11′ 24′ S and longitude 32º 18′ 0′ E. The soils at Mbarara are sandy-clay loams with a pH range of 5.2 - 7.0. The area receives a mean annual rainfall of 1373 mm and a temperature range of 21.6˚C to 24.1˚C (Muzira et al., 2020).

At anthesis, inflorescences of male parent genotypes were covered with cotton bags to avoid pollen contamination from natural pollinators such as birds, bats, or insects. Similarly, flowers of female parents were bagged in transparent plastic bags until the entire inflorescence was pollinated to prevent external pollination by unknown pollen sources (Ssebuliba et al., 2005).

Hand pollinations were carried out from December 2019 to March 2021 between 07:00 h and 09:30 h by gently rubbing the anthers of male parent flowers containing pollen against the stigmas of female parent flowers. Pollinations were performed daily and the bunches rebagged until the last set of flowers on the female inflorescence was pollinated (Ssebuliba et al., 2000; Waniale et al., 2021a). Thirty (30) inflorescences were pollinated for each female parent, i.e., 15 pollinations with 'TMB2x9722-1' and 15 pollinations with 'TMB2x9172', resulting in 15 replicates per female-male parent cross combination.

At full maturity, pollinated bunches were harvested and stored in a room at a temperature range of 18 - 30°C and relative humidity of 60 - 100% for one to two weeks to allow all the fruits to ripen. Seeds were then extracted from the ripe pulp, washed, counted, and taken to the tissue culture laboratory at the National Agricultural Research Laboratories (NARL), Kawanda, for embryo rescue. Hybrid seed and embryo profiles were examined, and images taken using a microscope camera (model ZEISS Axiocam 208 colour, Carl Zeiss Microscopy GmbH, Oberkochen, Germany). Standard laboratory procedures for banana embryo culture, involving embryo excision and subsequent germination, were followed (Vuylsteke et al., 1990) to generate seedlings.

5.2.3 Data collection and analysis

The following data were recorded: Total number of seeds per bunch, number of extracted embryos and number of germinated embryos. For each tetraploid x diploid cross combination, the total number of pollinated bunches without seeds, total number of pollinated bunches with seeds, the total number of seeds extracted, the highest number of seeds per bunch and the highest number of embryos were calculated. Pollination success was computed as follows:

$$\text{Pollination success} = \frac{\text{Total pollinated bunches with seeds x 100}}{\text{Total pollinated bunches}} \quad (5.1)$$

Means and standard errors of the number of seeds extracted, embryos extracted, and embryos germinated were calculated for each tetraploid-diploid cross combination. Subsequently, the seed embryo germination success over the experimental period was calculated as:

$$\text{Embryo germination success} = \frac{\text{Number of germinated seed embryos x 100}}{\text{Total number of seed embryos extracted}} \quad (5.2)$$

A Shapiro-Wilk test for normality was performed on all the data before analysis. Analysis of variance (ANOVA) was then performed at 95% confidence interval to determine the effect of genotype on pollination success using the Genstat® software 18th Edition (Payne et al., 2018). Correlation analyses were performed to determine the association between the number of seeds obtained, the number of embryos extracted and the embryo germination rate.

5.3 Results

5.3.1 Seed set rates and pollination success

Results from ANOVA revealed that the female parent, male parent, and female-male parent combinations had significantly different seed set (Table 5.2). The female parent, '917K-2' generated the highest seeds per pollinated bunch (95.9 seeds), followed by '222K-1' (79.4 seeds) and '1201K-1' (36.7 seeds). Female parent '660K-1' produced the least number of seeds, with an average of 16.3 seeds per pollinated bunch (Table 5.3). Regarding the two male parents, crosses with 'TMB2x9172' on average produced more seeds (110.5 seeds per pollinated bunch) compared to pollinations involving 'TMB2x9722-1' (3.7 seeds per pollinated bunch).

Table 5.2 Analysis of variance for seed set of banana parental lines inter-crossed from December 2019 to March 2021 at the germplasm collection in Mbarara, Uganda

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance	F pr
Female parent	3	122461	40820	4.03	0.009**
Male parent	1	341974	341974	33.8	< 0.001**
Female * Male parent	3	108849	36283	3.59	0.016*
Residual	112	1133063	10117		

*, $p < 0.05$; **, $p < 0.01$ level of significance

Table 5.3 Hybrid seed set, embryo recovery and germination success from the hybridization of improved *Musa* spp. genotypes

Female parent	Pollinated bunches without seeds	Pollinated bunches with seeds	Total seeds extracted	Seed set per bunch ± *SE*	Pollination success (%)	Total embryos extracted	Mean embryos per bunch ± *SE*	Total embryos germinated	Mean embryos germinated ± *SE*	Germination success (%)
660K-1	15	15	490	16.3 ± 4.4	50.0	124	4.1 ± 1.1	10	0.3 ± 0.3	8.1
917K-2	9	21	2878	95.9 ± 31.8	70.0	913	30.4 ± 10.8	108	3.6 ± 1.3	11.8
1201K-1	17	13	1102	36.7 ± 19.3	43.3	423	14.1 ± 9.4	3	0.1 ± 0.1	0.7
222K-1	8	22	2382	79.4 ± 20.4	73.3	806	26.9 ± 7.9	25	0.8 ± 0.5	3.1
Total	49	71	6852			2266		146		
Male parent										
TMB2x9722-1	39	21	223	3.7 ± 1.0	35	75	1.3 ± 0.4	0	0 ± 0	0
TMB2x9172	10	50	6629	110.5 ± 19.6	83.3	2191	36.5 ± 7.7	146	2.4 ± 0.7	6.7
Total	49	71	6852			2266		146		

Variation in mean seed set per bunch among crosses, ranging from 1.8 in ‘1201K-1’ x ‘TMB2x9722-1’ cross to 184.3 in ‘917K-2’ x ‘TMB2x9172’, was observed (Table 5.4). The highest number of seeds per pollinated bunch was 780, recorded in ‘917K-2’ x ‘TMB2x9172’ cross combination. Female parent ‘917K-2’ produced the highest number of seeds when crossed with either male parent, i.e., 114 and 2764 seeds for ‘TMB2x9722-1’ and ‘TMB2x9172’, respectively (Table 5.4).

Pollination success was higher in tetraploid crosses with ‘TMB2x9172’ (≥ 60%) than in crosses with ‘TMB2x9722-1’ (≤ 50%). All the ‘222K-1’ x ‘TMB2x9172’ hybridizations resulted in seed production i.e., 100% pollination success. The lowest pollination success was expressed by the ‘1201K-1’ x ‘TMB2x9722-1’ cross (20%) with an average of 1.8 seeds per pollinated bunch followed by the ‘660K-1’ x ‘TMB2x9722-1’cross (26.7%) with an average of 2.3 seeds per pollinated bunch.

Table 5.4 Hybridization success of four tetraploid *Musa* hybrids inter-crossed with two improved diploids from December 2019 to March 2021 at the banana germplasm collection in Mbarara, Uganda

Female parent	Male parent	Pollinated bunches without seeds	Pollinated bunches with seeds	Total seeds extracted	Highest seeds per bunch	Seed set per bunch ± *SE*	Pollination success (%)
660K-1	TMB2x9722-1	11	4	34	18	2.3 ± 1.3	26.7
660K-1	TMB2x9172	4	11	456	72	30.4 ± 7.0	73.3
917K-2	TMB2x9722-1	8	7	114	50	7.6 ± 3.6	46.7
917K-2	TMB2x9172	1	14	2764	780	184.3 ± 55.3	93.3
1201K-1	TMB2x9722-1	12	3	27	19	1.8 ± 1.3	20.0
1201K-1	TMB2x9172	5	10	1075	555	71.7 ± 37.0	66.7
222K-1	TMB2x9722-1	8	7	48	11	3.2 ± 1.0	46.7
222K-1	TMB2x9172	0	15	2334	470	155.6 ± 30.0	100.0
Total		49	71	6852			

The hybrid seeds varied in nature and development level (Figure 5.1): i) some were black with a hard seed coat (Figure 5.1A); ii) some were brownish, soft and without an endosperm or embryo (Figure 5.1B); iii) some had an embryo (brown in colour) but without an endosperm (Figure 5.1C); iv) some had a large endosperm but no embryo (Figure 5.1D); v) some contained both the endosperm and embryo (Figure 5.1E). These varying sectional hybrid seed profiles were observed across all the eight crosses.

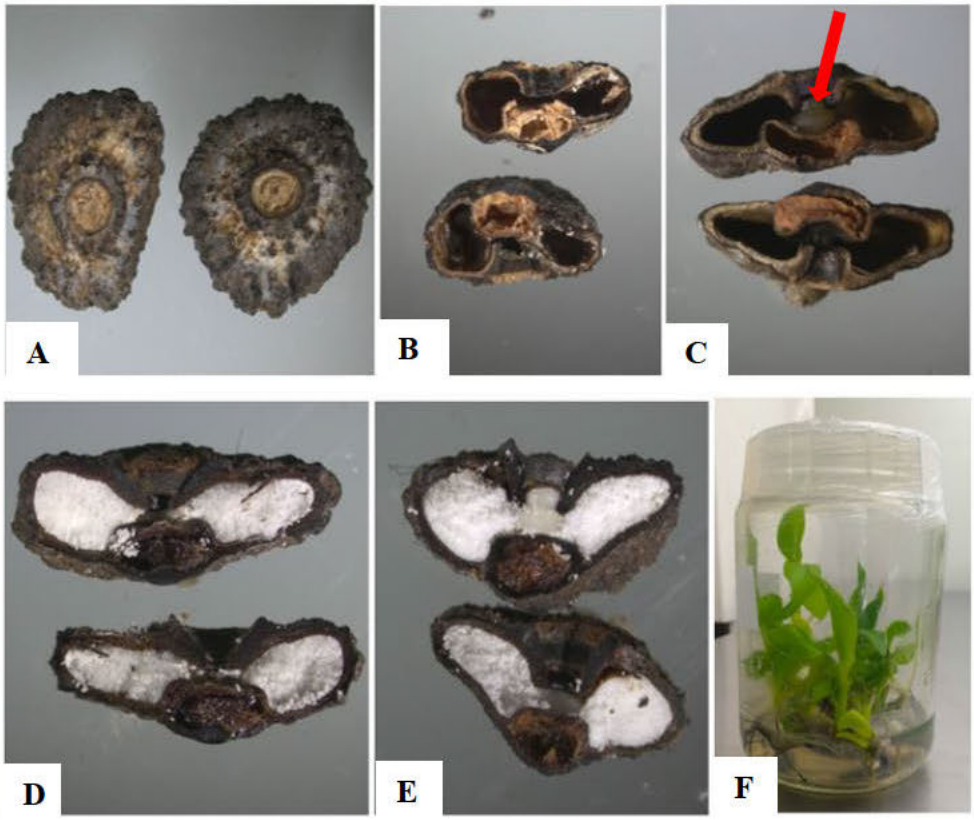


Figure 5.1 Nature of seeds extracted from bunches of hybrid tetraploid bananas crossed with improved diploids. A) Unopened seed with hard and rough seed coat (0.63x); B) Seed with both endosperm and embryo absent (0.63x); C) Seed with embryo (shown by red arrow) but endosperm absent (0.63x); D) Seed with endosperm (large white mass) present but embryo absent (0.63x); E) Seed with endosperm and embryo present (0.63x); F) *In vitro* seedlings of germinated embryos growing on proliferation medium.

5.3.2 Embryo recovery and germination

There was significant variation in embryo recovery when the two diploid male parents were each crossed with the four female tetraploids (Table 5.5). The highest number of embryos was observed in seeds obtained from crosses with ‘TMB2x9172’ (mean = 36.5 embryos), whereas ‘TMB2x9722-1’ seeds produced only 1.2 embryos on average (Table 5.6).

Although embryo recovery varied among female parental lines, the differences were non-significant ($p = 0.051$) (Table 5.5). The highest average number of embryos were obtained from ‘917K-2’ (30.4 embryos), followed by ‘222K-1’ (26.9 embryos), then ‘1201K-1’ (14.1 seeds) and the lowest from ‘660K-1’ (4.1 embryos). Despite the insignificant variations noted in embryo recovery due to the female x male parent interaction ($p = 0.075$) (Table 5.5), there was a great fluctuation in the number of embryos extracted among crosses, ranging from 4 to 280 embryos per cross. Seeds from the ‘917K-2’ x ‘TMB2x9172’ cross contained the highest number of embryos (58.3 embryos), closely followed by ‘222K-1’ x ‘TMB2x9172’ (52.7 embryos). The least embryos were obtained from the ‘660K-1’ x ‘TMB2x9722-1’ cross (0.5 embryos). The extracted embryos varied in structure and colour (Figure 5.2).

Although embryo germination rates were generally low, significant variations among crosses were noted (Table 5.6). The highest germination percentage was exhibited by embryos recovered from the ‘917K-2’ x ‘TMB2x9172’ seeds (12.3%), followed by ‘660K-1’ x ‘TMB2x9722-1’ seeds (8.5%). All the embryos obtained from crosses with ‘TMB2x9722-1’ had zero germination success. Overall, out of the 6852 seeds obtained from all the crosses, only 2266 (33.1%) contained embryos, of which only 146 (6.4%) germinated. There was a weak but significant relationship between the number of embryos recovered and those that germinated ($r = 0.426$, $p = 0.001$) and between the number of seeds extracted and germinated embryos ($r = 0.479$, $p = 0.001$). As expected, there was a strong correlation between the number of seeds extracted and embryos recovered ($r = 0.960$, $p = 0.001$).

Table 5.5 Analysis of variance for embryo recovery and embryo germination rates of parental banana hybrids inter-crossed from December 2019 to March 2021 at the *Musa* germplasm collection in Mbarara, Uganda.

	Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance	F pr
Number of embryos extracted	Female parent	3	13127	4376	2.67	0.051
	Male parent	1	37312	37312	22.75	< 0.001
	Female * Male parent	3	11614	3871	2.36	0.075
	Residual	112	183673	1640		
Number of germinated embryos	Female parent	3	235.63	78.54	7.1	< 0.001
	Male parent	1	177.63	177.63	16.05	< 0.001
	Female * Male parent	3	235.63	78.54	7.1	< 0.001
	Residual	112	1239.47	11.07		

Table 5.6 Performance of improved tetraploid-diploid *Musa* spp. crosses as determined by embryo recovery and embryo germination success

Female parent	Male parent	Total embryos extracted	Highest number of embryos per cross	Mean embryos extracted ± *SE*	Total embryos germinated	Mean embryos germinated ± *SE*	Germination success (%)
660K-1	TMB2x9722-1	7	4	0.5 ± 0.3	0	0 ± 0	0.0
660K-1	TMB2x9172	117	22	7.8 ± 1.7	10	1.7 ± 0.5	8.5
917K-2	TMB2x9722-1	38	16	2.5 ± 1.3	0	0 ± 0	0.0
917K-2	TMB2x9172	875	266	58.3 ± 19.2	108	4.5 ± 2.3	12.3
1201K-1	TMB2x9722-1	14	8	0.9 ± 0.9	0	0 ± 0	0.0
1201K-1	TMB2x9172	409	280	27.3 ± 18.4	3	0.2 ± 0.2	0.7
222K-1	TMB2x9722-1	16	7	1.1 ± 0.5	0	0 ± 0	0.0
222K-1	TMB2x9172	790	180	52.7 ± 12.8	25	1.7 ± 0.9	3.2
Total		2266			146		

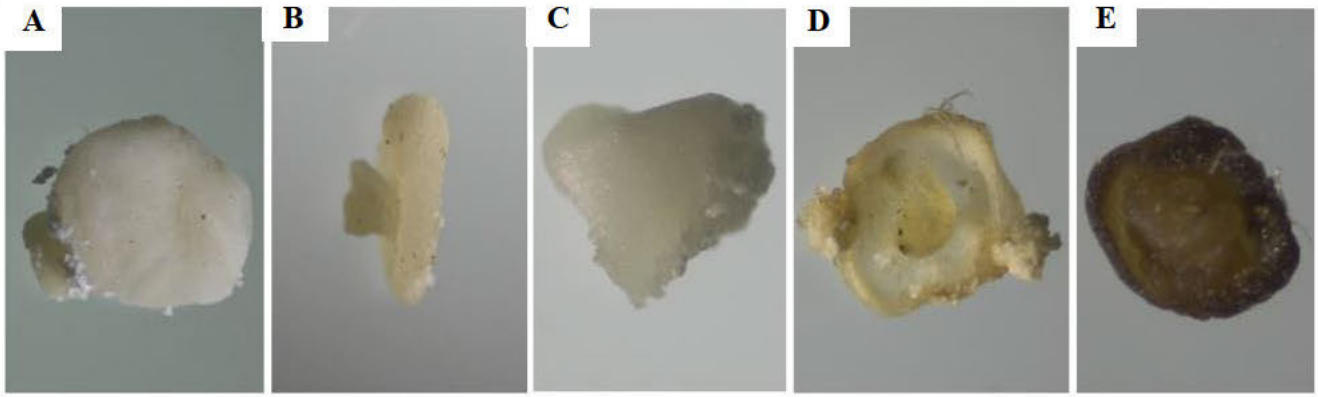


Figure 5.2 Morphology of embryos extracted from seeds of hybrid tetraploid bananas crossed with improved diploids. A) Normal embryo (1.6x); B) Embryo with deformed base and normal apex (1.6x); C) Embryo with deformed apex and base (2.0x); D) Embryo with no apex (2.0x); E) Embryo with oxidation (browning) (1.6x).

5.4 Discussion

In banana, successful development of intra- and inter-specific hybrids using conventional crossbreeding largely depends on the production of viable seeds. Previous reports have shown varying seed sets in *Musa* crosses depending on the maternal and paternal parents (Brown et al., 2017; Amah et al., 2020). For instance, Batte et al. (2019) reported higher mean seed sets in improved tetraploids than in their EAHB triploid parents. Based on their findings, four improved tetraploids with the highest hybridisation success were selected and used as female parents in this study. These tetraploids were crossed with two drought-tolerant diploid candidates (male parents) that were selected in a previous drought evaluation experiment (Chapter 4). The contrast in the pollination successes reported in Batte et al. (2019) and in this study may be due to the differences in the genotype of male parents. The huge difference in pollination success and average seed set observed in this study implies varying male fertility of the two improved diploid parents. 'TMB2x9172' crosses produced almost 30 times more seeds than pollinations with 'TMB2x9722-1' (Table 5.2). As 'TMB2x9722-1' showed more tolerance to water stress conditions than 'TMB2x9172' (Chapter 4), this accession is a more drought-tolerant candidate but results in rather poor seed set and production of non-viable embryos when used as a male parent in banana crosses. Its utilisation in crossbreeding would require extensive pollinations to increase the chances of obtaining enough progenies for use in genetic analyses. Moreover, the strong correlation between the number of seeds extracted and embryos recovered supports the need for more seeds to obtain more viable embryos, a process which is very costly and time-consuming. Therefore, the generation of segregating progeny populations would be faster using 'TMB2x9172' as a male parent than with 'TMB2x9722-1'. Among the four female parents, '222K-1' and '917K-2' were identified as the best performers, considering they had the highest hybridisation (Table 5.3). These two tetraploid hybrids can be deployed for banana crossbreeding.

The absence of seeds in some bunches indicates unsuccessful sexual reproduction, even after manual cross-pollination. Seed set is influenced by many factors, including seasonal (Ssebuliba et al., 2009; Waniale et al., 2021b) and biological factors (Ortiz and Vuylsteke, 1995; Waniale et al., 2021c). It varies considerably based on the fertility of the female parent, as does with male fertility (Uma and Arun, 2016). Regarding the female parent,

biological factors such as early withering of fertilised ovules (Waniale et al., 2022) and stigma receptivity have been shown to limit seed production in EAH bananas. Light brown stigmas with a drier mass (classified as stage III stigmas) were reported to be the most receptive and thus recommended for hand pollinations to improve seed production in *Musa* cultivars (Ssebuliba et al., 2006a). For the male parents, pollen quantity and quality are considered important measures of male fertility in bananas (Dumpe and Ortiz, 1996; Ssebuliba et al., 2008; Ssali et al., 2012). However, the non-significant association between pollen stainability and seed set reported by Ortiz et al. (1998) implies that pollen viability is not a reliable predictor of hybrid seed production. Rather the actual fertility of a male parent should be based on the number of viable seeds it produces when crossed with a female parent.

Embryo recovery varied after crossing tetraploid females with the two improved diploids, ranging from 0 to 280 embryos per pollinated bunch. The number of embryos recorded from each cross was consistently less than that of seeds extracted because not all the seeds contained embryos. This observation is similar to Ssebuliba et al. (2006b), who reported that only 59% of seeds with a black integument ('good seeds') contained embryos. The absence of embryos can be attributed to a disruption of endosperm-embryo relations or embryo abortion in the early stages of seed development (Vuylsteke and Swennen, 1992; Waniale et al., 2022). The current study also observed that some seeds contained brown embryos, but the endosperm was absent (Figure 5.1C). According to Silva et al. (2019), such browning is caused by oxidation. In angiosperms, the endosperm supplies nutrients and acts as a mechanical barrier for the embryo during seed development and germination (Yan et al., 2014). The embryo and endosperm are formed due to a double fertilization event between two female gametes and two male sperm cells (Figueiredo et al., 2016). Therefore, the absence of either the embryo or endosperm suggests failure of their formation or abortion during the early stages of seed development.

Embryo germination success varied among banana crosses (0 to 12.3%). Although embryo culture was done between two to four days after seed extraction, the germination percentage was very low (Table 5.6). Poor germination of banana seeds has previously been attributed to seed dormancy (Chin, 1996; Ssebuliba et al., 2006b; Fortescue and Turner, 2011) and desiccation during storage (Kallow et al., 2020). Uma et al. (2011),

investigating different factors influencing *in-vitro* regeneration in bananas, suggested that the success of embryo culture is also greatly dependent on the maturity stage of the embryos. They reported gradual increases in plantlet regeneration up to 90% embryo maturity, above which a drastic increase was observed. Despite the above suggestions, it is still unclear whether these very low germination rates are primarily caused by embryo dormancy or low viability.

All the brown embryos found in non-endospermic banana seeds (Figure 5.1C, Figure 5.2E) did not germinate. This browning could be due to their deterioration caused by oxidative stress (Pehlivan, 2017), which is manifested as browning. Although normal embryos seem to have a higher chance of germinating than others, the effect of embryo structure on germination success warrants further investigation. In this study, none of the embryos obtained from crosses with 'TMB2x9722-1' germinated, which makes 'TMB2x9172' a better male parent (in addition to having higher pollination success and seed set) for developing hybrid populations for subsequent banana drought tolerance studies.

5.5 Conclusions

The success of generating viable hybrid *Musa* seeds depends on the fertility of both male and female parent accessions. This study investigated the efficiency of two drought-tolerant diploid candidates in producing progenies after crossing them with female fertile improved tetraploid lines. The two diploid male parents showed high genotypic variations for studied sexual hybridisation traits. Higher pollination success, seed set embryo recovery and germination success were recorded in crosses with 'TMB2x9172' than in pollinations with 'TMB2x9722-1'. Consequently, 'TMB2x9172' appears to be the ideal male parent to use for generating progeny populations for drought tolerance genetic studies. However, despite 'TMB2x9172' being a suitable male parent for generating progenies, its usefulness in banana drought tolerance breeding will depend on its ability to transfer favourable genes/ alleles to its offspring as well as the ability to produce a considerable number of tolerant progenies. The discrepancy between the hybridization success of crosses with 'TMB2x9722-1' and 'TMB2x9172' necessitates further research on the potential causes of low male fertility of 'TMB2x9722-1'. Specifically, pollen quantity and pollen viability and embryo studies on 'TMB2x9722-1' are recommended.

Knowledge of the actual causes of male sterility in 'TMB2x9722-1' would be of value for overcoming pollen fertility barriers to enhance its usability in crossbreeding programs, particularly for banana drought tolerance improvement.

References

Aguilar-Morán, J.F. 2013. Improvement of Cavendish banana cultivars through conventional breeding. Acta Hortic. 986:205–208.

Akankwasa, K., P. Marimo, R. Tumuhimbise, M. Asasira, E. Khakasa, et al. 2021. The East African highland cooking bananas 'Matooke' preferences of farmers and traders: Implications for variety development. Int J. Food Sci. Technol. 56:1124–1134.

Amah, D., D.W. Turner, J.D. Gibbs, A. Waniale, G. Gram, and R. Swennen. 2020. Overcoming the fertility crisis in bananas (*Musa* spp.), p.1-50, *In* G. Kema, and A. Drenth, eds. Achieving sustainable cultivation of bananas Volume 2: Germplasm and genetic improvement. Burleigh Dodds Science Publishing, Cambridge, UK.

Batte, M., R. Swennen, B. Uwimana, V. Akech, A. Brown, et al. 2019. Crossbreeding East African Highland bananas: Lessons learnt relevant to the botany of the crop after 21 years of genetic enhancement. Front. Plant Sci. 10:1-9.

Batte, M., M. Nyine, B. Uwimana, R. Swennen, V. Akech, et al. 2020. Significant progressive heterobeltiosis in banana crossbreeding. BMC Plant Biol. 20:489.

Brown, A., R. Tumuhimbise, D. Amah, B. Uwimana, M. Nyine, et al. 2017. Bananas and Plantains (*Musa* spp.), p.219-240, *In* H. Campos, and P.D.S. Caligari, eds. Genetic improvement of tropical crops. Springer International Publishing, Cham, Switzerland.

Brown, A., S.C. Carpentier, and R. Swennen. 2020. Breeding climate-resilient bananas, p.91-115, *In* Kole C, eds. Genomic designing of climate-smart fruit crops. Springer Nature, Switzerland AG.

Chin, F.H. 1996. Germination and storage of banana seeds, p.218-229, *In* E.A. Frison, J.P. Horry, and D. De Waele, eds. New frontiers in resistance breeding for Nematode, *Fusarium* and Sigatoka. International Plant Genetic Resources Institute, International Network for the Improvement of Banana and Plantain (INIBAP), Rome, Italy.

Dumpe, B.B., and R. Ortiz. 1996. Apparent male fertility in *Musa* germplasm. HortScience. 31:1019–1022.

Figueiredo, D.D., R.A. Batista, P.J. Roszak, L. Hennig, and C. Köhler. 2016. Auxin production in the endosperm drives seed coat development in Arabidopsis. Elife. 5:1-23.

Fortescue, J.A., and D.W. Turner. 2011. Reproductive biology, p.145-179, *In* M. Pillay, and A. Tenkouano, eds. Banana breeding: Progress and challenges. CRC Press, Boca Raton, USA.

Iskra-Caruana, M., M. Chabannes, P.O. Duroy, and E. Muller. 2014. A possible scenario for the evolution of banana streak virus in banana. Virus Res. 186:155–162.

Kallow, S., K. Longin, N.F. Sleziak, S.B. Janssens, F. Vandelook, et al. 2020. Challenges for ex situ conservation of wild bananas: seeds collected in Papua New Guinea have variable levels of desiccation tolerance. Plants. 9:1-21.

Muzira, R., L. Turyagyenda, P. Kankwatsa, M. Kyomugisha, H. Mutenyo, and S. Natuha. 2020. Exploration of the growth performance of indigenous and exotic banana clones in the semi-arid climate of South-Western agro-ecological zone of Uganda. Open Access Lib J. 7:1-11.

Nansamba, M., J. Sibiya, R. Tumuhimbise, W. Ocimati, E. Kikulwe, et al. 2022. Assessing drought effects on banana production and on-farm coping strategies by farmers—A study in the cattle corridor of Uganda. Clim. Change. 173:21.

Nowakunda, K., A. Barekye, R.T. Ssali, J. Namaganda, W.K. Tushemereirwe, et al. 2015. 'Kiwangaazi' (syn 'KABANA 6H') Black Sigatoka nematode and banana weevil tolerant 'Matooke' hybrid banana released in Uganda. HortScience. 50:621–623.

Ortiz, R., and D.R. Vuylsteke. 1995. Factors influencing seed set in triploid *Musa* spp. L. and production of euploid hybrids. Ann. Bot. 75:151–155.

Ortiz, R., F. Ulburghs, and J. Okoro. 1998. Seasonal variation of apparent male fertility and 2n pollen production in plantain and banana. HortScience. 33:146–148.

Ortiz, R. 2000. Understanding the *Musa* genome: an update. Acta Hortic. 54:157–168.

Ortiz, R., and R. Swennen. 2014. From crossbreeding to biotechnology-facilitated improvement of banana and plantain. Biotechnol. Adv. 32:158-169.

Payne, R.W., D.A. Murray, and S.A. Harding. 2018. An introduction to GenStat command language. VSN international, Hampstead, UK.

Pehlivan, F.E. 2017. Free radicals and antioxidant system in seed biology, p.167-183, *In* J.C. Jimenez-Lopez, ed. Adv. Seed Biol. IntechOpen, London, UK.

Pillay, M., and L. Tripathi. 2007. Banana breeding, p.393-428, *In* M.S. Kang, and P.M. Priyadarshan, eds. Breeding major food staples. Blackwell Publishing, Iowa, USA.

Prohens, J., P. Gramazio, M. Plazas, H. Dempewolf, B. Kilian, et al. 2017. Introgressiomics: A new 743 approach for using crop wild relatives in breeding for adaptation to climate change. Euphytica. 213:1–9.

Shepherd, K. 1960. Seed fertility of edible bananas. J. Hortic. Sci. 35:6-20.

Silva, M. dos.S., T.C. Rodrigues, F.F. Aud, J.A. dos Santos-Serejo, and E.P. Amorim. 2019. Illustrated guide to the classification of banana seeds and embryos. Rev. Bras. Frutic. 41:1-5.

Ssali, R.T., M. Pillay, P. Rubaihayo, and W.K. Tushemereirwe. 2012. Male fertility in *Musa*: Pollen quality in diploid banana hybrids. Uganda J. Agric. Sci. 13:39-45.

Ssebuliba, R., D. Vuylsteke, J. Hartman, D. Makumbi, D. Talengera, et al. 2000. Towards improving highland bananas. Uganda J. Agric. Sci. 5:36-38.

Ssebuliba, R.N., P. Rubaihayo, A. Tenkouano, D. Makumbi, D. Talengera, and M. Magambo. 2005. Genetic diversity among East African Highland bananas for female fertility. Afr Crop Sci J. 13:13–26.

Ssebuliba, R., M. Magambo, D. Talengera, D. Makumbi, A. Tenkouano, et al. 2006a. Biological factors affecting seed production in East African Highland bananas. J. Crop Improv. 16:67-79.

Ssebuliba, R., D. Talengera, D. Makumbi, P. Namanya, A. Tenkouano, et al. 2006b. Reproductive efficiency and breeding potential of East African highland (*Musa* AAA-EA) bananas. Field Crops Res. 95:250–255.

Ssebuliba, R.N., A. Tenkouano, and M. Pillay. 2008. Male fertility and occurrence of 2n gametes in East African Highland bananas (*Musa* spp.). Euphytica. 164:53–62.

Ssebuliba, R., D. Makumbi, and M. Pillay. 2009. Patterns of Seed Set in East African Highland banana (*Musa* spp.) hybrids. J. New Seeds. 10:160–170.

Talengera, D., D. Vuylsteke, and E. Karamura. 1996. *In vitro* germination of Ugandan banana hybrids. *Musa*Africa 10:14.

Tenkouano, A., D. Vuylsteke, J. Okoro, D. Makumbi, R. Swennen, and R. Ortiz. 2003. Registration of TMB2x5105-1 and TMB2x9128-3 diploid banana hybrids with good combining ability, partial resistance to black Sigatoka and resistance to nematodes. HortScience. 38:468–472.

Tumuhimbise, R., A. Barekye, J. Kubiriba, K. Akankwasa, I.K. Arinaitwe, et al. 2018. New high-yield cooking banana cultivars with multiple resistances to pests and diseases ('NAROBan1', 'NAROBan2', 'NAROBan3', and 'NAROBan4') released in Uganda. HortScience. 53:1387–1389.

Tumuhimbise, R., H. Buregyeya, J. Kubiriba, W.K. Tushemereirwe, A. Barekye, et al. 2019. 'NABIO808' (Syn. 'NAROBAN5'): A tasty cooking banana cultivar with resistance to pests and diseases. Crop Breed. Appl. Biotechnol. 19:491-495.

Tushemereirwe, W., M. Batte, M. Nyine, R. Tumuhimbise, A. Barekye, et al. 2015. Performance of NARITA banana hybrids in the preliminary yield trial for three cycles in Uganda. Banana Technical Report, p.35. IITA-NARO, Uganda. www.musalit.org/seeMore.php?id=15482. Accessed February 27, 2022.

Uma, S., S. Lakshmi, M.S. Saraswathi, A. Akbar, and M.M. Mustaffa. Embryo rescue and plant regeneration in banana (*Musa* spp.). Plant Cell Tissue. Organ Cult. 105:105-111.

Uma, S., and K. Arun. 2016. Understanding the diversity and reproductive biology of banana – for improvement through basic research. Acta Hortic. 1114:1-12.

Van Asten, P.J.A., A.M. Fermont, and G. Taulya. 2011. Drought is a major yield loss factor for rainfed East African Highland Banana. Agric. Water Manage. 98:541–552.

Vuylsteke, D., R. Swennen, and D. De Langhe. 1990. Tissue culture technology for the improvement of Africa plantains, p.316-337, *In* R.A. Fullerton, and R.H. Stover, eds. Sigatoka leaf spot diseases of bananas. International Network for Improvement of Banana and Plantain (INIBAP), Montpellier, France.

Vuylsteke, D., and R. Swennen. 1992. Biotechnological approaches to plantain and banana improvement at IITA, p.143-150, *In* G. Thottappilly, L. Monti, D.R. Mohan Raj, and A.W. Moore, eds. Biotechnology: Enhancing research on tropical crops in Africa. CTA/ IITA, Ibadan, Nigeria.

Waniale, A., R. Swennen, S.B. Mukasa, A.K. Tugume, J. Kubiriba, et al. 2021a. Seed set patterns in East African Highland cooking bananas show asymmetric distribution in bunches and fruits. Agronomy. 11(763):1-20.

Waniale, A., R. Swennen, S.B. Mukasa, A.K. Tugume, J. Kubiriba, et al. 2021b. Seed set patterns in East African Highland cooking bananas are dependent on weather before, during and after pollination. Horticulturae. 7:1-12.

Waniale, A., R. Swennen, S.B. Mukasa, A.K. Tugume, J. Kubiriba, et al. 2021c. Application of pollen germination media on stigmas during pollination increases seed set in East African Highland cooking bananas (*Musa spp*.). Agronomy. 11(1085):1-9.

Waniale, A., S.B. Mukasa, A.K. Tugume, J. Kubiriba, W.K. Tushemereirwe, and R. Tumuhimbise. 2022. Early withering of enlarged ovules in pollinated fruits of bananas (*Musa* spp.) suggest abortion after fertilization. Horticulturae. 8:426.

Yan, D., L. Duermeyer, C. Leoveanu, and E. Nambara. 2014. The functions of the endosperm during seed germination. Plant Cell Physiol. 55:1521–1533.

Chapter 6: Genetic relationships and diversity analysis of banana germplasm using Diversity Array Technology (DArT)-based SNP markers: implications for banana drought tolerance breeding

Abstract

Knowledge of the genetic diversity and relationships between banana accessions is crucial for the genetic improvement of the crop. The aim of this study was to determine the genetic relationships and genetic diversity of 55 banana genotypes using diversity array technology (DArT)-based single nucleotide polymorphism (SNP) markers. We zoom into the genetic relationships of selected *Musa* progenies and their drought-sensitive female ('917K-2') and tolerant male ('TMB2x9172') parents. The study population included breeding lines, *Musa* progenies, wild species and landraces. A total of 1551 high-quality DArT markers were used to characterize and estimate the genetic diversity and show genetic relationships among the genotypes. Genetic dissimilarities between accessions were established based on the presence or absence of polymorphic DArT markers and phylogenetic relationships determined by Neighbour-joining (NJ) cluster analysis. The NJ tree was divisible into a major cluster comprising of all the breeding lines, progenies, 'Mpologoma' (AAA) and 'Calcutta-4' (AA) and a minor cluster consisting of '*Musa balbisiana'* and three landraces, all with a 'B' genome. It also allowed to identify sub-clusters of uniquely and genetically related *Musa* progenies. Principal component analysis (PCA) was consistent with NJ tree clustering. PCA also predicted that 'NM101F1', 'MNK-17-11' and 'MNK-17-12', were more related to their male parent, while the rest of the progenies were more related to the female parent. Thus, 'NM101F1', 'MNK-17-11' and 'MNK-17-12', might be carriers of favourable drought tolerance alleles from their tolerant male parent. Analysis of molecular variance revealed higher (88%) and lower (12%) genetic variation within and between the gene pools, respectively. Understanding the genetic relations of the progenies will be valuable in providing insight into the genetic diversity underlying the phenotypic diversity, especially regarding their responses to water stress.

Keywords: banana, cluster analysis, Diversity Arrays Technology markers, genetic diversity, *Musa* progenies, water stress

6.1 Introduction

Banana (*Musa* spp.) is the topmost grown fruit crop in the world and the eighth most important energy source in the least-developed countries (FAOSTAT, 2021). In Africa, banana is a key income source for millions of low-income rural farmers. The East and Central African countries collectively produce about 12.4 million tonnes of bananas per annum, valued at US$ 6.5 billion (FAOSTAT, 2021) and the crop is predominantly cultivated by smallholder farmers.

The average yield for bananas in the East African Great Lakes (EAGL) region is very low (<30 t ha^{-1} $year^{-1}$) compared to the potential average of >70 t ha^{-1} $year^{-1}$ (Tushemereirwe et al., 2001; van Asten et al., 2005). The low productivity of bananas is attributed to a complex of biotic and abiotic production constraints (Ocan et al., 2008; Ravi et al., 2013; Tumuhimbise et al., 2018), which threaten the sustainability of the crop's production in the region (Swennen et al., 2013). Drought is the leading abiotic stress factor affecting banana production in the EAGL region (Taulya et al., 2006; Uwimana et al., 2021; Nansamba et al., 2022), with estimated yield decreases of about 8% for every 100 mm reduction in water (van Asten et al., 2011). Therefore, this necessitates the need to develop cultivars that are drought-tolerant to improve productivity. The successful development and release of improved varieties greatly depend on appropriate breeding strategies and adequate genetic diversity within existing banana germplasm collections.

Genetic variation within domesticated edible bananas is derived from two main progenitors, *Musa acuminata* Colla (*M. acuminata*) ('A' genome) and *Musa balbisiana* Colla (*M. balbisiana*) ('B' genome), which are believed to have originated from Southeast Asia and Melanesia region (Simmonds, 1962; Waite, 1963; Lebot, 1999). From there, bananas were spread worldwide, including tropical and subtropical regions, where the crop has acquired great importance. Further inter- and intraspecific hybridizations and somatic mutations gave rise to a wide range of edible cultivars with varying genome groups, including diploids (AA, AB), and triploids (AAA, BBB, AAB, ABB) (De Langhe and de Maret, 1999; Ball et al., 2005; De Langhe et al., 2010). *M. acuminata* ('A' genome) is known to have better fruit and taste quality attributes, whereas *M. balbisiana* ('B' genome) contains several biotic and abiotic stress tolerance traits (D'Hont et al., 2012; Wang et al., 2019; van Wesemael et al., 2019).

The banana cropping system in the EAGL region is dominated by East African highland bananas (EAHB, AAA genome), which have evolved through numerous somatic mutations since their introduction, thereby increasing their diversity and number. In fact, the EAGL region is Africa's largest banana producer, with over 80 local varieties grown by smallholder farmers (Ssali, 2016). For instance, a typical banana farm in Uganda will constitute about 8 to 12 varieties (Karamura et al., 1996; Chapter 3). To this end, the East African highlands are regarded as a secondary centre of diversity for the EAHBs (Simmonds, 1966). Therefore, germplasm from this region complements the existing *Musa* gene pools and provides essential genetic diversity for banana breeding.

Knowledge of plant genetic diversity and the association between the phenotype and genetic markers is critical for crop improvement. Diversity studies in banana utilize both molecular and morphological markers (Sardos et al., 2016a; Nyine et al., 2017; Hinge et al., 2022). Nonetheless, morphological markers are greatly impacted by environmental variance, which lowers the selection efficiency during the development of improved cultivars. Conversely, molecular markers are stable and not affected by environmental conditions since their determination is largely automated. A wide range of molecular markers including random amplified polymorphic DNA (RAPDs) markers (Pillay et al., 2001), restriction fragment length polymorphism markers (RFLPs) (Carreel et al., 2002; Raboin et al., 2005), amplified fragment length polymorphism (AFLP) (Ahmad et al., 2014), microsatellites/ simple sequence repeats (SSRs) (Perrier et al., 2009; Irish et al., 2014; Karamura et al., 2016; Milton et al., 2022) have been used to study and characterize banana diversity. Diversity array technology (DArT) markers are also widely used (Jaccoud et al., 2001; Risterucci et al., 2009; Sardos et al., 2016b), given that whole-genome profiling is possible without prior knowledge of sequence information. Furthermore, DArT markers have been utilized in other banana research applications, including genetic mapping studies (Hippolyte et al., 2010; Mbanjo et al., 2012; D'Hont et al., 2012; Uwimana et al., 2024). Banana drought tolerance breeding programs in the East African highlands can benefit from analysing genetic diversity within the gene pools using DArT markers. DArT-based SNP markers not only provide an opportunity to assess the genetic diversity among EAHB populations and exotic banana germplasm (Sardos et al., 2016b; Nyine et al., 2019) but also offer sufficient variation to distinguish between closely

related genotypes. The potential of these DArT markers toward banana drought tolerance improvement is yet to be fully explored.

Understanding the association between the genotype and phenotype is crucial for identifying candidate genotypes with favourable alleles that are correlated to drought tolerance and identifying quantitative trait loci (QTLs) linked with drought tolerance traits. For instance, the polygenic nature of drought tolerance has been confirmed in well-investigated crops including rice (Barik et al., 2019; Ghazy et al., 2024), wheat (Gupta et al., 2017; Kumar et al., 2020; Schierenbeck et al., 2023), maize (Hao et al., 2010; Neiff et al., 2023), beans (Mukeshimana et al., 2014; Diaz et al., 2020; Mutari et al., 2023) and groundnut (Ravi et al., 2011; Pandey et al., 2020). On the other hand, for bananas, only one recent study (Sampangi-Ramaiah et al., 2023) has so far reported five major QTLs (on chromosomes 2, 5 and 8) for three drought tolerance-related traits, including leaf cuticular wax, adaxial stomatal density and leaf water retention capacity. Thus, although a great wealth of information on general families, pathways and putative candidate genes involved in alleviating the negative impacts of drought or osmotic stress in banana exists (Davey et al., 2009; Feng et al., 2015; Mattos-Moreira et al., 2018; Jangale et al., 2019; Brown et al., 2020), knowledge of the transfer of those favourable genes/ alleles into banana hybrids and genotype-phenotype associations (regarding drought tolerance) in the progenies is still lacking.

The objective of this study was to determine the genetic relationships and genetic diversity of 55 banana genotypes using DArT-based SNP markers. We zoom into the genetic relationships of the two parental genotypes and their progenies that were evaluated for their response to water deficit conditions in the subsequent thesis chapter (Chapter 7). This information will shed some light on the genetic diversity underlying the phenotypic variation among these progenies, particularly their performance under water stress conditions.

6.2 Materials and methods

6.2.1 Plant materials and ploidy level estimation

A population of 55 banana genotypes comprising of nine breeding lines (five diploids and four tetraploids), 38 progenies generated by crossing '917K-2' (female parent) and 'TMB2x9172' (male parent) (Chapter 5), two wild *Musa* species, and six landraces was used (Table 6.1). The genome constitutions and ploidy levels of the wild *Musa* species, landraces and the diploid breeding lines were retrieved from the *Musa* Germplasm Information System (MGIS) (https://www.crop-diversity.org/mgis/accession-search) while the ploidy levels of the tetraploid breeding lines were obtained from Batte et al. (2020). Further, a recent genetic diversity study by Akech et al. (2024) reported the genome composition of the improved diploid, 'TMB2x9172' to be 'AA'. The ploidy levels of the 38 progenies were estimated following the flow cytometry method (Doležel and Bartoš, 2005; Doležel et al., 2007). About 30 mg of fresh leaf tissue (from the middle of the youngest leaf) was finely cut with a razor blade in a petridish containing 0.5 ml of ice-cold OTTO I solution (0.5% v/v Tween 20, 0.1 M citric acid). The homogenate was then mixed and filtered through a 30 µm membrane of the Partec CellTrics® filter into a cuvette to remove the leaf debris. As an internal reference, chicken blood chicken red blood cell nuclei (CRBC), prepared in accordance with Galbraith et al. (1998), were added to the solution of banana nuclei. The solutions were then incubated for about 30 minutes at room temperature, after which 1 ml of OTTO II buffer (0.4 M Na_2-HPO_4) mixed with 2 µg/ml DAPI (4,6-diamidino-2-phenylindole) were added. The samples were analysed using the Cyflow ploidy analyser (Sysmex Partec GmbH, Goerlitz, Germany). The ploidy level of each progeny was then estimated by comparing peak positions of progeny nuclei and CRBC. The ratio between the G1-phase nuclei of the progeny accessions and relative DAPI fluorescence intensity of CRBC nuclei is approximately 0.5 and 0.75 for diploid and triploid plants, respectively (Doležel et al., 2007; Christelová et al. 2017).

Table 6.1 *Musa* spp. genotypes used in the study.

Genotype ID	Genome	Type	Pedigree
1201K-1	AAAA	Breeding line	Nakawere x Calcutta-4
917K-2	AAAA	Breeding line	Enzirabahima x Calcutta-4
660K-1	AAAA	Breeding line	Enzirabahima x Calcutta-4
222K-1	AAAA	Breeding line	Nfuuka x Calcutta-4

Genotype ID	Genome	Type	Pedigree
TMB2x6142-1	AA	Breeding line	Nyamwihogora x Long Tavoy
TMB2x9128-3	AA	Breeding line	Tjau lagada x Pisang Lilin
TMB2x8075-7	AA	Breeding line	SH 3362 x Calcutta-4
TMB2x9722-1	Unknown	Breeding line	Bobby Tannap x Calcutta-4
TMB2x9172	AA	Breeding line	Obino L'Ewai x Calcutta-4
NM101F1	Unknown	Progeny	917K-2 x TMB2x9172
NM201F1	Unknown	Progeny	917K-2 x TMB2x9172
NM202F1	Unknown	Progeny	917K-2 x TMB2x9172
NM203F1	Unknown	Progeny	917K-2 x TMB2x9172
NM601F1	Unknown	Progeny	917K-2 x TMB2x9172
NM602F1	Unknown	Progeny	917K-2 x TMB2x9172
NM603F1	Unknown	Progeny	917K-2 x TMB2x9172
NM604F1	Unknown	Progeny	917K-2 x TMB2x9172
NM801F1	Unknown	Progeny	917K-2 x TMB2x9172
NM802F1	Unknown	Progeny	917K-2 x TMB2x9172
NM803F1	Unknown	Progeny	917K-2 x TMB2x9172
NM804F1	Unknown	Progeny	917K-2 x TMB2x9172
NM805F1	Unknown	Progeny	917K-2 x TMB2x9172
NM806F1	Unknown	Progeny	917K-2 x TMB2x9172
NM807F1	Unknown	Progeny	917K-2 x TMB2x9172
NM808F1	Unknown	Progeny	917K-2 x TMB2x9172
NM809F1	Unknown	Progeny	917K-2 x TMB2x9172
MNK-16-1*	Unknown	Progeny	917K-2 x TMB2x9172
MNK-16-2*	Unknown	Progeny	917K-2 x TMB2x9172
MNK-16-3*	Unknown	Progeny	917K-2 x TMB2x9172
MNK-16-4*	Unknown	Progeny	917K-2 x TMB2x9172
MNK-16-5*	Unknown	Progeny	917K-2 x TMB2x9172
MNK-16-6*	Unknown	Progeny	917K-2 x TMB2x9172
MNK-16-8*	Unknown	Progeny	917K-2 x TMB2x9172
MNK-16-16*	Unknown	Progeny	917K-2 x TMB2x9172
MNK-17-4*	Unknown	Progeny	917K-2 x TMB2x9172
MNK-17-5*	Unknown	Progeny	917K-2 x TMB2x9172
MNK-17-6*	Unknown	Progeny	917K-2 x TMB2x9172
MNK-17-7*	Unknown	Progeny	917K-2 x TMB2x9172
MNK-17-9*	Unknown	Progeny	917K-2 x TMB2x9172
MNK-17-10*	Unknown	Progeny	917K-2 x TMB2x9172
MNK-17-11*	Unknown	Progeny	917K-2 x TMB2x9172
MNK-17-12*	Unknown	Progeny	917K-2 x TMB2x9172
NM8011F1	Unknown	Progeny	917K-2 x TMB2x9172
NM8012F1	Unknown	Progeny	917K-2 x TMB2x9172
NM8013F1	Unknown	Progeny	917K-2 x TMB2x9172
NM9015F1	Unknown	Progeny	917K-2 x TMB2x9172
NM9017F1	Unknown	Progeny	917K-2 x TMB2x9172

Genotype ID	Genome	Type	Pedigree
Calcutta-4	AA	Wild species	-
Musa balbibisiana	BB	Wild species	-
Mpologoma	AAA	Landrace	-
Sukali Ndiizi	AAB	Landrace	-
Vunapope	AB	Landrace	-
Monthan	ABB	Landrace	-
Kunnan	AB	Landrace	-

6.2.2 DNA extraction and Diversity Array Technology sequencing

Genomic DNA of the 55 banana genotypes was extracted from lyophilised (freeze-dried) leaf samples of three-month-old tissue culture-derived plantlets following the modified cetyl trimethylammonium bromide (CTAB) protocol from Doyle and Doyle (1990). An agarose gel electrophoresis was used to determine the quality of each DNA sample and the concentration estimated using a NanoDrop 2000 spectrophotometer (ND-2000 V3.5, NanoDrop Technologies, Inc.). The DNA samples were stored in a 96-well microtiter plate and shipped to Diversity Arrays Technology Pty Ltd., Canberra, Australia (DArT P/L), for destructive analysis.

6.2.3 Genotyping of *Musa* spp. genotypes using DArT-seq™ technology

The DNA samples were genotyped with a set of 26,363 silicoDArTs using the DArTseq genotype-based sequencing protocol. Polymorphic markers were identified and scored using DArTsoft v.7.4 software (DArT P/L, Canberra, Australia, 2014). The markers were evenly spread across all the 11 chromosomes of *Musa* spp. and given a score of '1' for presence and '0' if the genomic representation restriction fragment corresponding to the DArT probe was absent. The silicoDArTs used had reproducibility value of 1.0, polymorphic information content ranging between 0.03 and 0.5 and the mean call rate of 0.94, ranging from 0.8 to 1.0.

6.2.4 SNP calling and filtering

After demultiplexing, each of the raw fastq file was checked using FASTQC software, followed by cleaning to remove low-quality ends (Phred score > 20) and Illumina adapter

sequences with Cutadapt (Martin, 2011). After trimming, reads smaller than 30 bp were removed. The remaining reads were then mapped to the *Musa acuminata,* double haploid 'Pahang' genome v2 (D'Hont et al., 2012) obtained from the Banana Genome Hub (Droc et al., 2013) using BWA-MEM (Li and Durbin, 2010). Re-alignment was done using the IndelRealigner module from GATK v4.1 (McKenna et al., 2010). The GATK pipeline recommended for a non-model organism was then followed by adding the recalibration step. This procedure involved the following steps: conducting an initial round of SNP calling on the original uncalibrated data, choosing SNPs with the highest confidence and recalibrating the bases on the original aligned read files. For duplicate samples, the recalibrated bam alignment files were then merged using Sambamba software (Tarasov et al., 2015). SNPs and indel calling were then performed with the GATK module HaplotypeCaller v4.1. Finally, a script gVCF2vcf-gz.pl was written to combine the individual gVCF files obtained into a single VCF file. The snpcluster procedure was used to process SNP clusters, set for a threshold of three or more SNPs per 10 bp window. The pipeline used to conduct those analyses is available at https://github.com/CathyBreton/Genomic_Evolution.

The optimized Genotype Investigator for Genome-Wide Analyses (GIGWA) tool v2.6.1 (Sempéré et al., 2019) was used to eliminate markers with minor allele frequency (MAF) < 0.05, missing data of greater than 10%, and non-biallelic SNP markers. Genotypes exhibiting a read depth < 10 were set to missing while SNP loci located on putative mitochondrion were removed. After filtering, a total of 1551 SNP markers distributed across the 11 chromosomes were retained and used in subsequent genetic relationships and diversity analyses.

6.2.5 Data analysis

6.2.5.1 Genetic relationships

To enable combined analysis of ploidies (2x, 3x and 4x) and reduce complexity of data analysis, the genotypic data was coded as a binary absence ('0') and presence ('1') matrix. Using DARwin 6 program (Perrier and Jacquemoud, 2006; Perrier et al., 2009), a dice index was used to calculate the dissimilarities for all possible pairs of genotypes on the set of markers with less than 10% missing data for the two genotypes. As done in other studies

(Sardos et al., 2018; Perrier et al., 2019), a diversity tree was built using the weighted Neighbour-Joining (NJ) algorithm (Saitou and Nei, 1987) under the topological constraint of an NJ tree generated on the diploid genotypes and tree rooting based on the cluster containing genotypes with a known '*balbisiana'* ('B') genome as an outgroup. The 'influential unit detection' tool in DARwin software was used to check the robustness of the generated NJ tree. The 'influential unit detection' function is a procedure which removes one of the genotypes and then the resulting partial tree is compared with the whole tree in order to deduce the contribution of that genotype to the structure of the entire tree. A high contribution suggests a questionable genotype given that a completely different structure is obtained after removing it. We further zoomed into the genetic relationships between 16 *Musa* progenies and their two parental genotypes including '917K-2' (female) and 'TMB2x9172' (male). These progenies were phenotyped for drought in Chapter 7 and are highlighted with an asterisk (*) in Table 6.1.

Principal component analysis (PCA) was performed in STATISTICA version 14.0 (TIBCO Statistica) and plotted in ggplot2 package (version 3.4.4) (Wikham, 2016) implemented in R version 4.2.2 software.

6.2.5.2 Genetic diversity and differentiation parameters

The proportion of polymorphic loci (P) and expected heterozygosity (H_e) were calculated with GenAlEx software, version 6.5 (Peakall and Smouse, 2012). The minor allele frequency (MAF), inbreeding coefficients (F_{IS}) and observed heterozygosity (H_o) were estimated with R packages, "adegenet" (Jombart and Ahmed, 2011), "hierfstat" (Goudet, 2005) and "dartR" (Mijangos et al., 2022), respectively. To conduct analysis of molecular variance (AMOVA), the test genotypes were first grouped based on the gene pool as either wild species, landrace, progenies or breeding line. AMOVA was then performed using the GenAlEx software, version 6.5 (Peakall and Smouse, 2012) to partition the molecular variance among and within *Musa* spp. gene pools. The similarity of pairwise genotypes from the whole test collection was determined using the genetic differentiation parameter (PhiPT).

6.3 Results

6.3.1 Ploidies of *Musa* progenies

Ploidy analysis of the study's progenies resulted in a combination of two ploidy levels. Out of the 38 progenies, three including 'MNK-17-11', 'MNK-17-12' and 'NM101F1' were diploid (2x). The remaining 35 progenies were triploid (3x) (Appendix 6.1).

6.3.2 Diversity tree

An NJ tree generated from the dissimilarity matrix revealed two major clusters, I and II (Figure 6.1). The first large cluster (Cluster I) further branched into three sub-clusters (SC I, II and III). At the top of the tree was the largest sub-cluster (SC I, consisting of 48% of the population) grouping most of the progenies used in this study. All the progenies in this cluster were triploids. Within this cluster of only progenies were four sub-clusters, evidencing greater genetic similarity within specific sets of progenies.

The second sub-cluster (SC II), linked to the first large sub-cluster, comprised a mixture of four breeding lines (all tetraploid), an edible EAHB landrace ('Mpologoma'), and nine progenies. The grouping of 'Mpologoma' with the four tetraploid breeding lines ('1201K-1', '660K-1', '917K-2' and '222K-1') in the same cluster was expected since that these breeding lines are progenies of known EAHBs. Among the progenies, 'NM202F1' and 'NM603F1' clustered the closest to the female parent, '917K-2'.

The third sub-cluster (SC III) consisted of nine genotypes (all diploids) including all the diploid breeding lines, 'Calcutta-4' (a wild *Musa* species) and three *Musa* progenies, 'MNK-17-11', 'MNK-17-12' and 'NM101F1'. These progenies had the closest genetic proximity with the male parent ('TMB2x9172') compared to the rest of the 35 progenies.

Cluster II contained both diploid and triploid genotypes, all with a known 'B' genome (i.e. have either one or two 'B' copies). Four out of the five genotypes in this main group are landraces from different geographical origins, and one is a wild *Musa* species ('*M. balbisiana*').

Within the landraces, the dissimilarities ranged between 0.16 to 0.66. ‘Kunnan’ and ‘Sukali Ndiizi’ were the most identical while ‘Mpologoma’ and ‘Monthan’ were the most distant i.e had the highest dissimilarity. In fact, ‘Mpologoma’, an EAHB (AAA), was the most distantly isolated from the other four landraces, all of which carry at least one copy of the ‘B’ genome. The dissimilarities between ‘Mpologoma’ and the rest of the landraces ranged between 0.58 and 0.66. On the other hand, the dissimilarity between the two wild species, ‘Calcutta-4’ and ‘*M. balbisiana*’ was 0.81 while the dissimilarity between the male (‘TMB2x9172’) and female (‘917K-2’) parental genotypes of the progenies was 0.54.

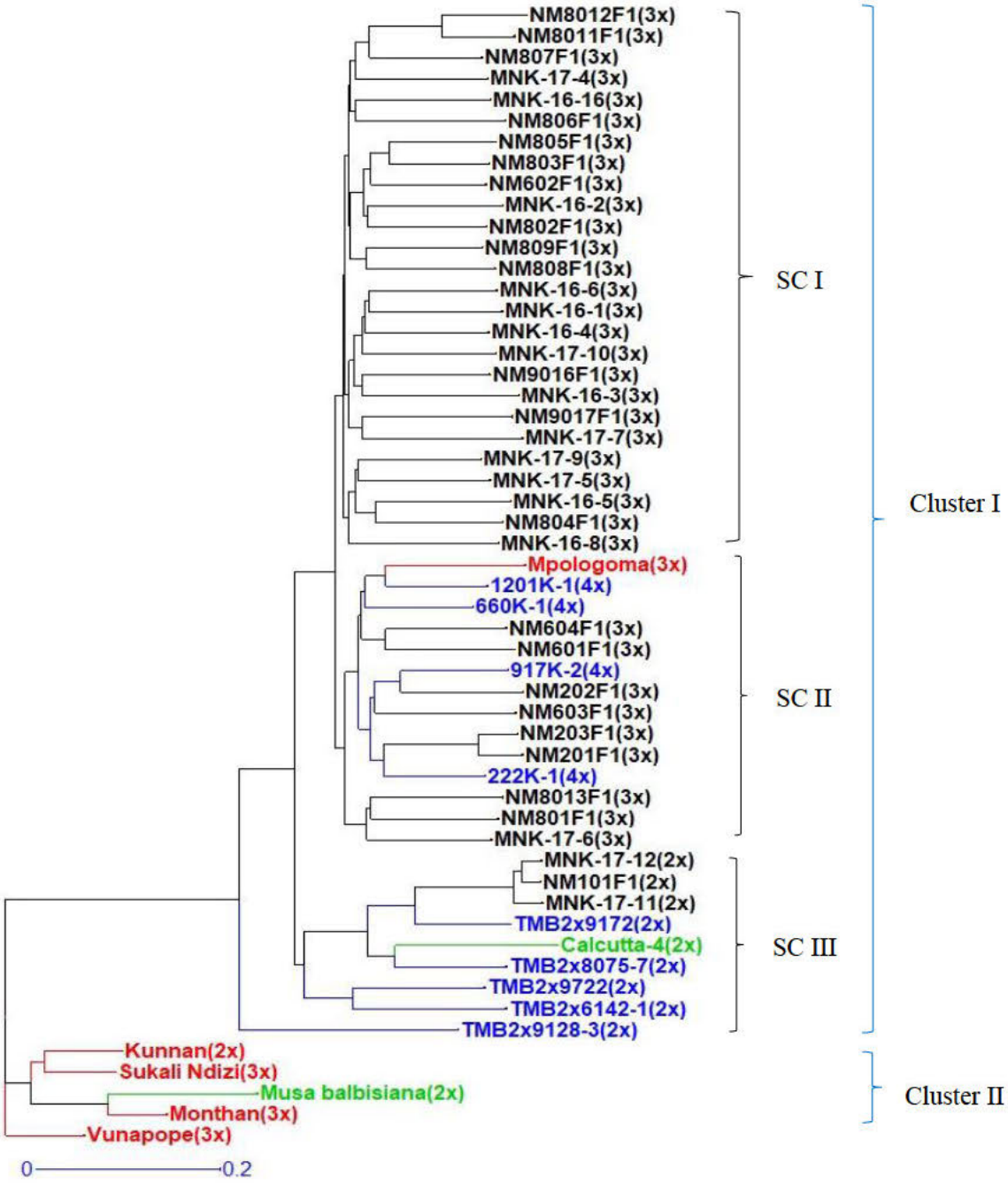


Figure 6.1 Weighted Neighbor-joining tree of 54 banana genotypes built under the topological constraint of the diploid genotypes and based on 1551 high quality DArT-based SNP markers. 2x, 3x and 4x (in brackets) represent diploid, triploid and tetraploid statuses, respectively. Breeding lines, landraces, progenies and wild *Musa* species are coloured in blue, red, black and green, respectively.

A closer look at the genetic relationships between 16 *Musa* progenies revealed that 14 progenies grouped together with the female parent ('917K-2') (Cluster 1) while two

progenies, ‘MNK-17-11’ and ‘MNK-17-12’ clustered in a small group with their diploid male parent, ‘TMB2x9172’ (Cluster II) (Figure 6.2). Cluster I was further divided into smaller subgroups. For instance, four progenies, ‘MNK-16-1’, ‘MNK-16-4’, ‘MNK-16-6’ and ‘MNK-17-10’ clustered together while ‘MNK-16-2’, ‘MNK-16-16’ and ‘MNK-17-4’ also grouped together. ‘MNK-16-5’, ‘MNK-17-5’, ‘MNK-17-6’ and ‘MNK-17-9’ clustered in a separate subgroup. ‘MNK-16-8’, ‘MNK-16-3’ and ‘MNK-17-7’ clustered individually and independently from the rest of the subgroups in cluster I.

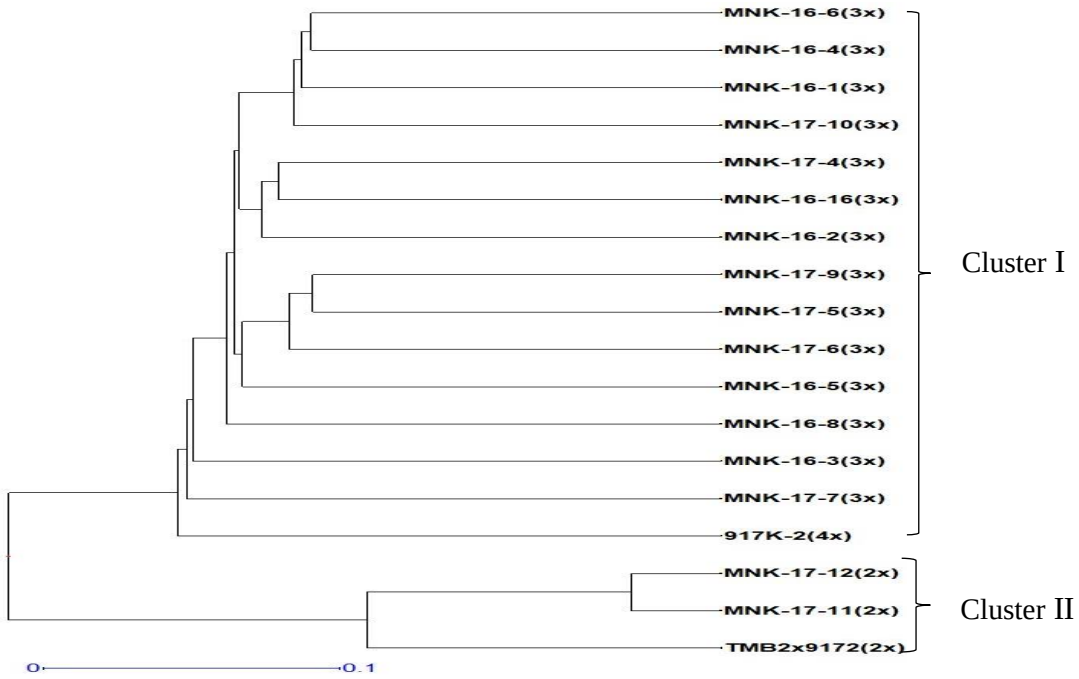


Figure 6.2 Genetic relationships of 16 *Musa* spp. progenies and their two parental genotypes, ‘917K-2’ (female) and ‘TMB2x9172’ (male). 2x, 3x and 4x represent diploid, triploid and tetraploid statuses, respectively.

6.3.3 Principal component analysis

PCA showed that genetic diversity exists in the study’s *Musa* spp. genotypes. The first two components accounted for 29.0% of the cumulative variation (Figure 6.3). ‘*M. balbisiana*’ (a wild *Musa* species) and four landraces, including ‘Monthan’, ‘Sukali Ndiizi’, ‘Vunapope’ and ‘Kunnan’) showed distinct grouping from the rest of the genotypes. The

PCA biplot also showed that almost all the progenies clustered closely with their female parent ('917K-2') except for 'MNK-17-11', 'MNK-17-12' and 'NM101F1', which clustered together and had the closest genetic distance to the male parent ('TMB2x9172'). The results are consistent with the NJ tree. PC 1 significantly correlated to '*balbisiana'* related markers (r = 0.97, $p < 0.01$) (Figure 6.4A) while PC 2 significantly correlated to 'Calcutta-4' specific markers (r = 0.67, $p < 0.01$) (Figure 6.4B). Of the 1551 markers used, 220 and 285 markers were specifically associated with '*M. balbisiana*' and 'Calcutta-4', respectively (Appendix 6.2). Among the progenies, 'MNK-17-11', 'MNK-17-12' and 'NM101F1' had the highest number of '*M. balbisiana'* specific SNPs (ranging between 40 and 43), while 'NM8012' and 'MNK-17-4' had the lowest number (both having only three '*M. balbisiana'* specific SNPs) (Appendix 6.2). On the other hand, 'NM603F1' and 'NM806' had the highest and lowest number of 'Calcutta-4' specific markers.

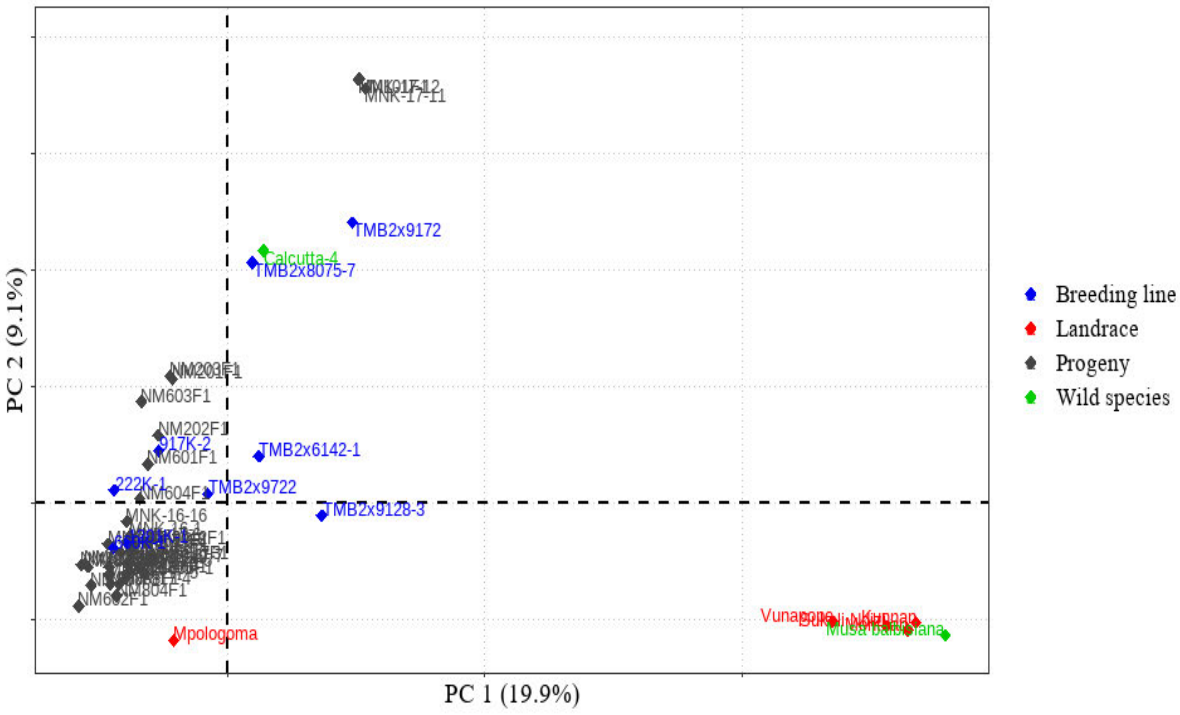


Figure 6.3 Genetic distance among 54 *Musa* spp. genotypes revealed by a principal component analysis (PCA) based on 1551 high quality DArT-based SNP markers.

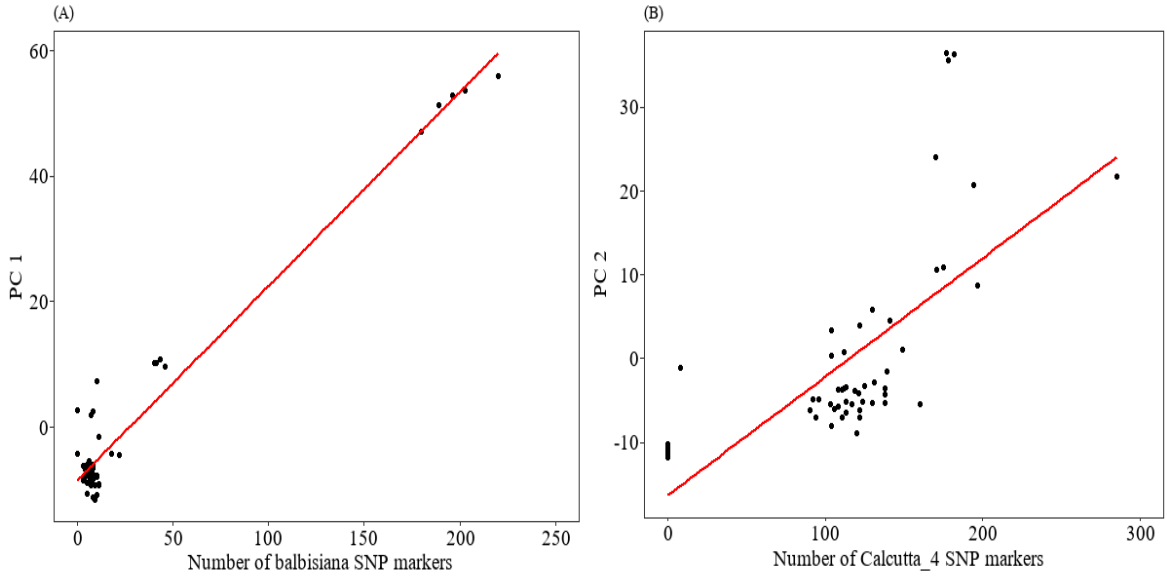


Figure 6.4 Correlation between (A) '*Musa balbisiana'* specific SNP markers and principal component 1 (PC 1) and (B) 'Calcutta-4' specific SNP markers and principal component 2 (PC 2). A significant strong positive correlation between the number of '*M. balbisiana'* specific SNP markers and PC 1 was observed (r = 0.97, $p < 0.01$). A significant positive correlation between the number of 'Calcutta-4*'* specific SNP markers and PC 2 (r = 0.67, $p < 0.01$) was observed.

6.3.4 Genetic differentiation among and within populations

The minor allele frequency (MAF) ranged from 0.01 to 0.5 while the proportion of polymorphic loci ranged between 0.37 and 0.85, with a mean of 0.65. The average expected heterozygosity was 0.24 and varied from 0 to 0.57 while the observed heterozygosity ranged between 0.34 and 0.37, with a mean value of 0.36 (Table 6.2). The overall inbreeding coefficient of the test population was -0.45.

The AMOVA revealed that the variation among gene pools accounted for 12%, while the variation within the gene pools accounted for 88% of the total variation (Table 6.3). The overall PhiPT was 0.124, associated with a permutation p-value < 0.05. The maximum PhiPT and Phi'PT values were 0.595 and 0.208, respectively.

Table 6.2 Genetic diversity indices of the 55 test *Musa* spp. genotypes based on SNP markers

Parameter	MAF	P (%)	H_e	H_o
Minimum	0.01	0.37	0.00	0.34
Maximum	0.5	0.85	0.57	0.37
Mean	0.12	0.65	0.24	0.36

MAF – minor allele frequency, P – proportion of polymorphic loci, H_e – expected heterozygosity, H_o - observed heterozygosity

Table 6.3 Analysis of molecular variance of *Musa* spp populations based on 1551 SNP markers

Source	df	SS	MS	Est.Var.	% Variance
Among gene pools	3	2230.08	743.36	43.24	12%
Within gene pools	55	16804.94	305.54	305.54	88%
Total	58	19035.02		348.79	100%

df = degrees of freedom, SS = sum of square, MS = mean square, Est. Var. = estimated variance, gene pools = wild species, landrace, F1 progeny or breeding lines

6.4 Discussion

6.4.1 Genetic relationships and structure among genotypes

6.4.1.1 Neighbour joining tree

The study population was majorly delineated into two distinct clusters based on the genetic distance calculated between all possible pairs of genotypes using the dice dissimilarity index (Figure 6.1). The two major clusters consisted of one large group (Cluster I) with genotypes solely containing '*acuminata*' ('A') genome (except for 'TMB2x9722-1' whose genome constitution is unknown) and a second smaller cluster (Cluster II) comprising accessions with a known 'B' genome. This grouping is expected given that the most genetic variability within bananas is predominantly derived from two wild major progenitors, *M. acuminata* Colla ('A' genome) and *M. balbisiana* Colla ('B' genome), which are believed to have originated from Southeast Asia and Melanesia regions (Simmonds, 1962; De Langhe et al., 2009; Perrier et al., 2011; Sardos et al., 2022). The grouping of genotypes

with similar genomic backgrounds into the same major cluster indicates the effectiveness of the SNP markers utilized in this study in allocating the test *Musa* spp. genotypes into homogenous clusters.

The study population consisted of wild *Musa* species, breeding lines, landraces and *Musa* progenies. Although the genome composition of the 38 progenies is yet to be established, the rest of the studied genotypes contain either the 'A', 'B' or both genomes (Table 6.1). Still, the genome constitution of the progenies can be presumed to be solely based on the 'A' genome given that both their parental genotypes i.e., '917K-2' (AAAA) and 'TMB2x9172' (AA), contain only the 'A' genome. A recent genetic diversity study by Akech et al. (2024) reported 'TMB2x9172' to be a diploid hybrid with an 'AA' genome constitution.

The joint grouping of the progenies, breeding lines (diploids and tetraploids), 'Mpologoma' (an EAHB landrace) and 'Calcutta-4' (in cluster I) could be interpreted as the existence of very similar genetic backgrounds resulting from at least one common ancestor in the pedigree of the genotypes. For instance, all the four tetraploid breeding lines (also including '917K-2', the female parent of the progenies) were developed from crosses between EAHB landraces and 'Calcutta-4' (Chapter 5; Table 5.1). Additionally, the diploid breeding line 'TMB2x6142-1' is an offspring of 'Nyamwihogora', which is also an EAHB landrace. EAHBs (a *Musa* sub-group to which 'Mpologoma' belongs) have been shown to have high genetic homogeneity despite having extensive morphological variation (Christelová et al., 2017; Pillay, 2024). Further, 'Calcutta-4' is the male parent of three diploid breeding lines in this major cluster including 'TMB2x9172', 'TMB2x8075-7' and 'TMB2x9722-1'. Consequently, 'Calcutta-4' is the 'grandfather' of all the 38 *Musa* progenies since both their parental genotypes are offsprings of 'Calcutta-4'. Tetraploid *Musa* spp. breeding lines have been widely used in banana improvement programs to develop secondary hybrids with enhanced pest and disease resistance as well as improved banana yields (Brown et al., 2017). For example, '917K-2', '660K-1', '222K-1' and '1201K-1' were used to generate triploid NARITA hybrids with enhanced resistance to black Sigatoka, banana weevil and the burrowing nematodes (Tushemereirwe et al., 2015; Madalla et al., 2022a; 2022b). Tumuhimbise et al. (2018; 2019) also reported that one of the parents to the NAROBan hybrids was an improved tetraploid, thereby confirming the

extensive utilization of these breeding lines in banana improvement. On the other hand, the diploid breeding lines have been used as important sources of resistance to several banana pests and diseases and for investigating the genetic basis of resistance of those biotic stresses (Kiggundu et al., 2003; Tenkouano et al., 2003; Ssali et al., 2013; Kimunye et al., 2021).

It is crucial to note that among the *Musa* progenies, 'NM101F', 'MNK-17-11' and 'MNK-17-12' clustered the closest with the male parent ('TMB2x9172'), suggesting high genetic similarity between 'TMB2x9172' and the above three progenies. 'TMB2x9172' was reported to be relatively tolerant to water stress conditions (Chapter 4), and hence 'NM101F', 'MNK-17-11' and 'MNK-17-12' might be suitable hosts of favourable alleles for drought tolerance. The clustering of majority of the progenies in Cluster I, SC I, points to high genetic uniformity among these progenies and even greater genetic uniformity within the smaller sub-groups. The sub-clustering of different progenies suggests weak genetic dissimilarities and closer genetic relationships within the respective sub-clusters. For instance, zooming into the genetic relationships of 16 selected progenies (highlighted with asterisk in Table 6.1) revealed high genetic homogeneity between 'MNK-17-11' and 'MNK-17-12' (both diploids), while 'MNK-16-1', 'MNK-16-4', 'MNK-16-6' and 'MNK-17-10' were more genetically similar with each other (Figure 6.2). Other specific, close genetic relationships were observed among the remaining progenies that were part of this subset. The sub-clustering of these progenies points to high genetic homogeneity of all particular progenies belonging to the same sub-cluster. On the contrary, the separate and individual sub-grouping of 'MNK-16-8', 'MNK-16-3' and 'MNK-17-7' (Figure 6.2) suggests stronger genetic dissimilarities among these progenies and less genetic homogeneity with the other 13 progenies in this subset. Knowledge of the genetic relationships between these progenies is critical as it provides some insight into the genetic diversity underlying the phenotypic diversity (in terms of response to water stress), which will be investigated and discussed in the next chapter of this thesis (Chapter 7).

In Figure 6.1, all the genotypes in the second major group (Cluster II) contain the 'B' genome (Ruas et al., 2017), which has been underutilised in banana breeding given its association with endogenous banana streak virus sequences that become activated under appropriate conditions (Iskra-Caruana et al., 2014). The distinct clustering of this group

from Cluster I in the NJ tree reflects the ancient divergence of '*M. balbisiana*' from '*M. acuminata'*, an event believed to have occurred before the creation of present day cultivated triploids or diploids. A clear discrimination of '*M. balbisiana*' from the '*M. acuminata'* using DArT markers has also been shown in other studies (Sardos et al., 2016b). Four of the five genotypes that co-localized with '*M. balbisiana*' in this group are landraces with a sweet taste and are usually eaten raw. However, 'Vunapope' and 'Monthan' are also consumed in cooked form.

6.4.1.2 Principal component analysis

The first two PCs explained 29% of the genetic variation among the studied *Musa* genotypes, which suggests that adequate genotype discrimination requires a higher number of components. The reduction of the effectiveness of PCs may be attributed to the limited range of genetic diversity resulting from the inclusion of closely related genotypes (e.g. breeding lines, progenies), which constituted the largest proportion of the study population. Reduced effectiveness of PCs due to narrow genetic diversity has also been reported in other crops (Somta et al., 2009; Nkhata et al., 2020).

PCA confirmed the results of the NJ tree as it separated the '*balbisaina'* cluster from the solely based '*acuminata'* genotypes (Figure 6.3). The discrimination displayed by PC 1 correlates to some extent to Cluster II while PC 2 corresponds to Cluster I of the NJ tree ((Figure 6.1, Figure 6.2). Notably, three of the *Musa* progenies (including 'NM101F1', 'MNK-17-11' and 'MNK-17-12') were in close genetic proximity with the male parent, 'TMB2x9172', while the remaining 35 F1 genotypes clustered together with their female parent, '917K-2'. As such, the above observation suggests a close genetic resemblance of 'NM101F1', 'MNK-17-11' and 'MNK-17-12' with their male parent, while the rest of the progenies are more genetically identical to the female parent.

The 'B' genome has previously been linked to resistance alleles and drought tolerance in banana (Ekanayake et al., 1994; Bakry et al., 2009; Vanhove et al., 2012). Therefore, considering that the 'B' genome containing genotypes had the largest (highest positive) PC 1 values, it can be hypothesized that the higher the PC 1 value (more positive), the more correlation towards drought tolerance and the smaller the PC 1 value (more negative), the less tolerance to drought. The genetic prediction of this observation is that 'NM101F1',

‘MNK-17-11’ and ‘MNK-17-12’ might be more tolerant to water stress than the rest of the progenies which had close genetic proximity with the female parent (‘917K-2’) (Carpentier, personal communication, November 2023[4]).

Despite the genome constitutions of the parents of the progenies being known to be ‘AAAA’ (‘917K-2’, female) and ‘AA’ (‘TMB2x9172’, male), this study has shown that these progenies, together with their male parent carry some ‘balbisiana’ specific SNPs (Figure 6.4, Figure 6.2). This ‘balbisiana’ background, although limited compared to the ‘Calcutta-4’ background, was not surprising since ‘TMB2x9172’ is a plantain hybrid whose female parent, ‘Obino L’Ewai’, is an ‘AAB’. The predominant ‘Calcutta-4’ background was also expected considering that ‘Calcutta-4’ is a common parent of ‘917K-2’ and ‘TMB2x9172’ (Table 6.1). Based on the above results and estimated ploidy levels (Figure 6.2), this study classifies ‘TMB2x9172’, the male parent, and its progenies as AAs (for ‘NM101F1’, ‘MNK-17-11’ and ‘MNK-17-12’) or AAAs (all the remaining 35 progenies) with a little ‘B’ background. Based on the separate clustering of the EAHB landrace, ‘Mpologoma’ and ‘Calcutta-4’ (Figure 6.1, Figure 6.3) and complete absence of ‘Calcutta-4’ specific SNPs in ‘Mpologoma’ (Figure 6.2), the DArT markers used in this study seem to be robust in detecting *M. acuminata* subspecies. ‘Calcutta-4’is a *M. acuminata* ssp. *burmannica* accession while molecular characterization of the EAHB sub-cluster to which ‘Mpologoma’ belongs revealed that the genomic composition of these endemic bananas is a combination of mainly *M. acuminata* subsp. *banksii, M. acuminata* ssp. *zebrina,* and *M. schizocarpa* (Perrier et al., 2011; Němečková et al., 2018; Sardos et al., 2025).

6.4.2 Analysis of genetic diversity

Genetic diversity analyses are crucial in identifying individual accessions among distantly and closely related groups for the initiation of new crop improvement efforts. This study utilized 1,551 high-quality DArT-based SNP markers to elucidate the genetic diversity of

[4] Principal Scientist, Banana drought tolerance researcher at the Alliance of Bioversity International and International Center of Tropical Agriculture, Leuven, Belgium

54 *Musa* spp. genotypes. On average, the study population had moderate to high proportions of polymorphic loci, expected and observed heterozygosity levels (Table 6.2), as well as an excess of heterozygotes (F_{is} = -0.45). The excess heterozygosity estimated in the current study's population could be attributed to the large proportion of breeding lines and *Musa* progenies that were generated from deliberate crossbreeding. Excess levels of heterozygosity in banana could be attributed to three mechanisms: the vegetative mode of propagation of the species, accumulation of somatic mutations over a long period and prohibited or limited self-pollination due to unsynchronized development of the female and male flowers in the inflorescence (Ge et al., 2005; Navascués et al., 2010; Bona et al., 2019). Excess levels of heterozygosity as low as -1.0 have been reported in banana (Mertens et al., 2021; Kallow et al., 2021). The difference in excess heterozygosity levels between this study and previous studies could be attributed to the different *Musa* spp. germplasm assessed. While this study assessed a combination of breeding lines, *Musa* progenies, landraces and wild species, previous studies evaluated banana wild relatives from different ecologies.

The moderate genetic diversity observed (mean H_o of 0.36) suggests moderately wide genetic diversity within the study's population, thereby making it suitable for selecting complementary genotypes for crop improvement purposes. The lower H_e estimates found in this study are expected given that bananas are mostly naturally clonally propagated and hence, most of the crop's loci would be homozygous (Brown et al., 2017; Mertens et al., 2021). The low average MAF of 0.12 observed in this study for the entire population indicates that most of the test genotypes shared common alleles. Kitavi et al. (2016) reported an even lower mean MAF (0.05) based on SSR microsatellite markers in a collection of EAHB varieties. The large variation within the gene pools of the current study revealed by the AMOVA (Table 6.3) confirms the heterogeneous nature of the *Musa* spp. species (Sardos et al., 2016).

6.5 Conclusions

Genetic analysis based on DArT-based SNP markers revealed genetic variation among the studied *Musa* spp. genotypes. Neighbour-joining (NJ) clustering highlighted the genetic relationships among the different genotypes. Principal component analysis (PCA) results

were consistent with the NJ tree. PCA also partially grouped the progenies into two heterotic groups based on the genetic distances from their parental genotypes. Genetic prediction based on the PCA revealed that three of the *Musa* progenies, including 'NM101F1', 'MNK-17-11' and 'MNK-17-12', were the most genetically close to the drought-tolerant candidate male parent ('TMB2x9172'). In contrast, the rest of the progenies had closer genetic proximity to the female parent ('917K-2') and hence had higher genetic uniformity with the female parent, '917K-2'. 'NM101F1', 'MNK-17-11' and 'MNK-17-12', all diploids, might be carriers of favourable genes/ alleles inherited from their drought-tolerant male parent. The genetic diversity of the study population was affected by the presence of common ancestors. Nonetheless, the DArT-based SNP markers used distinguished the germplasm. This study lays a foundation for further exploration and validation of drought tolerance, particularly within the *Musa* progenies. Thus, the study's progenies should be evaluated under water stress conditions to confirm whether 'NM101F1', 'MNK-17-11' and 'MNK-17-12' are indeed drought-tolerant and the rest of the progenies are sensitive to drought.

References

Akech, V., T. Bengtsson, R. Ortiz, R. Swennen, B. Uwimana, et al. 2024. Genetic diversity and population structure in banana (*Musa* spp.) breeding germplasm. The Plant Genome. p.e20497.

Ahmad, F., R. Megia, and Y.S. Poerba. 2014. Genetic diversity of *Musa balbisiana* Colla in Indonesia based on AFLP Marker. HAYAT J. Biosci. 21:39–47.

Ball, T., L. Vrydaghs, I. Van Den Hauwe, J. Manwaring, and E. De Langhe. 2006. Differentiating banana phytoliths: wild and edible *Musa acuminata* and *Musa balbisiana*. J. Archaeol. Sci. 33:1228-1236.

Bakry, F., F. Carreel, C. Jenny, and J.P. Horry. 2009. Genetic improvement of banana, p.3-50, *In* Jain, S.M., and P.M. Priyadarshan, eds. Breeding plantation tree crops: tropical species. Springer, New York, USA.

Barik, S.R., E. Pandit, S.K. Pradhan, S.P. Mohanty, and T. Mohapatra. 2019. Genetic mapping of morpho-physiological traits involved during reproductive stage drought tolerance in rice. PLoS One. 14:e0214979.

Batte, M., R. Swennen, B. Uwimana, V. Akech, A. Brown, et al. 2019. Crossbreeding East African highland bananas: lessons learnt relevant to the botany of the crop after 21 years of genetic enhancement. Front. Plant Sci. 10:81.

Blum, A. 2010. Plant breeding for water-limited environments. Springer, London, UK.

Bona, A., U. Kulesza, and K.A. Jadwiszczak. 2019. Clonal diversity, gene flow and seed production in endangered populations of *Betula humilis* Schrk. Tree Genet Genomes. 15.

Brown, A., R. Tumuhimbise, D. Amah, B. Uwimana, M. Nyine, et al. 2017. Bananas and Plantains (*Musa* spp.), p.219-240, *In* H. Campos, and P.D.S. Caligari, eds. Genetic improvement of tropical crops. Springer International Publishing, Cham, Switzerland.

Brown, A., S.C. Carpentier, and R. Swennen. 2020. Breeding climate-resilient bananas, p.91-115, *In* Kole, C, ed. Genomic designing of climate-smart fruit crops. Springer International Publishing, Cham, Switzerland.

Carreel, F., D.G. De Leon, P. Lagoda, C. Lanaud, C. Jenny, et al. 2002. Ascertaining maternal and paternal lineage within *Musa* by chloroplast and mitochondrial DNA RFLP analyses. Genome 45:679-692.

Chari, F., and B.S. Ngcamu. 2022. Climate change and its impact on urban agriculture in Sub-Saharan Africa: A literature review. Environ. Socio-econ. Studies 10:22-32.

Christelová, P., E. De Langhe, E. Hřibová, J. Čížková, J. Sardos, et al. 2017. Molecular and cytological characterization of the global *Musa* germplasm collection provides insights into the treasure of banana diversity. Biodivers Conserv. 26: 801-824.

D'Hont, A., F. Denoeud, J.M. Aury, F.C. Baurens, F. Carreel, et al. 2012. The banana (*Musa acuminata*) genome and the evolution of monocotyledonous plants. Nature 488:213-217.

Davey, M., N. Graham, B. Vanholme, R. Swennen, S. May et al. 2009. Heterologous oligonucleotide microarrays for transcriptomics in a non-model species; a proof-of-concept study of drought stress in *Musa*. BMC Genome 10:436.

De Langhe, E., and P. de Maret. 1999. Tracking the banana: its significance to early agriculture, p. 377-396, *In* C. Godsen., and J. Hather, eds. The Prehistory of Food. Appetites for Change. Routledge, London and New York.

De Langhe, E., L. Vrydaghs, P. de Maret, X. Perrier, and T. Denham. 2009. Why bananas matter: An introduction to the history of banana domestication. Ethnobot Res. Appl. 7:165–177.

De Langhe, E., E. Hřibová, S. Carpentier, J. Doležel, and R. Swennen. 2010. Did backcrossing contribute to the origin of hybrid edible bananas?. Ann Bot. 106:849-857.

Diaz, S., D. Ariza-Suarez, P. Izquierdo, J.D. Lobaton, J.F. de La Hoz, et al. 2020. Genetic mapping for agronomic traits in a MAGIC population of common bean (*Phaseolus vulgaris* L.) under drought conditions. BMC genomics. 21:1-20.

Doležel, J., and J. Bartoš. 2005. Plant DNA flow cytometry and estimation of nuclear genome size. Ann Bot. 95:99–110.

Doležel, J., J. Greilhuber, and J. Suda. 2007. Estimation of nuclear DNA content in plants using flow cytometry. Nat Protoc. 2:2233–2244.

Doyle, J., and J.I. Doyle. 1990. A rapid total DNA preparation procedure for fresh plant tissue. Focus. 12:13-15.

Droc, G., D. Larivière, V. Guignon, N. Yahiaoui, D. This, et al. 2013. The banana genome hub. Database, bat035.

Ekanayake, I.J., R. Ortiz, and D.R. Vuylsteke. 1994. Influence of leaf age, soil moisture, VPD and time of day on leaf conductance of various *Musa* genotypes in a humid forest-moist savanna transition site. Ann. Bot. 74:173–178.

FAOSTAT. 2021. Food and Agriculture Organization of the United Nations Database of agricultural production. FAO Statistical Databases. http:www.fao.org/faostat/ Accessed 5 September 2023.

Feng, X., Z. Lai, Y. Lin, G. Lai, and C. Lian. 2015. Genome-wide identification and characterization of the superoxide dismutase gene family in *Musa acuminata* cv. Tianbaojiao (AAA group). BMC Genome. 16:1–16.

Galbraith, D.W., J. Doležel, G. Lambert, and J. Macas. 1998. Nuclear DNA content and ploidy analyses in higher plants, p. 1–7, *In* J.P. Robinson, ed. Current protocols in cytometry. Wiley, New York.

Ge, X.J., M.H. Liu, W.K. Wang, B.A. Schaal, and T.Y. Chiang. 2005. Population structure of wild bananas, *Musa balbisiana*, in China determined by SSR fingerprinting and cpDNA PCR-RFLP. Mol Ecol. 14:933–944.

Ghazy, M.I., S.A. El-Naem, A.G. Hefeina, A. Sallam, and S. Eltaher. 2024. Genome wide association study of rice diversity panel reveals new qtls for tolerance to water deficit under the Egyptian conditions. Rice. 17:29.

Goudet, J. 2005. Hierfstat, a package for R to compute and test hierarchical F-statistics. Mol. Ecol. Notes. 5:184-186.

Gupta, P.K., H.S. Balyan, and V. Gahlaut. 2017. QTL analysis for drought tolerance in wheat: present status and future possibilities. Agronomy, 7:5.

Hao, Z., X. Li, X. Liu, C. Xie, M. Li, et al. 2010. Meta-analysis of constitutive and adaptive QTL for drought tolerance in maize. Euphytica. 174:165-177.

Hinge, V.R., I.M. Shaikh, R.I. Chavhan, A.S. Deshmukh, R.M. Shelake, et al. 2022. Assessment of genetic diversity and volatile content of commercially grown banana (*Musa* spp.) cultivars. Sci. Rep. 12:7979.

Hippolyte, I., F. Bakry, M. Séguin, L. Gardes, R. Rivallan, et al. 2010. A saturated SSR/DArT linkage map of *Musa acuminate* addressing genome rearrangements among bananas. BMC Plant Biol. 10: 65.

Irish, B.M., H.E. Cuevas, S.A. Simpson, B.E. Scheffler, J. Sardos, et al. 2014. *Musa* spp. germplasm management: microsatellite fingerprinting of USDA–ARS national plant germplasm system collection. Crop Sci. 54: 2140–2151.

Iskra-Caruana, M., M. Chabannes, P.O. Duroy, and E. Muller. 2014. A possible scenario for the evolution of banana streak virus in banana. Virus Res. 186:155–162.

Jaccoud, D., K. Peng, D. Feinstein, and A. Kilian. 2001. Diversity arrays: a solid state technology for sequence information independent genotyping. Nucleic Acids Res. 29: e25.

Jangale, B.L., R.S. Chaudhari, A. Azeez, P.V. Sane, A.P. Sane, et al. 2019. Independent and combined abiotic stresses affect the physiology and expression patterns of DREB genes differently in stress-susceptible and resistant genotypes of banana. Physiol Plant. 165: 303–318

Jombart, T., and I. Ahmed. 2011. Adegenet 1.3-1: new tools for the analysis of genome-wide SNP data. Bioinformatics. 27: 3070-3071.

Kallow, S., B. Panis, D.T. Vu, T.D. Vu, J. Paofa, et al. 2021. Maximizing genetic representation in seed collections from populations of self and cross-pollinated banana wild relatives. BMC Plant Biol. 21:1-14.

Karamura, D., M. Kitavi, M. Nyine, D. Ochola, W. Ocimati, et al. 2016. Genotyping the local banana landrace groups of East Africa. Acta Hortic. 1114: 67–74.

Karamura, D.A., E.B. Karamura, and C.S. Gold. 1996. Cultivar distribution in primary banana growing regions of Uganda. MusAfrica. 9: 3-5.

Kiggundu, A., M. Pillay, A. Viljoen, C. Gold, W. Tushemereirwe, et al. 2003. Enhancing banana weevil (*Cosmopolites sordidus*) resistance by plant genetic modification: A perspective. Afr J. Biotechnol. 2:563-569.

Kimunye, J., E. Were, R. Swennen, A. Viljoen, and G. Mahuku. 2021. Sources of resistance to *Pseudocercospora fijiensis*, the cause of black Sigatoka in banana. Plant pathol. 70:1651-1664.

Kitavi, M., T. Downing, J. Lorenzen, D. Karamura, M. Onyango, et al. 2016. The triploid East African Highland Banana (EAHB) genepool is genetically uniform arising from a single ancestral clone that underwent population expansion by vegetative propagation. Theoret. Appl. Genet. 129:547-561.

Kumar, A., G. Saripalli, I. Jan, K. Kumar, P.K. Sharma, et al. 2020. Meta-QTL analysis and identification of candidate genes for drought tolerance in bread wheat (*Triticum aestivum* L.). Physiol. Mol Biol Plants. 26:1713-1725.

Lebot, V. 1999. Biomolecular evidence for plant domestication in Sahul. Genet Resour. Crop Evol. 46: 619–628.

Li, H., and R. Durbin. 2010. Fast and accurate long-read alignment with burrows-wheeler transform. Bioinformatics. 26: 589-595.

Madalla, N.A., C. Massawe, M. Shimwela, D. Mbongo, G. Kindimba, et al. 2022a. TARIBAN1, TARIBAN2, TARIBAN3, and TARIBAN4 ‘Matooke’ cooking banana cultivars for the Great Lakes Region of Africa. HortScience. 57:1588-1592.

Madalla, N.A., R. Swennen, A. F. Brown, C. Massawe, M. Shimwela, et al. 2022b. Yield stability of East African Highland cooking banana ‘Matooke’ hybrids. J. Amer. Soc. Hort. Sci. 147:334–348.

Martin, M. 2011. Cutadapt removes adapter sequences from high throughput sequencing reads. EMBnet.journal. 17: 10-12.

Mattos-Moreira, L.A., C.F. Ferreira, E.P. Amorim, C.P. Pirovani, E.M. de Andrade, et al. 2018. Differentially expressed proteins associated with drought tolerance in bananas (*Musa* spp.). Acta Physiol Planta 40:1–15.

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

McKenna, A., M. Hanna, E. Banks, A. Sivachenko, K. Cibulskis, et al. 2010. The genome analysis toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 20: 1297–1303.

Mertens, A., Y. Bawin, S. Vanden Abeele, S. Kallow, D. Toan Vu, et al. 2021. Genetic diversity and structure of *Musa balbisiana* populations in Vietnam and its implications for the conservation of banana crop wild relatives. PloS One. 16:p.e0253255.

Mijangos, J.L., O.F. Berry, C. Pacioni, and A. Georges. 2022. dartR v2: An accessible genetic analysis platform for conservation, ecology and agriculture. Methods Ecol. Evol. 13: 2150:2158.

Milton, A., D. Muhanguzi, A. Male, A. Kajubi, S. Buah, et al. 2022. Analysis of genetic diversity of banana weevils (*Cosmopolites sordidus*) (Coleoptera: Curculionidae) using transcriptome-derived simple sequence repeat markers. J. Econ Entomol. 115: 637-646.

Mukeshimana, G., L. Butare, P.B. Cregan, M.W. Blair, and J.D. Kelly. 2014. Quantitative trait loci associated with drought tolerance in common bean. Crop Sci. 54: 923-938.

Mutari, B., J. Sibiya, A. Shayanowako, C. Chidzanga, P.M. Matova, et al. E., 2023. Genome-wide association mapping for component traits of drought tolerance in dry beans (*Phaseolus vulgaris* L.). PloS One. 18: e0278500.

Nansamba, M., J. Sibiya, R. Tumuhimbise, W. Ocimati, E. Kikulwe, et al. 2022. Assessing drought effects on banana production and on-farm coping strategies by farmers — a study in the cattle corridor of Uganda. Clim. Change 173: 21.

Navascués, M., S. Stoeckel, and S. Mariette. 2010. Genetic diversity and fitness in small populations of partially asexual, self-incompatible plants. Heredity (Edinb). 104: 482–492.

Neiff, N., L. González Pérez, J.A. Mendoza Lugo, C. Martínez, B.A. Kettler, et al. 2023. QTL and genomic prediction accuracy for grain yield and secondary traits in a maize population under heat and heat-drought stresses. J Crop Improv. 37: 709-734.

Němečková. A., P. Christelová, J. Čížková, M. Nyine, I. Van den Houwe, et al. 2018. Molecular and cytogenetic study of East African highland banana. Front. Plant Sci. 9:1371.

Nkhata, W., H. Shimelis, R. Melis, R. Chirwa, T. Mzengeza, et al. 2020. Population structure and genetic diversity analyses of common bean germplasm collections of East and Southern Africa using morphological traits and high-density SNP markers. PLoS One. 15:p.e0243238.

Nyine, M., B. Uwimana, R. Swennen, M. Batte, A. Brown, et al. 2017. Trait variation and genetic diversity in a banana genomic selection training population. PLoS One. 12: e0178734.

Ocan, D., H.H. Mukasa, P.R. Rubaihayo, W. Tinzaara, and G. Blomme. 2008. Effects of banana weevil damage on plant growth and yield of East African *Musa* genotypes. J. Appl Biosci. 9: 415.

Ortiz, R., and R. Swennen. 2014. From crossbreeding to biotechnology-facilitated improvement of banana and plantain. Biotechnol Adv. 32:158–169.

Pandey, M.K., S.S. Gangurde, V. Sharma, S.K. Pattanashetti, G.K. Naidu, et al. 2020. Improved genetic map identified major QTLs for drought tolerance-and iron deficiency tolerance-related traits in groundnut. Genes. 12:37.

Peakall R., and P.E. Smouse. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics. 28:2537-2539.

Perrier X, Jacquemoud-Collet JP. 2006. DARwin software. http://darwin.cirad.fr/

Perrier, X., F. Bakry, F. Carreel, C. Jenny, J.P. Horry, et al. 2009. Combining biological approaches to shed light on the evolution of edible bananas. Ethnobot Res. Appl. 7: 199–216.

Perrier, X., E. De Langhe, M. Donohue, C. Lentfer, L. Vrydaghs, et al. 2011. Multidisciplinary perspectives on (*Musa* spp.) domestication. Proceedings of the National Academy of Sciences of the United States of America. 108:11311–11318.

Pillay M., E. Ogundiwin, D. Nwakanma, G. Ude, and A. Tenkouano. 2001. Analysis of genetic diversity and relationships in East African banana germplasm. Theor. Appl. Genet. 102:965–970.

Pillay M., 2024. The Genetic homogeneity of Uganda's East African Highland Bananas (Mutika/Lujugira) does not match the extensive morphological variation identified in this subgroup. Int J Plant Biol. 15: 267-280.

Raboin, L.M., F. Carreel, J.L. Noyer, F.C. Baurens, J.P. Horry, et al. 2005. Diploid ancestors of triploid export banana cultivars: molecular identification of 2n restitution gamete donors and n gamete donors. Mol. Breed. 16: 333–341.

Ravi, I., S. Uma, M.M. Vaganan, and M.M. Mustaffa. 2013. Phenotyping bananas for drought resistance. Front. Physiol. 4:9.

Ravi, K., V. Vadez, S. Isobe, R.R. Mir, Y. Guo, et al. 2011. Identification of several small main effect QTLs and a large number of epistatic QTLs for drought tolerance related traits in groundnut (*Arachis hypogaea* L.). Theoret. Appl. Genet. 122:1119–1132.

Risterucci, A.M., I. Hippolyte, X. Perrier, L. Xia, V. Caig, et al. 2009. Development and assessment of diversity arrays technology for high-throughput DNA analyses in Musa. Theoret. Appl. Genet. 119:1093–1103.

Ruas, M., V. Guignon, G. Sempere, J. Sardos, Y. Hueber, et al. 2017. MGIS: managing banana (*Musa* spp.) genetic resources information and high-throughput genotyping data. Database. p.bax046.

Saitou, N., and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol. 4:406–425.

Sampangi-Ramaiah, M.H., K.S. Shivashankara, A. Rekha, R.H. Laxman, E. Dayanandhi, and K.V. Ravishankar. 2023. High-density GBS-based genetic linkage map construction and QTL identification associated with leaf cuticular wax, adaxial stomatal density and leaf water retention capacity in banana. Sci. Hortic. 321:112205.

Sardos, J., M. Rouard, Y. Hueber, A. Cenci, K.E. Hyma, et al. 2016a. A genome-wide association study on the seedless phenotype in banana (*Musa* spp.) reveals the potential of a selected panel to detect candidate genes in a vegetatively propagated crop. PLoS One. 11:p.e0154448.

Sardos, J., X. Perrier, J. Doležel, E. Hřibová, P. Christelová, et al. 2016b. DArT whole genome profiling provides insights on the evolution and taxonomy of edible banana (*Musa* spp.). Ann. Bot. 118:1269-1278.

Sardos, J., P. Christelová, J. Čížková, J. Paofa, G.L. Sachter-Smith, et al. 2018. Collection of new diversity of wild and cultivated bananas (*Musa* spp.) in the Autonomous Region of Bougainville, Papua New Guinea. Genet Resourc Crop Evol. 65: 2267-2286.

Sardos, J., C. Breton, X. Perrier, I. Van den Houwe, S. Carpentier, et al. 2022. Hybridization, missing wild ancestors and the domestication of cultivated diploid bananas. Front. Plant Sci. 13:969220.

Sardos, J., A. Cenci, G. Martin, C. Breton, V. Guignon, et al. 2025. Painting the diversity of a world's favourite fruit: A next generation catalog of cultivated bananas. Plants, People, Planet. 7: 263-283.

Schierenbeck, M., A.M. Alqudah, S.G. Thabet, U. Lohwasser, M.R. Simón, et al. 2023. Association mapping unravels the genetics controlling seedling drought stress tolerance in winter wheat. Front. Plant Sci. 14:1061845.

Sempéré, G., A. Pétel, M. Rouard, J. Frouin, Y. Hueber, et al. 2019. Gigwa v2—Extended and improved genotype investigator, GigaScience. 8:giz051.

Simmonds, N.W. 1962. The evolution of bananas. Longman, London, UK.

Simmonds, N.W. 1966. Bananas. 2nd ed. Longmans, London, UK.

Somta, P., W. Sommanas, and P. Srinives. 2009. Molecular diversity assessment of AVRDC–The World Vegetable Center elite-parental mungbeans. Breed Sci. 59:149–57.

Ssali, R.T., A. Kiggundu, J. Lorenzen, E. Karamura, W. Tushemereirwe, et al. 2013. Inheritance of resistance to *Fusarium oxysporum* f. sp. *cubense* race 1 in bananas. Euphytica. 194:425-430.

Ssali, R.T. 2016. The identification and characterization of resistance *Musa* to *Fusarium Oxysporum* f. sp *cubense* race 1. PhD thesis. Stellenbosch University, South Africa.

Swennen, R., G. Blomme, P. Van Asten, P. Lepoint, E. Karamura, et al. 2013. Mitigating the impact of biotic constraints to build resilient banana systems in Central and Eastern Africa, p.85-104, *In* Van lauwe, B., P. van Asten, and G. Blomme, eds. Agro-ecological intensification of agricultural systems in the African Highlands. Routledge, New York, USA.

Tarasov, A., A.J. Vilella, E. Cuppen, I.J. Nijman, and P. Prins. 2015. Sambamba: fast processing of NGS alignment formats. Bioinformatics, 31:2032-2034.

Taulya, G., P.J.A. van Asten, S. Okech, and C. Gold. 2006. Effect of drought on the yields of an East African highland banana (cv. 'Kisansa') in Uganda. The proceedings of the 23rd Soil Science Society of East Africa conference. Masaka, Uganda.

Tenkouano, A., D. Vuylsteke, J. Okoro, D. Makumbi, R. Swennen, et al. 2003. Diploid banana hybrids TMB2x5105-1 and TMB2x9128-3 with good combining ability, resistance to black Sigatoka and nematodes. HortScience. 38:468-472.

Tumuhimbise, R., A. Barekye, J. Kubiriba, K. Akankwasa, I.K. Arinaitwe, et al. 2018. New high-yield cooking banana cultivars with multiple resistances to pests and diseases ('NAROBan1','NAROBan2','NAROBan3', and 'NAROBan4') released in Uganda. HortScience. 53:1387-1389.

Tumuhimbise, R., H. Buregyeya, J. Kubiriba, W.K. Tushemereirwe, A. Barekye, et al. 2019. 'NABIO808' (Syn. 'NAROBAN5'): A tasty cooking banana cultivar with resistance to pests and diseases. Crop Breed. Appl. Biotechnol. 19:491-495.

Tushemereirwe, W.K., D. Karamura, H. Ssali, D. Bwamiki, I. Kashaija, et al. 2001. Bananas (*Musa* spp), *In* J.K. Mukiibi Joseph, ed. Agriculture in Uganda, vol. 11 (crops). Fountain Publishers, Technical Centre for Agricultural and Rural Cooperation/National Agricultural Research Organization, Kampala, Uganda.

Tushemereirwe W., M. Batte, M. Nyine, R. Tumuhimbise, A. Barekye, et al. 2015. Performance of NARITA banana hybrids in the preliminary yield trial for three cycles in Uganda. Banana technical report. www.musalit.org/seeMore.php?id=15482. Accessed 17 August 2023.

Uwimana, B., G. V. Nakato, R. Kanaabi, C. Nasuuna, G. Mwanje, et al. 2024. Identification of the loci associated with resistance to banana Xanthomonas Wilt (*Xanthomonas vasicola* pv. *musacearum*) using DArTSeq markers and continuous mapping. Horticulturae. 10:87.

Van Asten, P.J.A., C.S. Gold, J. Wendt, D. De Waele, S.H.O. Okech, et al. 2005. The contribution of soil quality to yield and its relation with other banana yield loss factors in Uganda, p. 100-115, Proceedings of a workshop held on farmer participatory testing of IPM options for sustainable banana production in Eastern Africa. Bioversity International, Montpellier, France.

Van Asten, P.J.A., A.M. Fermont, and G. Taulya. 2011. Drought is a major yield loss factor for rainfed East African Highland Banana. Agric. Water Manage. 98:541–552.

Van Wesemael, J., E. Kissel, D. Eyland, T. Lawson, R. Swennen, and S. Carpentier. 2019. Using growth and transpiration phenotyping under controlled conditions to select water efficient banana genotypes. Front. Plant Sci. 10:354.

Vanhove, A.C., W. Vermaelen, B. Panis, R. Swennen, and S.C. Carpentier. 2012. Screening the banana biodiversity for drought tolerance: Can an *in vitro* growth model and proteomics be used as a tool to discover tolerant varieties and understand homeostasis. Front. Plant Sci. 3:176.

Waniale, A., S.B. Mukasa, A.K. Tugume, J. Kubiriba, W.K. Tushemereirwe, and R. Tumuhimbise. 2022. Early withering of enlarged ovules in pollinated fruits of bananas (*Musa* spp.) suggest abortion after fertilization. Horticulturae. 8:426.

Wickham, H. 2016. ggplot2: Elegant graphics for data analysis. Springer-Verlag, New York, USA.

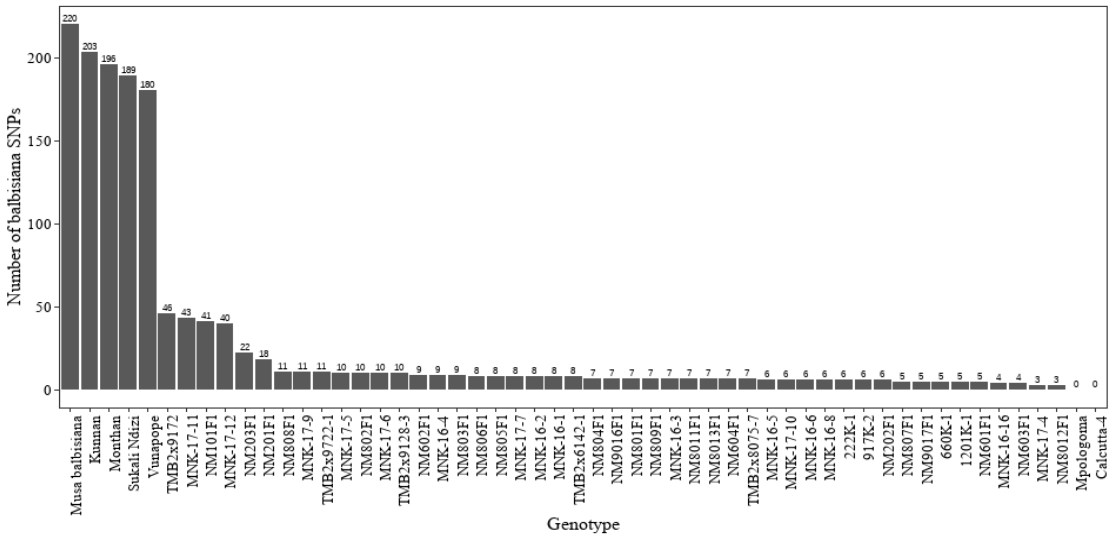
6.6 Appendices

Appendix 6.1 List of *Musa* genotypes used in this study with their respective ploidy levels and inferred clusters (major clusters and sub-clusters)

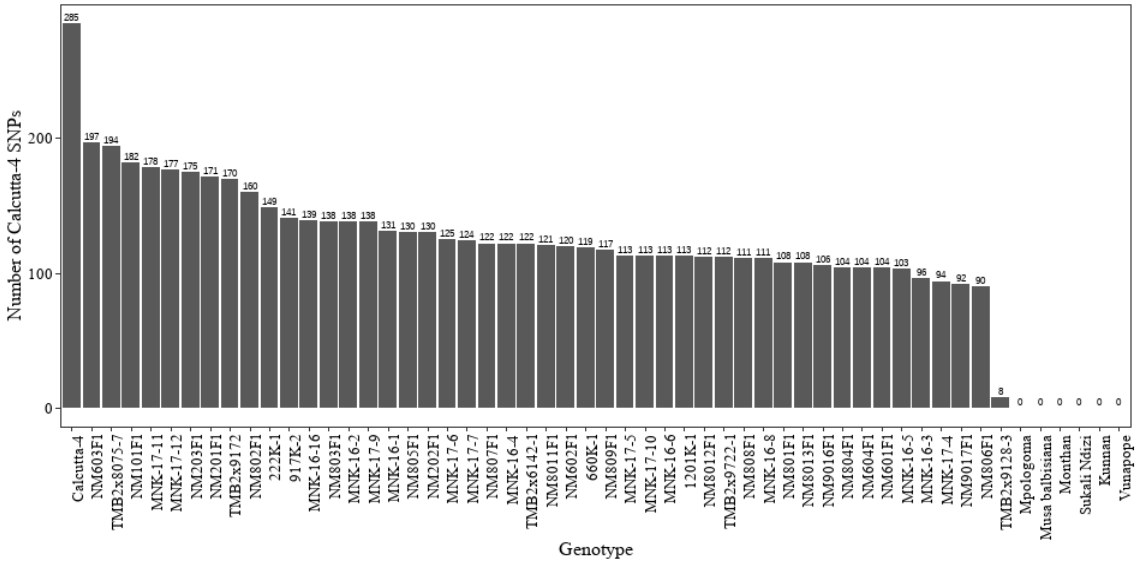
Genotype	Type	Genome	Ploidy	Major cluster	Sub-cluster
1201K-1	Breeding line	AAAA	4x	I	II
917K-2	Breeding line	AAAA	4x	I	II
660K-1	Breeding line	AAAA	4x	I	II
222K-1	Breeding line	AAAA	4x	I	II
TMB2x6142-1	Breeding line	AA	2x	I	III
TMB2x9128-3	Breeding line	AA	2x	I	III
TMB2x8075-7	Breeding line	AA	2x	I	III
TMB2x9722-1	Breeding line	Unknown	2x	I	III
TMB2x9172	Breeding line	AA	2x	I	III
NM101F1	Progeny	Unknown	2x	I	III
NM201F1	Progeny	Unknown	3x	I	II
NM202F1	Progeny	Unknown	3x	I	II
NM203F1	Progeny	Unknown	3x	I	II
NM601F1	Progeny	Unknown	3x	I	II
NM602F1	Progeny	Unknown	3x	I	I
NM603F1	Progeny	Unknown	3x	I	II
NM604F1	Progeny	Unknown	3x	I	II
NM801F1	Progeny	Unknown	3x	I	II
NM802F1	Progeny	Unknown	3x	I	I
NM803F1	Progeny	Unknown	3x	I	I
NM804F1	Progeny	Unknown	3x	I	I
NM805F1	Progeny	Unknown	3x	I	I
NM806F1	Progeny	Unknown	3x	I	I
NM807F1	Progeny	Unknown	3x	I	I
NM808F1	Progeny	Unknown	3x	I	I
NM809F1	Progeny	Unknown	3x	I	I
MNK-16-1	Progeny	Unknown	3x	I	I
MNK-16-2	Progeny	Unknown	3x	I	I
MNK-16-3	Progeny	Unknown	3x	I	I
MNK-16-4	Progeny	Unknown	3x	I	I
MNK-16-5	Progeny	Unknown	3x	I	I
MNK-16-6	Progeny	Unknown	3x	I	I
MNK-16-8	Progeny	Unknown	3x	I	I
MNK-16-16	Progeny	Unknown	3x	I	I
MNK-17-4	Progeny	Unknown	3x	I	I
MNK-17-5	Progeny	Unknown	3x	I	I
MNK-17-6	Progeny	Unknown	3x	I	II
MNK-17-7	Progeny	Unknown	3x	I	I
MNK-17-9	Progeny	Unknown	3x	I	I

Genotype	Type	Genome	Ploidy	Major cluster	Sub-cluster
MNK-17-10	Progeny	Unknown	3x	I	I
MNK-17-11	Progeny	Unknown	2x	I	III
MNK-17-12	Progeny	Unknown	2x	I	III
NM8011F1	Progeny	Unknown	3x	I	I
NM8012F1	Progeny	Unknown	3x	I	I
NM8013F1	Progeny	Unknown	3x	I	II
NM9015F1	Progeny	Unknown	3x	I	I
NM9017F1	Progeny	Unknown	3x	I	I
Calcutta-4	Wild species	AA	2x	I	III
M. balbibisiana	Wild species	BB	2x	II	-
Mpologoma	Landrace	AAA	3x	I	II
Sukali Ndiizi	Landrace	AAB	3x	II	-
Vunapope	Landrace	AB	3x	II	-
Monthan	Landrace	ABB	3x	II	-
Kunnan	Landrace	AB	2x	II	-

A)



B)



Appendix 6.2 Number of diversity array technology (DArT)-based single nucleotide polymorphic (SNP) markers specifically associated with two wild *Musa* species found within the study population. A) Number of '*Musa balbisiana*' specific markers found in each of the 54 *Musa* spp. genotypes. B) Number of 'Calcutta-4' specific markers found in each of the 54 *Musa* spp. genotypes.

Chapter 7: Differential growth and transpiration responses among banana hybrids - hope for banana drought tolerance breeding?

Abstract

Genetic diversity in East African highland bananas (EAHBs) is very limited and only resulting from frequent somatic mutations and continuous farmer selections. Considering the large market demand and drought sensitive nature of EAHBs, it is important to improve the resilience of these endemic bananas to future climatic conditions through breeding. The study was set up to determine the variability of water usage and assess the growth behaviour and transpiration responses of 16 secondary banana hybrids and their parental lines to a declining soil water content (SWC) in relation to vapour pressure deficit (VPD) and light intensity. By monitoring plant transpiration, daily and hourly, genotype-specific transpiration rates were modelled in relation to SWC, VPD and light. Well-watered plants were maintained at a SWC of 2.1 g g^{-1}, while the SWC of water stressed plants was allowed to drop from 2.1 g g^{-1} to 0.7 g g^{-1} by depriving them of water. The experiment was set up in a completely randomized design with one plant per pot and three replications per genotype. Significant genotype, water treatment and genotype by water treatment interaction effects were observed in water usage (i.e. number of days for SWC to drop from 2.1 g g^{-1} to 0.7 g g^{-1}), total leaf area (functional), total leaf damage, and transpiration rates across the progenies and their parental lines. The study detected critical genotype-specific SWC thresholds at which drought avoidance mechanisms were initiated. The diploid progenies, 'MNK-17-11' and 'MNK-17-12', achieved heterosis for either water usage, total leaf area or both. The contrasting phenotypic behaviour among genetically close progenies highlighted the significance of the environmental impact on a plant's phenotypic expression. The variability in water usage, plant growth and transpiration among the studied progenies suggests that the suitability of a given genotype will depend on the prevailing drought events and illustrates that drought tolerance improvement in EAHB populations is possible.

Keywords: banana hybrids, light intensity, plant growth, transpiration response, vapour pressure deficit, water stress

7.1 Introduction

Climate change presents a huge threat to global food and agricultural production systems. Increased crop yield losses resulting from changes in atmospheric temperatures, carbon dioxide levels and precipitation amounts are expected (Blanc, 2012; Gadedjisso-Tossou et al., 2021; Chari and Ngcamu, 2022). Considering the rapid global population growth rate (Melorose et al., 2015), the demand for food is set to increase (IPCC, 2014; Raza et al., 2019; Wang et al., 2021); hence there is an urgent need to mitigate the aggravating effects of climate change on crop production. This climate change has intensified the severity and occurrence of major abiotic stresses, including drought (Hepworth and Goulden, 2008; Gizaw and Gan, 2017; Haile et al., 2020; Mohammed et al., 2022).

Drought stress greatly impacts banana production (Taulya et al., 2006; van Asten et al., 2011; Uwimana et al.,2021; Nansamba et al., 2022), thus limiting its growth and yield. In the East African Great Lakes region, East African highland bananas (EAHBs) (AAA-EA genome) dominate the agricultural production systems (Karamura et al., 2012; Marimo et al., 2019; Akankwasa et al., 2020). They are considered endemic to the region with no clear analogue elsewhere in the world and are mainly grown on smallholder farms where they provide food- and income security (Frison et al., 1999; Lynam, 2000; Gambart et al., 2020).

Banana is a very ancient crop. Human migration and deforestation activities aided the dispersal of crop wild relatives during the mid-Holocene period (7,000 years ago), allowing spontaneous hybridizations between different *Musa* (sub)species and resulting in edible hybrids (De Langhe et al., 2009; Perrier et al., 2011). The existing banana diversity originates from a few meiosis events, followed by somaclonal mutations and continuous farmer selection (Sardos et al., 2016). EAHBs are AAA hybrids, originating from an inter-crossing between two *M. acuminata* subspecies, *M. acuminata subsp. banksii* and subsp. *zebrina*, and *M. schizocarpa* (Perrier et al., 2011; Němečková et al., 2018; Sardos et al., 2025). These hybridizations are believed to have occurred near Papua New Guinea and Java, from where the resulting edible hybrids (AAs and AAAs) were introduced to the East coast side of the African mainland by travellers via the Indian Ocean islands approximately 1,200 years ago (Perrier et al., 2019). This was a huge bottle neck in genetic variability

since no crop wild relatives exist in Africa. Only subsequent somaclonal mutations and continuous farmer selections gave rise to the current diversity in EAHB. To date, 115 different varieties have been described (Tugume et al., 2002), consisting of a wide phenotypic diversity (Karamura et al., 2016; Perrier et al., 2019) and differences in chromosome structure (Šimoníková et al., 2020).

For optimal growth, it has been estimated that EAHBs require in their agroecological native zone at least 1200 mm of water per year (Carr, 2009; Mustaffa and Kumar, 2012). They occur at high altitudes (900-2000 meters above sea level), which are associated with cool temperatures and hence a lower evaporative demand (Wortmann and Eledu, 1999; Karamura et al., 2012). EAHBs have a high-water usage, low water use efficiency, a weak drought avoidance mechanism (van Wesemael et al., 2019; Eyland et al., 2021) and tend to be absent on farms at low altitudes and/ or during longer dry seasons. Over the past half century, despite Uganda being the biggest EAHB producing and consuming country, banana production has shifted with seasonal variation in precipitation, rising mean annual temperature, limited soil nutrient replenishment, and disease pressure being the main contributing factors (Gold et al., 1999; Ochola et al., 2022). Successful crop production in the Great Lakes region has so far been sustained by the region's annual bimodal rainfall regimes regulated by topographical and coastal influences (Mutai et al., 1998; Conway et al., 2005; Becker et al., 2010). However, due to climate change, the weather patterns have become more unpredictable and variable (MFA-NL, 2018; Girvetz et al., 2019), thereby affecting the current and future suitability of some agroecological zones for banana production (Sabiti et al., 2018; Manners et al., 2021; Vandamme et al., 2022).

It is predicted that vapor pressure deficits (VPD) will continue to increase due to rising atmospheric temperatures, thereby causing a significant risk for production and yield (Rippke et al., 2016; Varma and Bebber, 2019; Abdoussalami et al., 2023). Sabiti et al. (2018) also envisaged that the moisture deficits resulting from expected temperature rises and hence increased evaporative demand could retard future banana growth in specific areas in Uganda. Considering the preferred market demand (Akankwasa et al., 2020) and the water-spending nature of the EAHB (Eyland et al., 2021; Kissel et al., 2015), it is crucial to improve the resilience of these endemic banana varieties to future environmental conditions through breeding. A scenario analysis by Vandamme et al. (2022) estimated

high suitability indices for drought-tolerant varieties (0.54 - 0.71) but low to moderate suitability for unimproved varieties under future climatic conditions. Therefore, improving the drought tolerance of EAHBs through breeding is the most effective and sustainable way to safeguard the crop against the expected daunting effects of climate change.

Drought tolerance is a complex quantitative trait controlled by several genes, each with additive and non-additive effects (Bernardo, 2008; Ravi et al., 2013). The polygenic nature of drought tolerance, coupled with banana's inherent sterility (male or female) and polyploidy nature, complicate and ultimately slow the crop's improvement through crossbreeding (Ssebuliba et al., 2006; Fortescue et al., 2011; Batte et al., 2019; Waniale et al., 2022). Despite these challenges, the existing genetic diversity within the gene pool remains the basis for elucidating and improving drought tolerance. Wild banana relatives could lend valuable abiotic tolerance to improved genotypes (Prohens et al., 2017; Dempewolf et al., 2017; Eyland et al., 2020). For instance, when deprived of water, the wild banana progenitor, *Musa balbisiana*, displayed adaptive traits that could be beneficial under conditions of high transpiration demands (Eyland et al., 2022). However, wild species also often possess multiple undesirable agronomic and fruit traits, which limit their utilization in crop breeding (Ramirez et al., 2011; Nansamba et al., 2020). To minimize the co-inheritance with poor traits and consequently reduce the banana drought tolerance breeding pipeline, it is important to identify and utilize relatively tolerant genotypes with 'less compromised' genomic backgrounds in banana crosses.

The objectives of the study were to determine the variability of water usage and assess the growth behaviour and transpiration responses of secondary banana hybrids and their parental lines to declining soil water content (SWC) in function of VPD and light intensity. We hypothesized that despite the narrow genetic diversity within EAHB varieties and the high-water usage, the meiotic recombination would lead to the segregation of reactions of transpiration responses and resulting growth to fluctuating VPD, light (photosynthetic active radiation, PAR) and SWC. This study evaluates the performance of bananas hybrids developed purposely for improved drought avoidance and tolerance.

7.2 Materials and methods

Two phenotyping experiments were performed concurrently at the Bioversity International phenotyping facility hosted in Belgium at the Katholieke University, Leuven (KU-Leuven). One experiment involved monitoring and taking daily plant growth and transpiration measurements. The second experiment involved continuous phenotyping of eight *Musa* progenies to model genotype-specific transpiration rate responses to declining SWC and fluctuating VPD and light intensity at higher resolution (i.e. one-hour interval).

7.2.1 Plant materials and experimental set up

Eighteen *Musa* spp. genotypes comprising 16 *Musa* progenies and their two parental lines, '917K-2' (female) and 'TMB2x9172' (male), were phenotyped (Table 7.1). 'TMB2x9172' is an improved diploid (AA-genome) hybrid that came from a cross between 'Obino L'Ewai' (plantain) and the wild banana relative, 'Calcutta-4' (AA genome), which is resistant to black sigatoka (Brown et al., 2017). 'TMB2x9172' previously showed some drought tolerance traits (Chapter 4). '917K-2' is a tetraploid hybrid (AAAA-genome) derived from a cross between the EAHB cultivar, 'Enzirabahima' (AAA genome) and 'Calcutta-4'. 'Enzirabahima' is one of the cooking bananas grown by farmers in the cattle corridor of Uganda (Chapter 3, Table 3.6). '917K-2' has been shown to have a relatively high pollination success, a desirable breeding trait considering the high female sterility rates of EAHBs (Batte et al., 2019).

In-vitro seedlings were generated in the tissue culture laboratory at the National Agricultural Research Laboratories (NARL), Kawanda, following the standard procedure elaborated in Vuylsteke et al. (1990) before transportation to the Bioversity International phenotyping facility in Belgium for evaluation. At the phenotyping facility, plantlets were acclimated to the greenhouse conditions for at least seven weeks before starting data collection. Plants were grown in 10-litre pots filled with a peat-based compost mixture (Levingtons F2S, UK) and watered daily until they reached a total leaf area (TLA) of 3000 cm^2 to normalise differential growth vigour. Once each genotype reached this threshold leaf area, three plants were maintained under well-watered conditions (control treatment), while the other three plants were deprived of water (water stress treatment). The water

treatments and genotypes were set up in a completely randomized design with one plant per pot. The relative humidity and air temperature were recorded every 5 mins with TROTEC climate data loggers (model BL30, Heinsberg, Germany) placed at eight points in the greenhouse. Light intensity was also measured every five minutes via a sensor (Skye instruments, Llandrindod Wells, UK) at the centre of the greenhouse. A 12-hr photoperiod was set, and an additional 14 W m^{-2} of light was provided when the external solar radiation fell below 250 W m^{-2} during the day. Vapour pressure deficit (VPD) and PAR varied over the course of the experiment (Appendix 7.1).

Table 7.1 *Musa* spp. genotypes evaluated for their response to water stress at the Bioversity International phenotyping facility hosted at KU-Leuven, Belgium.

Genotype	Type	Ploidy level	Measurements done
917K-2	F1 hybrid (female parent)	4x	
TMB2x9172	F1 hybrid (male parent)	2x	
MNK-16-1	Progeny	3x	Daily plant growth and transpiration rate Modelling of daily transpiration response to declining SWC
MNK-16-2	Progeny	3x	
MNK-16-3	Progeny	3x	
MNK-16-4	Progeny	3x	
MNK-16-5	Progeny	3x	
MNK-16-6	Progeny	3x	
MNK-16-8	Progeny	3x	
MNK-16-16	Progeny	3x	
MNK-17-4	Progeny	3x	Daily plant growth and transpiration rate Modelling of daily and hourly transpiration responses to declining SWC and fluctuating VPD and PAR
MNK-17-5	Progeny	3x	
MNK-17-6	Progeny	3x	
MNK-17-7	Progeny	3x	
MNK-17-9	Progeny	3x	
MNK-17-10	Progeny	3x	
MNK-17-11	Progeny	2x	
MNK-17-12	Progeny	2x	

7.2.2 Daily manual measurements

7.2.2.1 Total leaf area and leaf damage

The daily total leaf area was calculated as follows. For each plant, the length (cm) and width (cm) at the widest part of every fully opened leaf were measured with a tape measure. Measurements were only taken on functional leaves (i.e. with greater than 70 % green area). Single leaf areas were computed according to Summerville (1944) (Eq. 7.1).

$$\text{Leaf Area} = \text{Leaf length} \times \text{Leaf width} \times 0.83 \quad (7.1)$$

Where leaf area factor = 0.83

Individual plant total leaf areas (TLA) were computed by summing up all the single leaf areas. It is important to note that TLA was the total functional leaf area of the plant i.e. it does not include the leaf damage calculated below.

To check for the growth potential of the studied genotypes under well-watered and water stress conditions, the TLAs at the third week were plotted in a ranking quadrant. Considering that ploidy level affects a plant's growth attributes (Vandenhout et al., 1995), TLAs were normalised by computing the difference between the mean TLA of each genotype (i.e. individual genotype means under each water treatment) and the median of the corresponding water treatment. Subsequently, a genotype with a negative normalised TLA value was considered to have slower growth than the median plant for this water treatment. On the other hand, a positive normalised TLA value suggested faster growth than the median plant of that water treatment.

The leaf damage was determined by recording the area of each dying leaf (in cm^2) two days after foliage yellowing/ senescing commenced. Subsequently, total leaf damage (cm^2) was computed for each plant as the sum of the single leaf areas of all dead or dying leaves recorded during the first 21 days of drought assessment. Note that the TLA above was the functional leaf area and hence did not include the leaf damage (i.e. TLAs were independent of area(s) of dead leaves).

7.2.2.2 Water usage

To determine the soil moisture depletion of each genotype, the daily gravimetric SWC of plants was monitored and calculated from the total daily weights (W_{tot}) following the procedure elaborated by Eyland et al. (2022). Briefly, W_{tot} consisted of pot weight (W_{pot}), the weight of dry soil ($W_{dry\text{-}soil}$), the weight of water retained in the soil (W_{soil_water}) and plant weight (W_{plant}).

$$W_{tot} = W_{pot} + W_{dry\_soil} + W_{soil\_water} + W_{plant} \quad (7.2)$$

The pot weight (W_{pot}) was recorded prior to the start of the experiment. The weight of the dry soil (W_{dry_soil}) was calculated by determining the weight of the soil at the pot-filling stage ($W_{soil,start}$) and then taking a representative portion of which the fresh weight was recorded prior to drying. After two weeks of drying, the ratio of dry to fresh weight of the soil sample was calculated and used to estimate the W_{dry_soil}.

$$W_{dry\_soil} = W_{soil\_start} * \frac{W_{dry\ sample}}{W_{fresh\ sample}} \quad (7.3)$$

The plant weight (W_{plant}) was estimated from the calculated leaf areas using genotype-specific correlations ($R^2 > 0.7$).

With the W_{tot}, W_{pot}, $W_{dry\text{-}soil}$ and W_{plant} known, the amount of water retained in the soil (W_{soil_water}) was calculated from Eq 7.2. The gravimetric soil water content (SWC; g g^{-1}) was then calculated by dividing W_{soil_water} with the weight of the dry soil (W_{dry_soil}).

$$SWC = \frac{W_{soil\_water}}{W_{dry\_soil}} \quad (7.4)$$

The duration of moisture depletion (i.e. water usage) was expressed as the number of days it took for the SWC of each genotype to drop from 2.1 g g^{-1} (-0.01 MPa) to 0.7 g g^{-1} (-1.92 MPa).

7.2.2.3 Measurement of daily transpiration

Daily plant transpiration rates were determined by weighing the pots every 24 hours during the experiment. Water loss by evaporation was prevented by covering the top of the pots with plastic. Therefore, the difference in the pot weight between the two subsequent days was attributed to plant transpiration. Unlike water stress plants, well-watered plants were rewatered daily to a specific target weight to attain a SWC of 2.1 g g^{-1} (-0.01 MPa). Because the study's genotypes had different ploidy levels (2x, 3x and 4x), transpiration rates were normalised. Normalised daily transpiration rates (E_{rate_daily}; kg m^{-2} day^{-1}) were calculated by dividing the difference in the pot weight between two subsequent days (i.e. the amount of water lost by transpiration) with the estimated daily TLA (TLA_{daily}) retrieved from Eq. 7.1.

$$E_{rate\_daily} = \frac{W_{tot1} - W_{tot2}}{TLA_{daily}} \quad (7.5)$$

Where W_{tot1} represents the initial total weight, W_{tot2} is the total weight after 24 hrs.

7.2.3 Continuous transpiration phenotyping

To investigate transpiration response to increasing VPD and PAR under higher resolution, real-time transpiration rates were determined by monitoring the weights of plants of eight genotypes kept on high-precision balances (Phenospex, Heerlen, Netherlands). The Phenospex system records weight measurements every 60 seconds. SWC and whole-plant transpiration responses were determined by breaking down the total weight as described in sections 7.2.2.2 and 7.2.2.3. Dynamic whole-plant transpiration rates were determined by calculating and differentiating the average total weight every 15 minutes. Relative transpiration rates (E_{rate}; kg m^{-2} h^{-1}) were calculated as follows.

$$E_{rate} = \frac{W_{tot,t1} - W_{tot,t2}}{TLA} \quad (7.6)$$

Where $W_{tot,t1}$ represents the initial total weight, and $W_{tot,t2}$ the total weight after every 15 minutes. TLA is the total leaf area.

7.2.4 Modelling daily plant transpiration

The Jarvis-Stewart model (Jarvis, 1976; Whitley et al., 2009; Eyland et al., 2022) was modified to estimate individual genotype E_{rate_daily} in relation to SWC, light intensity and VPD. E_{rate_daily} was modelled (E_{model}) as a function of the following scaling terms.

$$E_{model} = E_{max} * f_1(Q_{in}) * f_2(VPD) * f_3(SWC) \quad (7.7)$$

With E_{max} as a scaling factor for the transpiration rate, f_1 is an asymptotic saturating function:

$$f_1(Q_{in}) = \frac{Q_{in}}{Q_{max}} * \frac{Q_{max} + k_1}{Q_{in} + k_1} \quad (7.8)$$

Where Q_{in} is the incoming radiation (W m^{-2}), Q_{max} is the maximum radiation recorded during the whole experiment and k_1 is the sensitivity of the light response.

f_2 defines the VPD response:

$$f_2(VPD) = VPD * e^{(-k_2 * VPD)} \quad (7.9)$$

With f_2 normalized by dividing Eq. 7.9 by the maximum value of the function and k_2 describes the curvature of the response of transpiration rate towards increasing VPD.

f_3 defines the transpiration response to the soil water content:

$$f_3(SWC) = \begin{cases} 0, & SWC \leq SWC_{wilt} \\ \frac{SWC - SWC_{wilt}}{SWC_{crit} - SWC_{wilt}}, & SWC_{wilt} < SWC < SWC_{crit} \\ 1, & SWC \geq SWC_{crit} \end{cases} \quad (7.10)$$

Where SWC_{crit} is the threshold soil water content at which stomatal closure begins under declining soil water conditions, SWC_{wilt} is the soil water content at which plant water uptake and transpiration cease.

Model parameterization was achieved by minimizing the root-mean-square error (RMSE) following the Fletcher variable metric method based on 1000 unique initial values (Nash, 2018). Bootstrapping was applied to calculate 95% confidence intervals (Canty and Ripley, 2019). Non-overlapping bootstrapped confidence intervals indicated significant differences in parameter estimates.

7.2.5 Heterosis

Because ploidy affects plant vigour, parent heterosis (i.e. considering the diploid male drought-tolerant parent ['TMB2x9172']) for TLA (under both water treatments) and water usage were only calculated for the two diploid progenies, including 'MNK-17-11' and 'MNK-17-12'. Male parent-diploid offspring heterosis for TLA was calculated as follows.

$$\text{Heterosis (\%)} = 100 * (TLA_{offspring} - TLA_{male\ parent}) / (TLA_{male\ parent}) \quad (7.11)$$

In a similar way, heterosis was calculated for water usage.

7.2.6 Data analysis

All data processing and statistical analysis were performed in R software version 4.2.2 (R Core Team, 2022). To account for repeated measurements (such as data obtained from the high-resolution experiment), date- and plant-specific factors were included as random effects in the linear mixed model. Analysis of variance (ANOVA) was performed to test for significant parameter mean differences due to the genotype, water treatment and genotype-water treatment interactions followed by a Tukey's Honest Significant Difference (HSD) post hoc test to compare parameter means. A Kruskal Wallis non-parametric test was performed for non-normally distributed parameters (e.g., duration of soil water content decline), followed by a Dunn's post hoc test.

Multivariate analysis (partial least squares (PLS)) was performed to investigate the relation between the observed transpiration behaviour and the effect of 21 days of no watering (acquired leaf damage). PLS analysis was performed in STATISTICA 14.0 (TIBCO Statistica). An artificial X variable was created for the transpiration behaviour using the

maximum transpiration rate (E_{max}), critical SWC threshold (SWC_{crit}), and slope of transpiration reduction (SWC_{slope})), and the correlation was investigated in function of the Y variable (leaf damage). The loadings for X were -0.32 for E_{max}, -0.72 for SWC_{crit} and 0.64 for SWC_{slope}. Genotype classification was made based on the transpiration behaviour (X) and leaf damage (Y) variables.

7.3 Results

7.3.1 Significant differences observed in water usage, total leaf area and leaf damage among the *Musa* progenies and their parental lines

Significant genotypic variations in the average number of days it took for the SWC to drop from 2.1 g g^{-1} (-0.01 MPa) to 0.7 g g^{-1} (-1.92 MPa) were observed ($p < 0.001$) (Table 7.2). The female parent, '917K-2' reached the 0.7 g g^{-1} SWC six days earlier than the drought-tolerant candidate male parent, 'TMB2x9172'. *Musa* progenies, 'MNK-17-12' and 'MNK-17-5', had the highest and lowest number of days, respectively, for their SWC to drop to -1.92 MPa. The rest of the progenies took 18.3 to 32.0 days to reach that target SWC. In terms of male parent-diploid offspring heterosis between 'TMB2x9172' and its diploid two offsprings, 'MNK-17-11' and 'MNK-17-12' showed 13% and 23% heterosis, respectively, for water usage.

The total leaf area (TLA) at the third week differed significantly across the studied banana genotypes ($p < 0.05$), water treatments ($p < 0.001$) and significant genotype x water treatment interactions were observed ($p < 0.05$) (Table 7.2). Water stress caused significant TLA reductions ranging between 8.1 and 59.8 %. The female parent ('917K-2') experienced a twice more TLA reduction than the male parent ('TMB2x9172'). 'MNK-16-16' and 'MNK-17-12', had the highest and least reductions in TLA, respectively. Under well-watered conditions, 'MNK-17-6' and 'MNK-17-11' were characterized by the highest and lowest TLAs, respectively, while 'MNK-16-6' and 'MNK-16-16' had the highest and lowest TLAs, respectively, under water stress conditions. Although the average reduction in TLA was 33.8 %, 'MNK-16-16' displayed almost 60% TLA reduction. Regarding the male parent-diploid offspring heterosis, the TLA of 'MNK-17-12' exceeded that of its male parent by 8 and 31% under well-watered and water stress conditions,

respectively, while ‘MNK-17-11’ had a negative heterosis of 9 and 16% under well-watered and water stress conditions, respectively.

The total leaf damage varied significantly among genotypes ($p < 0.05$), water treatments ($p < 0.01$) and the genotype x water treatment interactions ($p < 0.01$) (Table 7.2). The average leaf damage was 36.6 cm^2 and 946.6 cm^2 for well-watered and water stressed plants, respectively. The male parent ‘TMB2x9172’ and its offspring ‘MNK-16-5’, had the least total leaf damages of 157.9 cm^2 and 124.7, respectively, under water stress conditions. The female parent (‘917K-2’) had eight times more damage than the male parent. ‘MNK-17-9’ had the highest leaf area damage of 2087.4 cm^2 under water stress conditions. Unlike under well-watered conditions, leaf damage varied significantly across genotypes under water deficit conditions ($p < 0.05$). Leaf damage increased with decreasing soil water content over time (Appendix 7.2).

The TLAs at the third week were normalised and plotted in a ranking quadrant to check whether the studied genotypes were rather fast or slow growers under well-watered and water stress conditions (Figure 7.1). The female parent, ‘917K-2’, is one of the five genotypes situated in the fourth quadrant, which discriminates a relatively high TLA under well-watered conditions but a relatively low TLA under water stress conditions. On the other hand, the male parent ‘TMB2x9172’ and two progenies, ‘MNK-17-9’ and ‘MNK-17-11’, as shown in quadrant III, were characterized by low TLAs relative to all tested genotypes under both water treatments. Six progenies were situated in the first quadrant, which differentiates for relatively high TLA under both well-watered and water stress conditions. Four progenies, including ‘MNK-17-12’, ‘MNK-16-6’, ‘MNK-17-4’ and ‘MNK-17-7’, were represented in quadrant II, which discriminates for a relatively higher TLA under water stress treatment but relatively low TLAs under well-watered conditions.

Table 7.2 Duration of soil water content (SWC) decline (-0.01 to -1.92 MPa), total leaf area (at three weeks) and total leaf damage (cumulative damage over the first three weeks of drought assessment) of 18 hybrid banana genotypes (two parents ['917K-2' and 'TMB2x9172'] and their offsprings) grown under well-watered and water stress conditions.

		Days for SWC decline	Total leaf area (cm^2)			Total leaf damage (cm^2)	
Genotype	Ploidy	Water stress	Well-watered	Water stress	Reduction (%)	Well-watered	Water stress
917K-2	4x	22.0 $^{a-d}$	8125.6 $^{e-i}$	3992.6 ^{a}	50.9	40.3 ^{a}	1270.1 ^{bc}
TMB2x9172	2x	28.3 $^{b-e}$	5878.2 $^{a-i}$	4471.2 $^{a-c}$	23.9	12.2 ^{a}	157.9 ^{a}
MNK-17-11	2x	32.0 ^{de}	5359.7 $^{a-i}$	3768.7 ^{a}	29.7	62.9 ^{a}	404.1 ^{ab}
MNK-17-12	2x	34.7 ^{e}	6364.7 $^{a-i}$	5849.5 $^{a-i}$	8.1	24.2 ^{a}	280.9 ^{a}
MNK-16-1	3x	20.3 $^{a-c}$	7541.5 $^{b-i}$	4600.9 $^{a-d}$	38.9	22.8 ^{a}	868 $^{a-c}$
MNK-16-2	3x	20.7 $^{a-c}$	7716.6 $^{d-i}$	5321.5 $^{a-h}$	31.0	31.8 ^{a}	444.1 ^{ab}
MNK-16-3	3x	18.3 $^{a-c}$	8483.9 $^{g-i}$	4260.4 $^{a-d}$	49.8	123.4 ^{a}	600.1 $^{a-c}$
MNK-16-4	3x	22.0 $^{a-c}$	8365.3 $^{g-i}$	5028.3 $^{a-e}$	39.9	23.8 ^{a}	474.6 ^{ab}
MNK-16-5	3x	22.3 $^{a-e}$	7622.2 $^{c-i}$	5542.5 $^{a-i}$	27.3	5.3 ^{a}	124.7 ^{a}
MNK-16-6	3x	23.7 $^{a-e}$	7114.5 $^{a-i}$	5926.4 $^{a-i}$	16.7	10.1 ^{a}	1641.3 ^{cd}
MNK-16-8	3x	19.3 $^{a-c}$	8112.2 $^{e-i}$	5659.0 $^{a-h}$	30.2	69.7 ^{a}	1236.6 ^{bc}
MNK-16-16	3x	18.7 $^{a-c}$	8869.5 ^{hi}	3565.6 ^{a}	59.8	10.5 ^{a}	970.3 ^{bc}
MNK-17-4	3x	24.3 $^{a-e}$	6356.9 $^{a-i}$	4875.5 $^{a-i}$	23.3	6.1 ^{a}	1826.9 ^{d}
MNK-17-5	3x	17.0 ^{a}	8285.8 $^{f-i}$	5128.9 $^{a-f}$	38.1	10.3 ^{a}	1491.9 ^{cd}
MNK-17-6	3x	21.3 $^{a-c}$	8926.9 ^{i}	5294.0 $^{a-g}$	40.7	64.2 ^{a}	605.6 $^{a-c}$
MNK-17-7	3x	33.0 ^{de}	6842.9 $^{a-i}$	5105.9 $^{a-f}$	25.4	67.6 ^{a}	667.2 $^{a-c}$
MNK-17-9	3x	24.7 $^{b-e}$	6752.5 $^{a-i}$	4386.9 $^{a-d}$	35.0	33.9 ^{a}	2087.4 ^{d}
MNK-17-10	3x	31.0 ^{de}	7670.2 $^{b-i}$	4650.4 $^{a-d}$	39.4	14.0 ^{a}	1496.9 ^{cd}

Data represent the mean (n = 3). Different letters in the same column indicate significant genotypic differences. The total leaf area is the functional leaf area at three weeks i.e. it does not include the leaf damage.

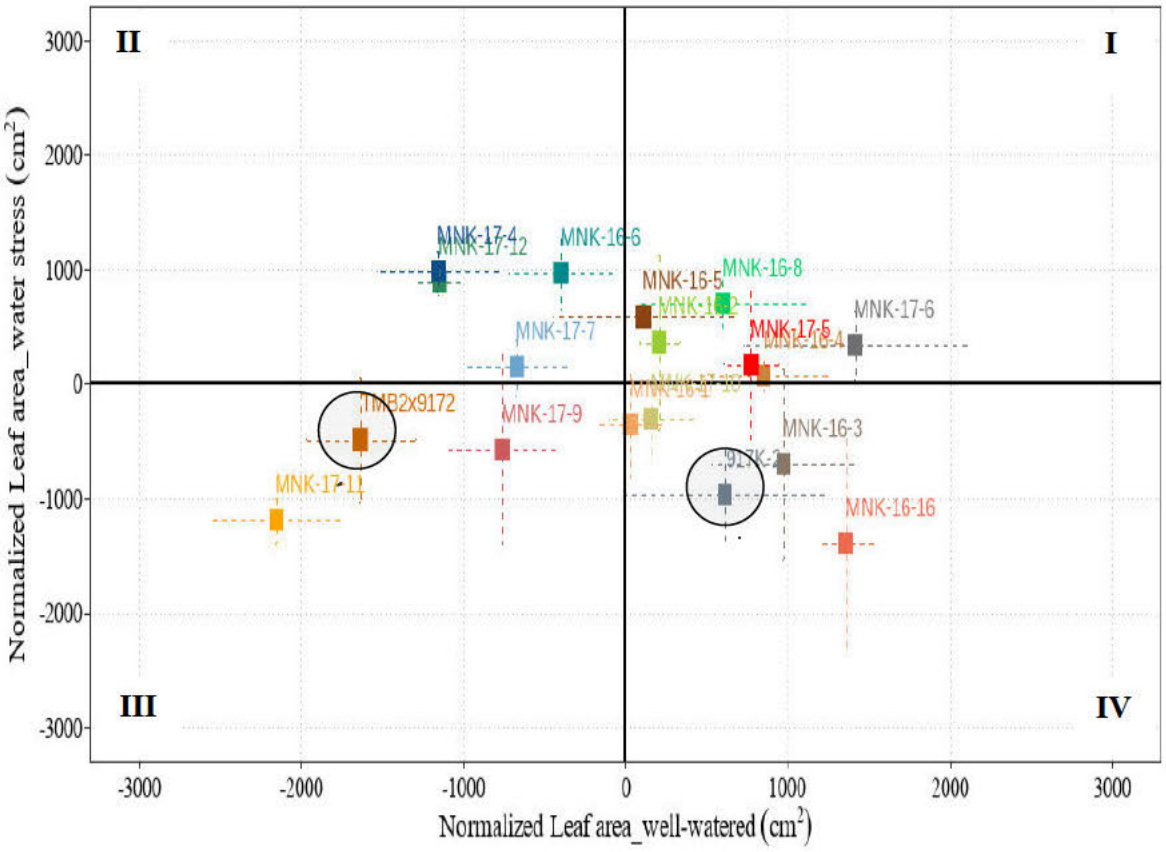


Figure 7.1 Quadrant rank of 18 banana hybrids (two parents ['917K-2' and 'TMB2x9172'] and their offsprings) at the third week, according to the median total leaf area (cm^2) for each water treatment. Horizontal and vertical lines differentiate the growth potential of the plants under well-watered or water stress conditions, respectively. Dotted lines represent the standard error. The parental genotypes are highlighted with a black circle.

7.3.2 Daily transpiration rates of banana hybrids differ under well-watered and water stress conditions

Normalised daily transpiration rates (E_{rate_daily}) under well-watered (SWC 2.1 g g^{-1}, -0.01 MPa) and water stress (SWC 1.2 g g^{-1}, -0.13 MPa) conditions differed significantly across genotypes ($p < 0.001$), water treatments ($p < 0.001$) and genotype by water treatment interactions ($p < 0.001$) (Figure 7.2). The overall mean E_{rate_daily} under well-watered (-0.01 MPa) and water stress conditions (-0.13 MPa) were 0.902 and 0.274 kg m^{-2} day^{-1}, respectively. Under well-watered conditions, the female ('917K-2') and male ('TMB2x9172') parents had mean E_{rate_daily} of 1.02 and 0.74 kg m^{-2} day^{-1}, respectively.

However, E_{rate_daily} values of these parents did not differ significantly under water stress conditions. The progenies, 'MNK-17-4' and 'MNK-16-3', had the highest E_{rate_daily} under well-watered and water stress conditions, respectively (Figure 7.2). The lowest E_{rate_daily} values during the experiment were observed in 'MNK-17-12' under both water treatments. 'MNK-17-9' was the only genotype of which the observed difference between E_{rate_daily} under well-watered and water stress conditions was not significant. For the rest of the genotypes, the E_{rate_daily} was significantly higher under well-watered conditions than water stress conditions. E_{rate_daily} reductions caused by water stress ranged between 54.6 to 79.9%, with 'MNK-16-3' displaying the lowest reduction and 'MNK-17-4' the highest.

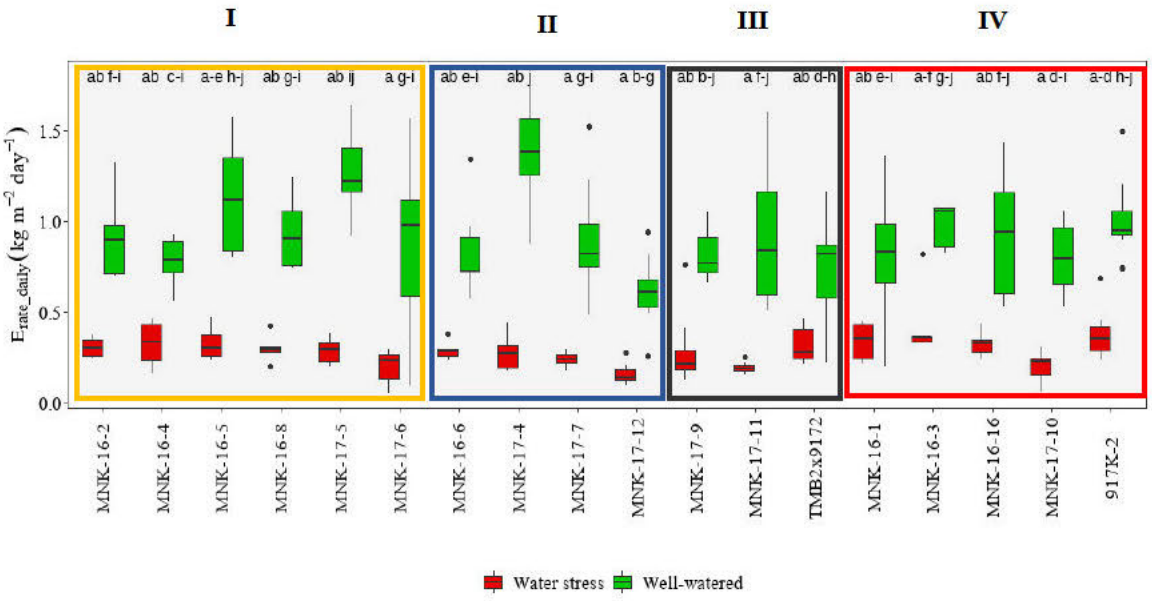


Figure 7.2 Daily transpiration rates of 18 banana genotypes (two parents ['917K-2' and 'TMB2x9172'] and their offsprings) at SWC of 2.1 g g^{-1}, -0.01 MPa (well-watered) and SWC of 1.2 g g^{-1}, -0.13 MPa (water stress). Different letters indicate significant genotypic differences ($p <$ 0.05; a<b<c<d<e<f<g<h<i<j). The genotypes are grouped (as enclosed in boxes) according to their respective ranking quadrants, including I, II, III and IV.

Environmental impacts were disentangled by modelling the daily E_{rate} of all studied genotypes as a function of SWC, VPD and net photosynthetic active radiation following the Jarvis-Stewart model (Eq. 7.7 - 7.10). The model RMSE ranged between 0.128 and 0.330 kg m^{-2} day^{-1} and the R^2 of both modelled and observed transpiration rates were greater than 0.7 for all genotypes. The critical threshold (SWC_{crit}) is the soil water content

at which stomatal closure starts, and that SWC triggers a decline in the transpiration rate to avoid drought stress. The slope of the decline is the rate at which the transpiration was limited by a decrease of stomatal and /or hydraulic conductance. The estimated SWC_{crit} threshold and the slope of decline in transpiration rate below this threshold varied among genotypes (Table 7.3).

The highest SWC_{crit} was observed in the 'MNK-17-6' genotype (SWC_{crit} 4.92 g g^{-1}, -0.0002 MPa) (Table 7.3). The SWC_{crit} values were lowest in 'MNK-17-4' and 'MNK-17-5' (SWC_{crit} 2.33 g g^{-1}, -0.006 MPa). Subsequently, 'MNK-17-4' showed the highest slope of transpiration rate reduction (SWC_{slope}) of 1.03 kg m^{-2} day^{-1}, while 'MNK-17-12' had the lowest SWC_{slope} (0.36 kg m^{-2} day^{-1}) (Table 7.3). The rest of the genotypes had SWC_{crit} thresholds between 2.47 and 3.79 g g^{-1} (-0.005 and -0.001 MPa). The modelled maximum transpiration rate (E_{max}) ranged between 0.77 and 1.87 kg m^{-2} day^{-1}.

Table 7.3 Modelled critical soil water content threshold (SWC_{crit}), slope of transpiration reduction (SWC_{slope}) and maximum transpiration rate (E_{max}) for 18 banana genotypes (two parents ['917K-2' and 'TMB2x9172'] and their offsprings). The parameters were modelled according to a modified Jarvis-Stewart model (Eq. 7.7 – 7.10). Values between squared brackets represent the lower and upper values of the 95 % confidence intervals.

Quadrant	Genotype	SWC_{crit} (g g^{-1})	SWC_{slope} (kg m^{-2} day^{-1})	E_{max} (kg m^{-2} day^{-1})
I	MNK-16-8	2.60 [2.06, 3.02]	0.60 [0.52, 0.68]	1.22 [0.95, 1.43]
I	MNK-17-5	2.33 [1.69, 2.79]	0.93 [0.75, 1,17]	1.43 [1.04, 1.73]
I	MNK-16-4	3.26 [3.04, 3.46]	0.54 [0.41, 0.65]	1.47 [1.25, 1.67]
I	MNK-16-5	3.34 [3.07, 3.64]	0.59 [0.50, 0.65]	1.62 [1.44, 1.79]
I	MNK-16-2	3.43 [3.17, 3.90]	0.58 [0.45, 0.70]	1.67 [1.63, 2.11]
I	MNK-17-6	4.92 [4.69, 5.16]	0.55 [0.41, 0.67]	2.32 [2.13, 2.43]
II	MNK-17-12	2.77 [2.39, 3.10]	0.36 [0.22, 0.45]	0.77 [0.63, 0.86]
II	MNK-17-7	2.62 [2.17, 3.12]	0.60 [0.40, 0.75]	1.15 [0.97, 1.34]
II	MNK-16-6	3.21 [2.90, 3.64]	0.49 [0.40, 0.57]	1.34 [1.16, 1.57]
II	MNK-17-4	2.33 [1.81, 2.79]	1.03 [0.52, 1.48]	1.42 [1.20, 1.62]
III	MNK-17-9	2.47 [1.33, 3.13]	0.69 [0.52, 0.93]	1.20 [0.72, 1.51]
III	MNK-17-11	3.32 [3.15, 3.46]	0.57 [0.44, 0.70]	1.41 [1.29, 1.54]
III	TMB2x9172	3.34 [3.12, 3.56]	0.56 [0.49, 0.62]	1.53 [1.43, 1.63]
IV	MNK-16-16	2.71 [2.27, 3.15]	0.68 [0.58, 0.77]	1.44 [1.23, 1.63]
IV	MNK-17-10	3.44 [3.24, 3.82]	0.55 [0.45, 0.64]	1.46 [1.38, 1.72]
IV	MNK-16-3	2.95 [2.81, 2.97]	0.72 [0.63, 0.81]	1.72 [1.57, 1.78]
IV	MNK-16-1	3.47 [3.18, 3.55]	0.60 [0.51, 0.68]	1.75[1.48, 1.78]
IV	917K-2	2.89 [2.49, 3.20]	0.82 [0.72, 0.92]	1.85 [1.63, 2.02]

7.3.3 Correlation between the transpiration behaviour and the drought associated phenotype

A significant positive correlation between the artificial variable X (transpiration behaviour) and associated leaf damage ($r = 0.57$, $p < 0.05$) was observed (Figure 7.3). Hence, the higher the transpiration behaviour, the more damage it acquired when irrigation was stopped. Despite the significant correlation between transpiration behaviour and leaf damage, some genotypes deviated significantly. For instance, 'TMB2x9172' (male parent) and five progenies, including 'MNK-16-3', 'MNK-16-5', 'MNK-17-7', 'MNK-17-11' and 'MNK-17-12' and had less leaf damage than predicted by the transpiration behaviour while 'MNK-16-6', 'MNK-17-9' and 'MNK-17-10' acquired more leaf damage than estimated by the transpiration behaviour (Figure 7.3). The leaf damage of the rest of the genotypes did not deviate significantly from the anticipated damage estimates at the 95% confidence interval.

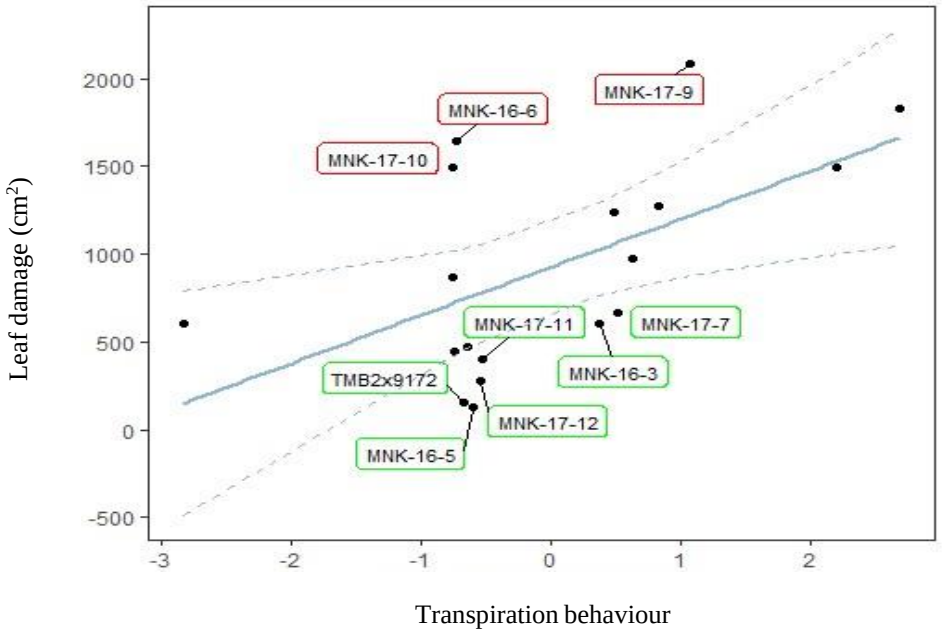


Figure 7.3 Relationship between modelled transpiration behaviour and the observed leaf damage of 18 test *Musa* spp. genotypes (two parents ['917K-2' and 'TMB2x9172'] and their offsprings) deprived of water for 21 days. The area between the dotted lines represents the region where the observed leaf damage does not deviate from the predicted leaf damage based

on the transpiration behaviour at 95% confidence interval. Genotypes in red and green have more and less damage, respectively, than predicted based on the transpiration behaviour.

The E_{max} strongly and positively correlated with the SWC_{crit} threshold ($r = 0.71$, $p < 0.001$) (Figure 7.4A). Thus, the higher the genotypic transpiration, the higher the SWC at which the transpiration was reduced. Despite the strong significant correlation between E_{max} and SWC_{crit} threshold, some genotypes deviated from this trend. For instance, the observed SWC_{crit} of the female parent ('917K-2') and four of its progenies ('MNK-16-3', 'MNK-16-16', 'MNK-17-4', 'MNK-17-5') was lower than the value estimated from the E_{max} (Figure 7.4A). On the other hand, 'MNK-17-6', 'MNK-17-10', 'MNK-17-11' and 'MNK-17-12' had higher SWC_{crit} values than predicted by the E_{max}. The observed SWC_{crit} values for the male parent ('TMB2x9172') and the rest of the progenies did not deviate significantly from the values predicted by their E_{max}.

The SWC_{crit} and the SWC_{slope} were negatively and significantly correlated ($r = -0.49$, $p < 0.05$). Based on the recorded SWC_{crit}, the observed SWC_{slope} of five of the 18 tested genotypes deviated from the estimated SWC_{slope} (Figure 7.4B). While '917K-2', 'MNK-17-5' and 'MNK-17-4' had stronger slope reductions than predicted, lower SWC_{slope} values were observed in 'MNK-16-6' and 'MNK-17-12'. Genotype classification based on the SWC_{crit} threshold, the SWC_{slope} and the leaf damage estimated from the transpiration behaviour is presented in Figure 7.3.

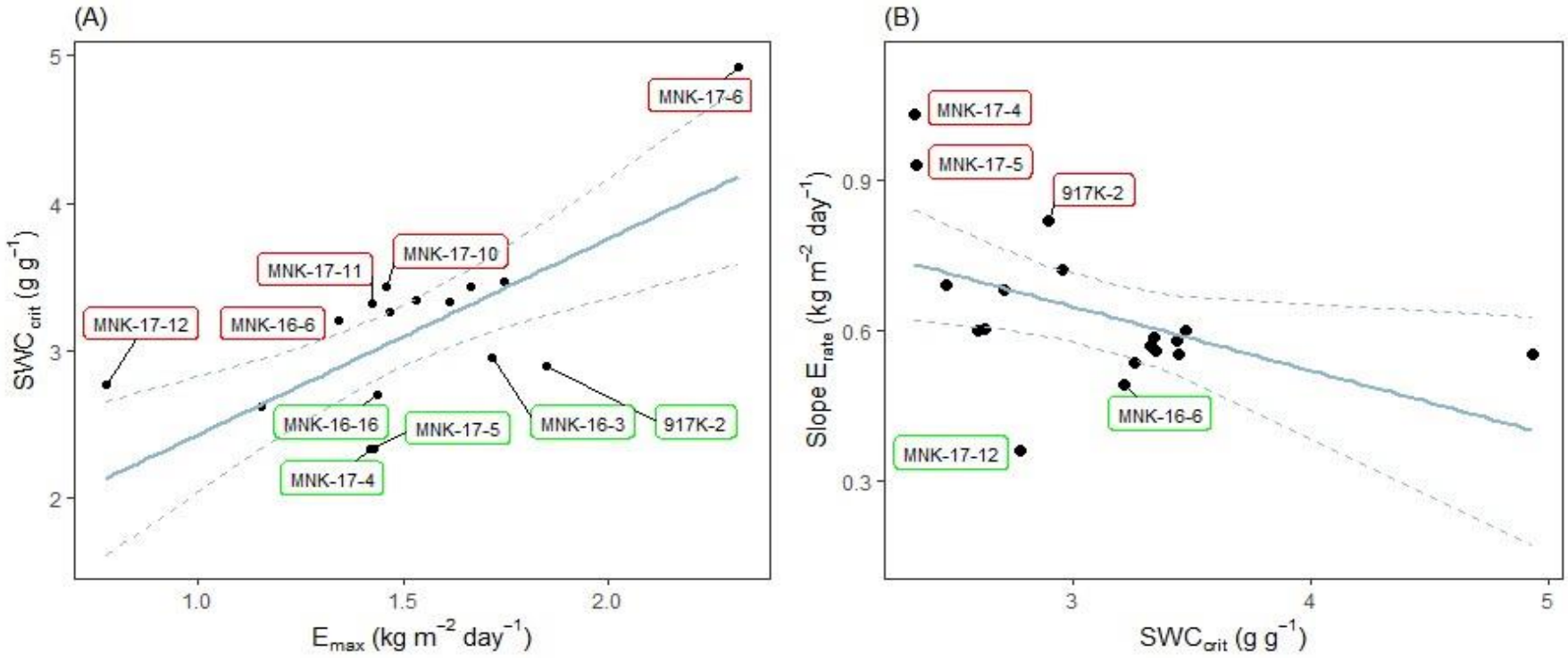


Figure 7.4 Relationship between (A) maximum transpiration rate (E_{max}) and critical soil water content threshold (SWC_{crit}) and (B) critical soil water content threshold and slope of transpiration reduction of 18 test *Musa* spp. genotypes (two parents ['917K-2' and 'TMB2x9172'] and their offsprings) deprived of water for 21 days. The area between the dotted lines represents the region where the observed values of the SWC_{crit} (for A) and slope of transpiration reduction (Slope E_{rate}) (for B) do not deviate from the predicted values based on the E_{max} (for A) and SWC_{crit} (for B) at 95% confidence interval. Genotypes in red and green had higher and lower SWC_{crit}, respectively than predicted by the E_{max} (for A), while genotypes in red and green had higher and lower Slope E_{rate}, respectively, than predicted by the SWC_{crit} (for B).

7.3.4 VPD and light triggered responses are at the basis of genotype-specific transpiration responses under well-watered conditions

To investigate transpiration response to increasing VPD and PAR under higher resolution (i.e., mean E_{rate} over one-hour intervals (kg m^{-2} h^{-1})), eight genotypes were selected based on the observed mean daily transpiration rates under well-watered conditions (Figure 7.2) and continuously phenotyped by keeping them on high precision balances. This representative set comprised genotypes with relatively low E_{rate} ('MNK-17-12', 0.61 kg m^{-2} day^{-1}), moderate E_{rate} ('MNK-17-6','MNK-17-7','MNK-17-9', 'MNK-17-10' and 'MNK-17-11' with mean E_{rate} ranging between 0.8 and 0.9 kg m^{-2} day^{-1}) and high E_{rate} ('MNK-17-4' and 'MNK-17-5', E_{rate} greater than 1.2 kg m^{-2} day^{-1}). Plant transpiration rate responses to fluctuating VPD and PAR were estimated by the Jarvis-Stewart model (Eq. 7.7 -7.9).

The transpiration rates of all genotypes continued to increase with increasing VPD (Figure 7.5A). No significant genotypic differences were observed at low VPD values, but the slope of transpiration rate as a function of VPD varied across the genotypes. 'MNK-17-4', followed by 'MNK-17-5', had the highest transpiration rates with increasing VPD. 'MNK-17-12' showed the lowest transpiration rate increase in response to increasing VPD.

A similar trend was observed in transpiration response to increasing light intensity, whereby at low light intensities, no significant differences in transpiration rate responses were observed across the genotypes (Figure 7.5B). Significant genotypic differences were triggered at higher light intensities. In the same way as increasing VPD, 'MNK-17-4' attained the highest transpiration rates with increasing light intensity, while 'MNK-17-12' maintained the lowest transpiration rates.

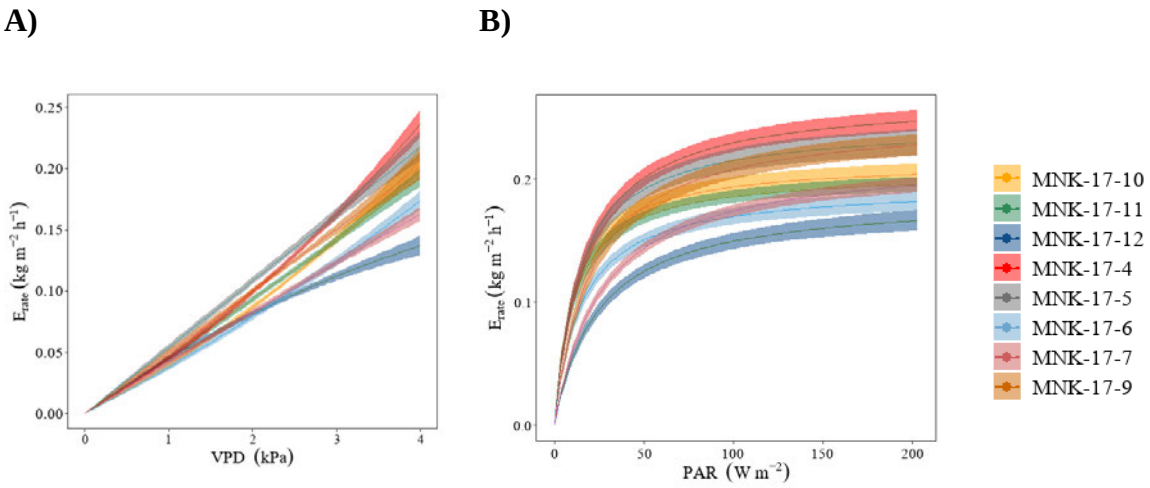


Figure 7.5 Modelled transpiration rate (E_{rate}) response of eight banana genotypes as a function of (A) vapor pressure deficit (VPD) (at PAR = 100 Wm^{-2}, SWC = 3 g g^{-1}, -0.002 MPa) and (B) photosynthetic active radiation (PAR) (at VPD = 2 kPa, SWC = 3 g g^{-1}, -0.002 MPa) (n = 3). Data represent the modelled transpiration rate response based on Equations 7.7 – 7.9 with 95% confidence interval. E_{rate} are considered significantly different if the confidence intervals do not overlap.

7.4 Discussion

Transpiration and growth responses to limiting water availability and fluctuating environmental conditions, including VPD and light intensity, have been shown to vary between and within plant species (Robinson and Bower, 1988; Leonardi et al., 2000; Hsiao et al., 2019; Hayat et al., 2020; Eyland et al., 2022). In this study, we crossed a drought-sensitive 'AAAA' parent ('917K-2') with a drought-tolerant 'AA' parent ('TMB2x9172') (Chapter 4). By monitoring plant growth (functional TLA) and transpiration rates of *Musa* progenies and their parental genotypes, we detected significant genotype-specific responses to declining SWC, varying VPD and light intensity (Table 7.2, Figure 7.1, Figure 7.2). These results confirm that a plant's response to water stress is determined by both the genotype and prevailing environmental conditions.

7.4.1 *Musa* progenies show differential growth and transpiration behaviour in response to declining SWC and fluctuating VPD and light intensity

The significant reduction in total leaf area observed under water stress conditions can be explained by a lower stomatal conductance, lower transpiration rate and accelerated leaf death or aging. Banana plants are quite sensitive to high VPD and/ or low SWC and lower their stomatal conductance to avoid water stress (Eyland et al., 2022). However, this is often insufficient and consequently, accelerated leaf damage occurs (Table 7.2, Appendix 7.2). Water stress is known to induce leaf death of the older leaves, which plays a major role in plant survival by avoiding huge transpiration losses, hence contributing to the maintenance of a favourable water balance at whole plant level as well as the redistribution of nutrient resources to younger plant tissues (Munné-Bosch and Alegre 2004; Martin-StPaul et al., 2013; Blackman et al., 2019; Guo et al., 2021). Water stress due to extended periods without precipitation can be minimized by low water usage, particularly in slow-growing genotypes with small leaf areas (Vadez, 2014).

Classification of the parental lines and their progenies in different quadrants indicated a broad range of growth behaviour (Figure 7.1). Genotypes grouped into quadrants II and III were slow growers and some showed relatively good tolerance to soil moisture deficit. For instance, the diploid male parent 'TMB2x9172' (quadrant III) and its two diploid progenies, 'MNK-17-12' (quadrant II) and 'MNK-17-11' (quadrant III), induced the isohydric drought avoidance mechanisms relatively early (at a high SWC) (Table 7.2, Figure 7.4) and acquired relatively low leaf damage (Figure 7.3, Appendix 7.3). The similar phenotypic performance of 'TMB2x9172', 'MNK-17-11' and 'MNK-17-12' matches their close genetic distance (Chapter 6; Figure 6.2). This study has however shown that a low leaf area is no guarantee for low leaf damage during water stress conditions. For example, 'MNK-17-9' (quadrant III) postponed its isohydric drought avoidance mechanism until a relatively low SWC (Table 7.3) and consequently acquired a higher amount of damage (Figure 7.3, Appendix 7.3). Moreover, quadrant II genotypes also showed varying degrees of water usage (Table 7.2) and drought avoidance (Table 7.3). For instance, despite its low acquired leaf area, 'MNK-17-4' acquired a lot of damage (Table 7.2). The model predicted 'MNK-17-4' (triploid) to induce the isohydric drought avoidance mechanism late but strongly (Figure 7.4). This risk-taking behaviour (i.e.

delayed induction of the drought avoidance mechanism) and high transpiration behaviour of 'MNK-17-4' resulted in high leaf damage (Table 7.2, Appendix 7.3). On the other hand, although the triploid progeny, 'MNK-16-6', had relatively low transpiration rates (Figure 7.2), it acquired a high leaf damage (Figure 7.3). The model predicted an early but weak induction of the isohydric drought avoidance mechanism in 'MNK-16-6' (Figure 7.4). Among quadrant II genotypes, 'MNK-16-6' and 'MNK-17-4' (both triploids) have the closest genetic proximity (Chapter 6; Figure 6.2) to which their similar phenotype (i.e. both acquired low leaf area, high leaf damage and moderately fast soil water depletion (~ 24 days)) can be linked. The variations in the SWC_{crit} thresholds and the slope of decline in transpiration rate indicate genotype-specific stomatal and /or hydraulic control levels (Table 7.3).

Nevertheless, slow growers are generally of less interest to farmers as they tend to bring forth low yields during optimal conditions (Stevens et al., 2020). Consequently, the adoption of such drought-tolerant but slow-growing quadrant II and III genotypes would be rather limited to certain agroecological zones. In contrast, quadrant I and IV genotypes are expected to be preferred by farmers as they show good growth potential under optimal conditions.

The ranking of the tetraploid female parent, '917K-2' and triploid offsprings, 'MNK-16-1', 'MNK-16-16', 'MNK-16-3', 'MNK-17-10', in quadrant IV indicates that they are fast-growing plants under well-watered conditions but show a considerable fall-out with reductions in TLA during water stress conditions. The genotypes in quadrant I are fast growers with a limited fallback in growth under water stress conditions. A reduction in growth under water stress can be attributed to several scenarios: (i) the plant had a high water usage, a low drought avoidance (reaction at low SWC and/or low slope), reached critical SWC early, and experienced accelerated leaf damage (parent '917K-2', 'MNK-16-3', 'MNK-16-8', 'MNK-16-16', 'MNK-17-5'), (ii) the plant has a high water usage but avoids drought (reaction at high SWC and/or high slope) and hence slows down transpiration and growth and limits damage in this way ('MNK-16-2', 'MNK-16-4', 'MNK-16-5', 'MNK-17-6'), (iii) the drought avoidance reaction (i.e. reaction at high SWC and/or slope) is not sufficient and damage accumulates ('MNK-16-1' and 'MNK-16-6'). Irrespective of some phenotypic differences, the quadrant ranking of some progenies was

consistent with the genetic clustering or relationships reported in the previous chapter (Chapter 6). For instance, close genetic relationships include: 'MNK-16-5', 'MNK-17-5' and 'MNK-17-6' (quadrant I), 'MNK-17-11' and 'TMB2x9172' (quadrant III) and 'MNK-17-10' (quadrant IV).

This study's test genotypes displayed relatively high mean daily transpiration rates (0.61 - 1.38 kg m^{-2} day^{-1}) under well-watered conditions (at soil water potential -0.01 MPa) (Figure 7.2). These high transpiration rates can be linked to the pedigree of the female parent, '917K-2', which is an offspring of the EAHB cultivar, 'Enzirabahima' (Brown et al., 2017; Tumuhimbise et al., 2018). Although plant transpiration varies with the environment, EAHBs are generally characterized by high transpiration rates (Kissel et al., 2015; Kayongo et al., 2015; van Wesemael et al., 2019) due to their rapid and prolonged stomatal conductance (Eyland et al., 2021). Nonetheless, multiple reports in literature have shown varying whole-plant transpiration rates among several *Musa* species, including wild banana relatives and edible varieties (van Wesemael et al., 2019; Eyland et al., 2021; 2022; Gambart, 2023).

Significant differences in daily transpiration rates were detected among genotypes (two parents and their progenies), water treatments and genotype by water treatment interactions ($p < 0.001$). As expected from its relatively low daily transpiration rates under both water treatments, 'MNK-17-12' took the longest time to reach a water potential of -1.92 MPa (Table 7.2). The relatively low leaf area of 'MNK-17-12' (even under well-watered conditions) is a crucial drought avoidance trait that limits absolute water usage, thereby resulting in slower water depletion (Tardieu, 2012; Eyland et al., 2022). 'MNK-17-12' can benefit from sustained carbon uptake and consequently continued photosynthesis during water stress conditions, given that its stomata close more gradually (as indicated by the lowest slope of reduction) than drastically, as seen in risk-taking triploid progenies such as 'MNK-17-4' and 'MNK-17-5' (Figure 7.4B). The late reaction of 'MNK-17-12' to declining SWC but low slope of transpiration reduction can be associated to its low transpiration rate.

Similar to Eyland et al. (2022), genotype-specific SWC_{crit} thresholds below which the maximum transpiration rates decline drastically were observed (Table 7.3). Between the two parental genotypes, 'TMB2x9172' (male) had a higher SWC_{crit} threshold and lower

E_{max} (SWC_{crit} = 3.34 g g^{-1}, E_{max} = 1.53 kg m^{-2} day^{-1}) than '917K-2' (female) (SWC_{crit} = 2.89 g g^{-1}, E_{max} = 1.85 kg m^{-2} day^{-1}). This result confirms 'TMB2x9172' as the more drought-tolerant and "conservative" parent and '917K-2' as the drought-sensitive "risk-taking" parent. With the lowest SWC_{crit} and the shortest period to reach a water potential of -1.92 MPa (Table 7.2), 'MNK-17-5' (triploid) was the least "conservative" and most "risk-taking" among the studied banana genotypes. On the other hand, the relatively high SWC_{crit} value observed in the 'MNK-17-6' triploid progeny indicates an early stomata control response (i.e., increase in stomatal resistance) to declining SWC compared to the rest of the genotypes (Table 7.3).

Among the eight genotypes studied for their response to increasing VPD and light intensity, 'MNK-17-4' had the fastest E_{rate} increase (Figure 7.5). Previous studies showed that genotypes with the most rapid E_{rate} increase under a gradual increase in VPD and light intensity also showed a risk-taking water consuming behaviour (Eyland et al., 2021; Eyland et al., 2022). 'MNK-17-12' had the lowest E_{rate} in response to increasing VPD and light (Figure 7.5), we deduce that this genotype thrives best in environments with high atmospheric evaporative demand. The online transpiration phenotyping on a smaller subset confirmed the first experiment except for 'MNK-17-6'.

7.4.2 Transpiration behaviour variables are correlated

This study revealed significant correlations between several transpiration variables (Figure 7.3, Figure 7.4). The significant positive correlation between the transpiration behaviour and leaf damage (r = 0.57) observed in this study implies that genotypes with high transpiration behaviour have a high-water usage and consequently, high leaf damage due to water stress can be expected. These genotypes are 'risk takers' which continue to consume water by maintaining their maximum transpiration rate (E_{max}) until a low soil water content and /or keeping a relatively high transpiration rate after the drought avoidance mechanism kicks in (Table 7.3, Appendix 7.3). Such 'risk-taking' behaviour suggests that generally, genotypes with high water consumption (i.e. high transpiration behaviour) will not do well in environments with frequent and severe droughts as they will experience high damage. However, the advantage of genotypes that have high transpiration potentials is that they tend to be fast growers and, hence, will bring forth more vigorous

vegetative growth and yield compared to slow-growing genotypes (Stevens et al., 2020), considering that photosynthesis is dependent on transpiration (von Caemmerer and Baker, 2007). This association between transpiration and growth potential was confirmed by the significant positive correlation between E_{max} and growth observed in this study ($r^2 = 0.25$, $p < 0.036$). Fast-growing cultivars are particularly ideal for environments with short rain seasons. However, as indicated earlier, the observed leaf damage of some genotypes deviated from the damage predicted from their transpiration behaviour (Figure 7.3). As such, triploid *Musa* progenies, including 'MNK-16-6', 'MNK-17-9' and 'MNK-17-10', are very sensitive to water stress as they acquired a higher leaf damage than predicted based on their transpiration. Ideally, a genotype that grows fast but is conservative when drought strikes and saves itself from damage is desirable.

The strong positive correlation between E_{max} and SWC_{crit} ($r = 0.71$, $p < 0.001$) implies that if a genotype has a high transpiration potential (high E_{max}) it will experience water deficit at a high soil water content (i.e., high SWC_{crit}). This result is in accordance with an earlier report on banana wild banana relatives by Eyland et al. (2022). For this study, however, deviations from this trend were also observed (Figure 7.4A). For instance, 'MNK-16-6', 'MNK-17-6', 'MNK-17-10', 'MNK-17-11' and 'MNK-17-12' had higher SWC_{crit} values than predicted by their respective E_{max}, making them conservative in that sense. Conversely, 'MNK-16-3', 'MNK-17-4', 'MNK-17-5' and '917K-2' (female parent) displayed a risk-taking behaviour given that they had lower SWC_{crit} values than predicted by their E_{max}.

The significant negative correlation between SWC_{crit} and the slope of reduction of transpiration ($r = -0.49$, $p < 0.05$) indicates that if a genotype reacts early to declining soil moisture (e.g., increases stomatal resistance at high SWC), it will not react drastically (has a low slope of reduction) because there is still plenty of water available in the soil. However, if a genotype maintains its E_{max} for longer (i.e., has low SWC_{crit}), the slope of transpiration reduction will be high because, at that point, the soil water is more limited. Contrary to this trend, two progenies, 'MNK-16-6' and 'MNK-17-12', showed a risk-taking behaviour by having lower slopes of transpiration reduction than anticipated by their SWC_{crit} (Figure 7.4B).

The above plant behaviours are inherent genotypic responses to mitigate water stress effects. However, the effectiveness and benefits of these different responses will depend on how quickly water becomes available through rain, considering that EAHB production systems are predominantly rain-fed. Further, knowledge of the above correlations is useful in determining the most suitable environment for the growth of each progeny evaluated in this study, depending on the drought scenario, particularly in the EAHB region where drought frequencies are becoming higher (Haile et al., 2020). For instance, considering their high growth potential but rapid soil moisture depletion, 'MNK-16-2', 'MNK-16-4', 'MNK-16-5' and 'MNK-17-6' are suited for environments with sufficient rainfall or short drought periods. The good growth potential exhibited by 'MNK-16-3', 'MNK-16-8', 'MNK-16-16', 'MNK-17-5' and 'MNK-17-10', under well-watered conditions but considerable growth reduction during water deficit conditions suggests that these genotypes are only suited for environments with sufficient rainfall. On the other hand, 'MNK-17-11' and 'MNK-17-12' had slower soil water depletion and were relatively tolerant to water stress conditions and hence will sustain growth in EAHB areas with a longer drought period. Moreover, the slow E_{rate} increase of 'MNK-17-12' upon gradual increase in light and VPD (Figure 7.5) reinforces its conservative nature and suitability in harsher environments. 'MNK-17-12' also had the lowest percentage reduction in TLA under water stress conditions (Table 7.2). Contrary to 'MNK-17-12', 'MNK-17-4' had the fastest E_{rate} increase response to increasing light and VPD (Figure 7.5), making it less suited for dry environments with high evaporative demand.

The contrasting phenotypic behaviour of some progenies which genetically grouped together (Chapter 6; Figure 6.2) could partly be attributed to the significant impact of the environment on the expression of evaluated phenotypic traits. Phenotypic expression in plants is the result of a complex interaction between the genotype and environment (De Leon et al. 2016; Napier et al., 2023). A recent study by Eyland et al. (2022) reported a discord between the phenotype and genotype of some banana wild relatives evaluated for their transpiration responses under optimum and water stress conditions. For instance, despite *M.acuminata* ssp. *errans* being genetically close to *M.acuminata* ssp. *banksii* and *microcarpa*, a distinct phenotype was observed.

7.5 Conclusions

This study reports the performance of banana hybrids developed purposely for enhanced drought tolerance and avoidance. Although the number of progenies is rather low for segregation studies, the differential phenotypic behaviour of study's progenies indicates that genes for drought tolerance or sensitivity may be segregating. The total leaf area (functional), leaf damage and transpiration rates were influenced by the genotype, water treatment and genotype by water treatment interaction. The suitability of a given improved genotype will, however, depend on the prevailing drought events (Tardieu, 2012; Vadez, 2014; Eyland et al., 2022). Given their good growth under both well-watered and stress conditions but relatively fast soil water depletion, triploid progenies 'MNK-16-2', 'MNK-16-4', 'MNK-16-5' and 'MNK-17-6' are suited for environments with relatively short dry periods. While the female parent line, '917K-2' and its triploid offspring, 'MNK-16-3', 'MNK-16-8', 'MNK-16-16' and 'MNK-17-5' have high growth potentials under optimum conditions, their growth is significantly and negatively impacted even during short dry periods. Such genotypes can only thrive well in environments with sufficient precipitation. The male parent, 'TMB2x9172' and two progenies, 'MNK-17-11' and 'MNK-17-12', are quite tolerant to water stress and seem to have good potential in areas with a longer dry season. The suitability of MNK-17-11' and 'MNK-17-12' in areas with a prolonged dry season was further exemplified by their expression of heterosis for water usage. Moreover, 'MNK-17-12' had positive heterosis for TLA under both well-watered and water stress conditions. Lastly, further research is required to check whether the results from these pot assays with small plants provide an accurate account of the response of older plants that have a more developed corm. Despite the challenges posed by the complexity of drought tolerance, this study gives hope that developing banana cultivars that can withstand water stress conditions is possible.

References

Abdoussalami, A., Z. Hu, A.R.T. Islam, and Z. Wu. 2023. Climate change and its impacts on banana production: a systematic analysis. Environ. Dev. Sustain. 1-30.

Akankwasa, K., P. Marimo, R. Tumuhimbise, M. Asasira, E. Khakasa, et al. 2020. The East African highland cooking bananas 'Matooke' preferences of farmers and traders: Implications for variety development. Int. J. Food Sci. Technol. 56:1124–1134.

Batte, M., R. Swennen, B. Uwimana, V. Akech, A. Brown, et al. 2019. Crossbreeding East African highland bananas: lessons learnt relevant to the botany of the crop after 21 years of genetic enhancement. Front. Plant Sci.10: 81.

Becker, M., W. LLovel, A. Cazenave, A. Güntner, and J. Crétaux. 2010. Recent hydrological behaviour of the East African Great Lakes region inferred from GRACE, satellite altimetry and rainfall observations. Comptes Rendus Geosci. 342:223-233.

Bernardo, R. 2008. Molecular markers and selection for complex traits in plants: learning from the last 20 years. Crop Sci. 48:1649-1664.

Blackman, C.J., X. Li, B. Choat, P.D. Rymer, M.G. De Kauwe, et al. 2019. Desiccation time during drought is highly predictable across species of Eucalyptus from contrasting climates. New Phytol. 224:632-643.

Blanc, E. 2012. The impact of climate change on crop yields in Sub-Saharan Africa. Am. J. Clim. Change. 1:1–13.

Brito, C., L-T. Dinis, H. Ferreira, J. Moutinho-Pereira, and C. Correia. 2018. The role of nighttime water balance on *Olea europaea* plants subjected to contrasting water regimes. J. Plant Physiol. 226:56-63.

Brown, A., R. Tumumbise, D. Amah, B. Uwimana, M. Nyine, et al. 2017. Bananas and plantains (*Musa* spp.), p.219-240, *In* Campos, H., and P.D.S. Caligari, eds. Genetic improvement of tropical crops. Springer International Publishing, Switzerland.

Buckley, T.N., T.L. Turnbull, S. Pfautsch, and M.A. Adams. 2011. Nocturnal water loss in mature subalpine *Eucalyptus delegatensis* tall open forests and adjacent *E. pauciflora* woodlands. Ecol. Evol. 1:435-50.

Caird, M.A., J.H. Richards, and L.A. Donovan. 2007. Nighttime stomatal conductance and transpiration in C_3 and C_4 plants. Plant Physiol. 143:4–10.

Canty, A., and B. Ripley. 2019. Boot: Bootstrap R (S-Plus) Functions. https://cran.rproject.org/web/packages/boot/boot.pdf Accessed 21 December 2022.

Cao, K.A.L., F. Rohart, S. Dejean, A. Abadi, B. Gautier, et al. 2021. Package *'mixOmics':* Omics data integration project.

Carr, M.K.V. 2009. the water relations and irrigation requirements of banana (*Musa* spp.). Exp. Agric. 45:333–371.

Chari, F., and B.S. Ngcamu. 2022. Climate change and its impact on urban agriculture in Sub-Saharan Africa: A literature review. Environ. Socio-econ. Studies 10:22-32.

Chaves, M.M., J.M. Costa, O. Zarrouk, C. Pinheiro, C.M. Lopes, and J.S. Pereira. 2016. Controlling stomatal aperture in semi-arid regions—The dilemma of saving water or being cool? Plant Sci. 251:54-64.

Conway, D., E. Allison, R. Felstead, and M. Goulden. 2005. Rainfall variability in East Africa: implications for natural resources management and livelihoods. Philos. Trans. Royal. Soc. 363:49–54.

Costa, J.M., F. Monnet, D. Jannaud, N. Leonhardt, B. Ksas, et al. 2015. Open all night long: the dark side of stomatal control. Plant Physiol. 167:289-94.

Dawson, T.E., S.S.O. Burgess, K.P. Tu, R.S. Oliveira, L.S. Santiago, et al. 2007. Nighttime transpiration in woody plants from contrasting ecosystems. Tree Physiol. 24:561-575.

De Langhe, E., L. Vrydaghs, P. De Maret, X. Perrier, and T. Denham. 2009. Why bananas matter: an introduction to the history of banana domestication. Ethnobot. Res. Appl. 7:165-77.

De Leon, N., J.L. Jannink, J.W. Edwards, and S.M. Kaeppler. 2016. Introduction to a special issue on genotype by environment interaction. Crop Sci. 56:2081-2089.

Dempewolf, H., G. Baute, J. Anderson, B. Kilian, C. Smith, and L. Guarino. 2017. Past and future use of wild relatives in crop breeding. Crop Sci. 57:1070-82.

Escalona, J.M., S. Fuentes, M. Tomás, S. Martorell, J. Flexas, and H. Medrano. 2013. Responses of leaf night transpiration to drought stress in *Vitis vinifera* L. Agric. Water Manage. 118:50–58.

Eyland, D., J. van Wesemael, T. Lawson, and S. C. Carpentier. 2021. The impact of slow stomatal kinetics on photosynthesis and water use efficiency under fluctuating light. Plant Physiol. 186:998–1012.

Eyland, D., C. Breton, J. Sardos, S. Kallow, B. Panis, et al. 2020. Filling the gaps in gene banks: Collecting, characterizing, and phenotyping wild banana relatives of Papua New Guinea. Crop Sci. 61:137–149.

Eyland, D., N. Luchaire, L. Cabrera-Bosquet, B. Parent, S.B. Janssens, et al. 2022. High-throughput phenotyping reveals differential transpiration behaviour within the banana wild relatives highlighting diversity in drought tolerance. Plant, Cell. Environ. 45:1647–1663.

Fortescue, J.A., and D.W. Turner. 2011. Reproductive Biology, p.145-179, *In* M. Pillay, and A. Tenkouano, eds. Banana breeding: progress and challenges. CRC Press, Boca Raton, USA; CRC Press, London, UK.

Frison, E.A., W.W. Collins, and S.L. Sharrock. 1999. ProMusa: A first experience of global programme in horticulture. Acta Hortic. 495:381-398.

Gadedjisso-Tossou, A., K.I. Adjegan, and A.K. Kablan. 2021. Rainfall and temperature trend analysis by mann-kendall test and significance for rainfed cereal yields in Northern Togo. Science 3:17.

Gambart, C., R. Swennen, G. Blomme, J.C. Groot, R. Remans, and W. Ocimati. 2020. Impact and opportunities of agroecological intensification strategies on farm performance: A case study of banana-based systems in Central and South-western Uganda. Front. Sustain. Food Syst. 4:87.

Gambart, C. 2023. The quest for climate smart bananas. phenotyping the banana diversity from plant to cell level. PhD thesis, Katholieke Universiteit Leuven, Belgium.

Girvetz, E., J. Ramirez-Villegas, L. Claessens, C. Lamanna, C. Navarro-racines, et al. 2019. Future climate projections in Africa: where are we headed?, p.15-27, *In* T. Rosenstock, A. Nowak, and E. Girvetz, eds. The Climate-Smart Agriculture Papers: Investigating the business of a productive, resilient and low emission future. Springer Nature, Cham, Switzerland.

Gizaw, M.S., and T.Y. Gan. 2017. Impact of climate change and El Niño episodes on droughts in sub-Saharan Africa. Clim. Dyn. 49:665–682.

Gold, C.S., E.B. Karamura, A. Kiggundu, F. Bagamba, and A.M. Abera. 1999. Geographic shifts in the highland cooking banana (*Musa* spp., group AAA-EA) production in Uganda. Int. J. Sustain Dev. World Ecol. 6:45–59.

Guo, Y., G. Ren, K. Zhang, Z. Li, Y. Miao, and H. Guo. 2021. Leaf senescence: progression, regulation, and application. Mol. Hortic. 1:1-25.

Haile, G.G., Q. Tang, S.M. Hosseini-Moghari, X. Liu, T.G. Gebremicael, et al. 2020. Projected impacts of climate change on drought patterns over East Africa. Earth's Future. 8:e2020EF001502.

Hayat, F., M.A. Ahmed, M. Zarebanadkouki, M. Javaux, G. Cai, and A. Carminati. 2020. Transpiration reduction in maize (*Zea mays* L) in response to soil drying. Front. Plant Sci. 10:1695.

Hepworth, N., and M. Goulden. 2008. Climate change in Uganda: Understanding the implications and appraising the response. LTS International, Edinburgh, United Kingdom.

Hoagland, D.R., and T.C. Broyer. 1936. General nature of the process of salt accumulation by roots with description of experimental methods. Plant Physiol. 11:471–507.

Howard, A.R., and L.A. Donovan. 2009. Helianthus nighttime conductance and transpiration respond to soil water but not nutrient availability. Plant Physiol. 143:145–155.

Hsiao, J., A.L.S. Swanna, and S-H. Kim. 2019. Maize yield under a changing climate: The hidden role of vapor pressure deficit. Agric. Forest Meteorol. 279:107692.

Hughes, R.N., and P. Brimblecombe. 1994. Dew and guttation formation and environmental significance: agricultural and forest meteorology. Agric. Meteorol. 67:173–190.

IPCC. 2014. Climate change 2014: Synthesis report, *In* R.K. Pachauri, and L.A. Meyer, eds. Core writing team, contribution of working groups I, II and III to the fifth assessment report of the Intergovernmental Panel on Climate Change (IPCC). IPCC, Geneva, Switzerland.

Jarvis, P.G. 1976. The interpretation of the variations in leaf water potential and stomatal conductance found in canopies in the field. Philos. Trans. Royal Soc. London. B, Biol. Sci. 273:593–610.

Karamura, D.A., E.B. Karamura, and W. Tinzaara. 2012. Banana cultivar names, synonyms and their usage in Eastern Africa. Bioversity International, Kampala, Uganda.

Kayongo, S.N., J.M. Sebuliba, and K. Nyombi. 2015. Responses of East African highland banana (EAHB-AAA) cultivars to drought stress. Uganda J. Agric. Sci. 16:55-81.

Kissel, E., P. van Asten, R. Swennena, J. Lorenzen, and S.C. Carpentier. 2015.Transpiration efficiency versus growth: Exploring the banana biodiversity for drought tolerance. Sci. Hortic. 185:175–182.

Kitavi, M., T. Downing, J. Lorenzen, D. Karamura, M. Onyango, et al. 2016. The triploid East African Highland Banana (EAHB) genepool is genetically uniform arising from a single ancestral clone that underwent population expansion by vegetative propagation. Theor. Appl. Genet. 129:547-561.

Leonardi, C., S. Guichard, and N. Bertin. 2000. High vapor pressure deficit influences growth, transpiration and quality of tomato fruits. Sci. Hortic. 84:285-296.

Lynam, J.K. 2000. Market development and production potential for banana and plantain. Acta Hortic. 540:39-53.

Manners, R., E. Vandamme, J. Adewopo, P. Thornton, M. Friedmann, et al. 2021. Suitability of root, tuber, and banana crops in Central Africa can be favored under future climates. Agric. Syst. 193:103246.

Marimo, P., D. Karamura, R. Tumuhimbise, M.M. Shimwela, I. Van den Bergh, et al. 2019. Post-harvest use of banana in Uganda and Tanzania: Product characteristics and cultivar preferences of male and female farmers. CGIAR Research Program on Roots, Tubers and Bananas (RTB). RTB Working Paper. No.3.

Martin-StPaul, N.K., J.M. Limousin, H. Vogt-Schilb, J. Rodríguez-Calcerrada, S. Rambal, et al. 2013. The temporal response to drought in a Mediterranean evergreen tree: comparing a regional precipitation gradient and a throughfall exclusion experiment. Global Change Biol. 19:2413-2426.

McNellis, B., and A.R. Howard. 2015. Phylogenetic and ecological patterns in nighttime transpiration among five members of the genus Rubus co-occurring in western Oregon. Ecol. Evol. 5:3557-69.

Melorose, J., R. Perroy, and S. Careas. 2015. World population prospects. United Nations 1:587–592.

MFA-NL. 2018. Climate Change Profile: East African Great Lakes and Ruzizi Plain. Ministry of Foreign Affairs of the Netherlands (MFA-NL), The Hague, The Netherlands.

Mohammed, U.D., S.A. Legesse, A.B. Berlie, and M.A. Ehsan. 2022. Climate change repercussions on meteorological drought frequency and intensity in South Wollo, Ethiopia. Earth Syst. Environ. 6:645-55.

Munné-Bosch, S., and L. Alegre. 2004. Die and Let Live: Leaf senescence contributes to plant survival under drought stress. Funct. Plant Biol. 31:203–216.

Mustaffa, M.M., and V. Kumar. 2012. Banana production and productivity enhancement through spatial, water and nutrient management. J. Hortic. Sci. 7: 1–28.

Mutai, C.C., M.N. Ward, and A.W. Colman. 1998. Towards the prediction of the East Africa short rains based on sea-surface temperature–atmosphere coupling. Int. J. Climatol: A J. Royal Meteorol. Soc. 18:975-97.

Nansamba, M., J. Sibiya, R. Tumuhimbise, W. Ocimati, E. Kikulwe, et al. 2022. Assessing drought effects on banana production and on-farm coping strategies by farmers — a study in the cattle corridor of Uganda. Clim. Change. 173:21.

Napier, J.D., R.W. Heckman, and T.E. Juenger. 2023. Gene-by-environment interactions in plants: Molecular mechanisms, environmental drivers, and adaptive plasticity. The Plant Cell. 35:109-124.

Nash, J. 2018. Rvmmin: Variable Metric Nonlinear Function Minimization. https://cran.rproject.org/package=Rvmmin . Accessed 12 February 2023.

Němečková. A., P. Christelová, J. Čížková, M. Nyine, I. Van den Houwe, et al. 2018. Molecular and cytogenetic study of East African highland banana. Front. Plant Sci. 9:1371.

Ochola, D., B. Boekelo, G.W. van de Ven, G. Taulya, J. Kubiriba, et al. 2022. Mapping spatial distribution and geographic shifts of East African highland banana (*Musa* spp.) in Uganda. Plos One 17(2):e0263439.

Paine, C.E.T., T.R. Marthews, D.R. Vogt, D. Purves, M. Rees, et al. 2012. How to fit nonlinear plant growth models and calculate growth rates: an update for ecologists. Methods. Ecol. Evol. 3:245–256.

Perrier, X., E. De Langhe, M. Donohue, C. Lentfer, L. Vrydaghs, et al. 2011. Multidisciplinary perspectives on banana (*Musa* spp.) domestication. Proc. Natl. Acad. Sci. 108:11311-11318.

Perrier, X., C. Jenny, F. Bakry, D. Karamura, M. Kitavi, et al. 2019. East African diploid and triploid bananas: a genetic complex transported from South-East Asia. Ann. Bot. 123:19-36.

Pillay, M., E. Ogundiwin, D. Nwakanma, G. Ude, and A. Tenkouano. 2001. Analysis of genetic diversity and relationships in East African banana germplasm. Theor. Appl. Genet. 102:965–970.

Prohens, J., P. Gramazio, M. Plazas, H. Dempewolf, B. Kilian, et al. 2017. Introgressiomics: a new approach for using crop wild relatives in breeding for adaptation to climate change. Euphytica. 213:1-9.

R Core Team. 2022. R: A language and environment for statistical computing. R Foundation

for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Ramirez, J., A. Jarvis, I. Van den Bergh, C. Staver, and D. Turner. 2011. Chapter 20: Changing climates: Effects on growing conditions for banana and plantain (*Musa* spp.) and possible responses, p.426-438, *In* S. Yadav, B. Redden, J.L. Hattfield, and H. Lotze-Campen, eds. Crop adaptation to climate change. Wiley-Blackwell Publishing Ltd, Chichester, United Kingdom.

Ravi, I., S. Uma, M.M. Vaganan, and M.M. Mustaffa. 2013. Phenotyping bananas for drought resistance. Front. Physiol. 4:1-15.

Raza, A., A. Razzaq, S.S. Mehmood, X. Zou, X. Zhang, et al. 2019. Impact of climate change on crops adaptation and strategies to tackle its outcome: A review. Plants. 8:34.

Rippke, U., J. Ramirez-Villegas, A. Jarvis, S.J. Vermeulen, L. Parker, et al. 2016. Timescales of transformational climate change adaptation in sub-Saharan African agriculture. Nat. Clim. Change. 6:605-609.

Robinson, J.C., and J.P. Bower. 1988. Transpiration from banana leaves in the subtropics in response to diurnal and seasonal factors and high evaporative demand. Scientia Hortic. 37:129–143.

Sabiiti, G., J.M.Ininda, L.A. Ogallo, J. Ouma, G. Artan, et al. 2018. Adapting agriculture to climate change: suitability of banana crop production to future climate change over Uganda. Limits to climate change adaptation. 175-190.

Sardos, J., X. Perrier, J. Doležel, E. Hřibová, P. Christelová, et al. 2016. DArT whole genome profiling provides insights on the evolution and taxonomy of edible banana (*Musa* spp.). Ann. Bot. 118:1269-78.

Sardos, J., A. Cenci, G. Martin, C. Breton, V. Guignon, et al. 2025. Painting the diversity of a world's favourite fruit: A next generation catalog of cultivated bananas. Plants, People, Planet. 7: 263-283.

Shepard, D. 1968. A two-dimensional interpolation function for irregularly-spaced data, p.517-524. Proceedings of the 1968 23rd ACM National Conference.

Šimoníková, D., A. Němečková, J. Čížková, A. Brown, R. Swennen, et al. 2020. Chromosome painting in cultivated bananas and their wild relatives (*Musa* spp.) reveals differences in chromosome structure. Int. J. Mol. Sci. 21:7915.

Singh, S. 2016. Guttation: Mechanism, momentum and modulation. Bot. Rev. 82:149-82.

Ssebuliba, R., M. Magambo, D. Talengera, D. Makumbi, A. Tenkouano, et al. 2006. Biological factors affecting seed production in East African Highland Bananas. J. Crop Improv. 16:67-79.

Stevens, B., J. Diels, A. Brown, S. Bayo, P.A. Ndakidemi, and R. Swennen. 2020. Banana biomass estimation and yield forecasting from non-destructive measurements for two contrasting cultivars and water regimes. Agronomy. 10:1435.

Summerville, W.A.T. 1944. Studies on nutrition as qualified by development in *Musa* Cavendishii Lamb. J. Agric. Meteorol. 12: 361–389.

Tardieu, F. 2012. Any trait or trait-related allele can confer drought tolerance: just design the right drought scenario. J. Exp. Bot. 63:25–31.

Taulya, G., P.J.A. van Asten, S. Okech, and C. Gold. 2006. Effect of drought on the yields of an East African highland banana (cv. 'Kisansa') in Uganda. The proceedings of the 23rd Soil Science Society of East Africa conference. Masaka, Uganda.

Tugume, A.K., G.W. Lubega, and P.R. Rubaihayo. 2002. Genetic diversity of East African Highland bananas using AFLP. InfoMusa. 11:28–32.

Tumuhimbise, R., A. Barekye, J. Kubiriba, K. Akankwasa, I.K. Arinaitwe, et al. 2018. New high-yield cooking banana cultivars with multiple resistances to pests and diseases ('NAROBan1','NAROBan2','NAROBan3', and 'NAROBan4') released in Uganda. HortScience. 53:1387-1389.

Uwimana, B., Y. Zorrilla-Fontanesi, J. van Wesemael, H. Mduma, A. Brown, et al. 2020. Effect of seasonal drought on the agronomic performance of four banana genotypes (*Musa* spp.) in the East African Highlands. Agronomy. 11:4.

Vadez, V. 2014. Root hydraulics: the forgotten side of roots in drought adaptation. Field Crops Res. 165:15–24.

Van Asten, P.J.A, A.M. Fermont, and G. Taulya. 2011. Drought is a major yield loss factor for rainfed East African Highland Banana. Agric. Water Manage. 98:541–552.

Van Wesemael, J., E. Kissel, D. Eyland, T. Lawson, R. Swennen, and S. Carpentier. 2019. Using growth and transpiration phenotyping under controlled conditions to select water efficient banana genotypes. Front. Plant Sci. 10:354.

Vandamme, E., R. Manners, J. Adewopo, G. Thiele, M. Friedmann, and P. Thornton. 2022. Strategizing research and development investments in climate change adaptation for root, tuber and banana crops in the African Great Lakes Region: A spatial prioritization and targeting framework. Agric. Syst. 202:103464.

Vandenhout, H., R. Ortiz, D. Vuylsteke, R. Swennen, and K.V. Bai. 1995. Effect of ploidy on stomatal and other quantitative traits in plantain and banana hybrids. Euphytica. 83:117-122.

Varma, V., and D.P. Bebber. 2019. Climate change impacts on banana yields around the world. Nat. Clim. Change. 9:752–757.

Von Caemmerer, S., and N. Baker. 2007. The biology of transpiration. From guard cells to globe. Plant Physiology. 143:3.

Vuylsteke, D.R., R. Swennen, and D. de Langhe. 1990. Tissue culture technology for the improvement of Africa plantains. Sigatoka leaf spot diseases of bananas, *In* Proceedings of an international workshop held by the International Network for Improvement of Bananas and Plantain (INIBAP). INIBAP, Montpellier, France.

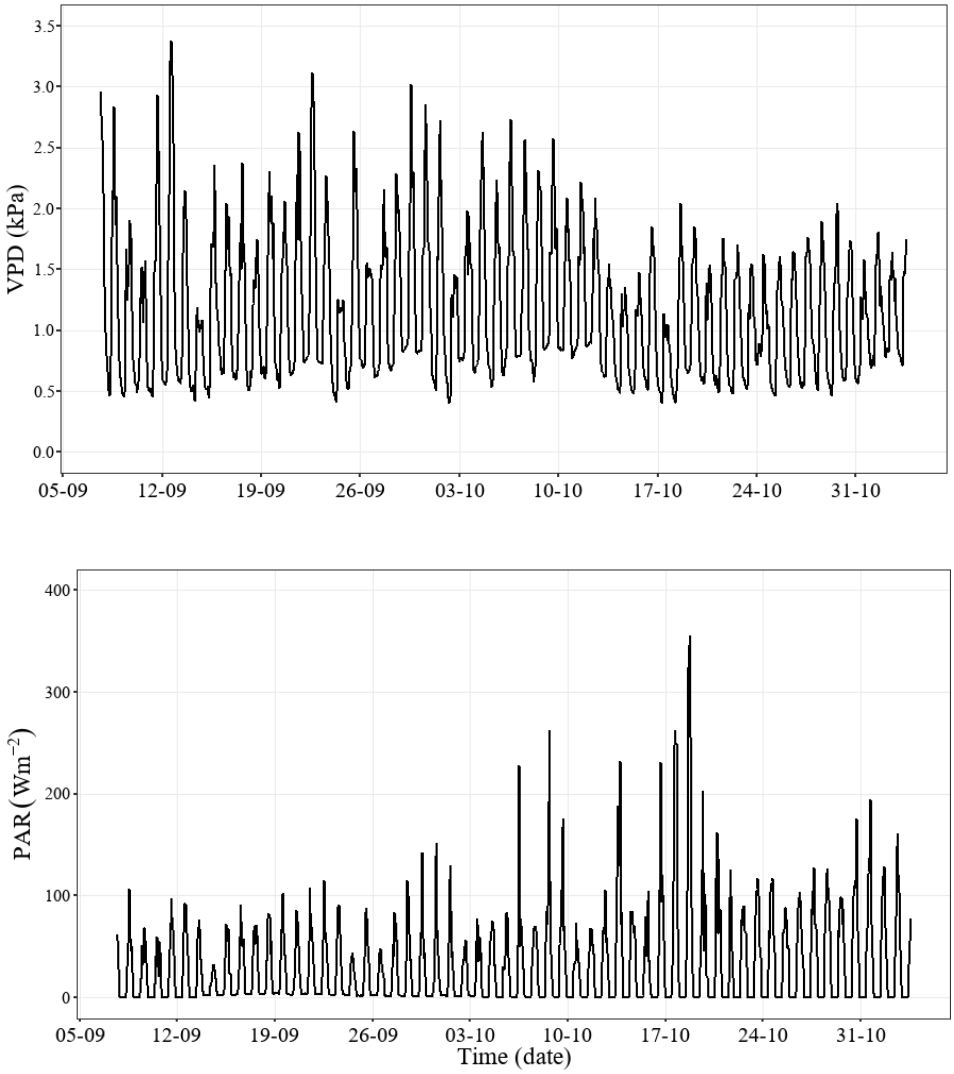
Wang, Z., T.Q. Zhang, C.S. Tan, L. Xue, M. Bukovsky, and Z.M. Qi. 2021. Modelling impacts of climate change on crop yield and phosphorus loss in a subsurface drained field of Lake Erie region, Canada. Agric. Syst. 190:103110.

Waniale, A., S.B. Mukasa, A.K. Tugume, J. Kubiriba, W.K. Tushemereirwe, and R. Tumuhimbise. 2022. Early withering of enlarged ovules in pollinated fruits of bananas (*Musa* spp.) suggest abortion after fertilization. Horticulturae. 10:426.

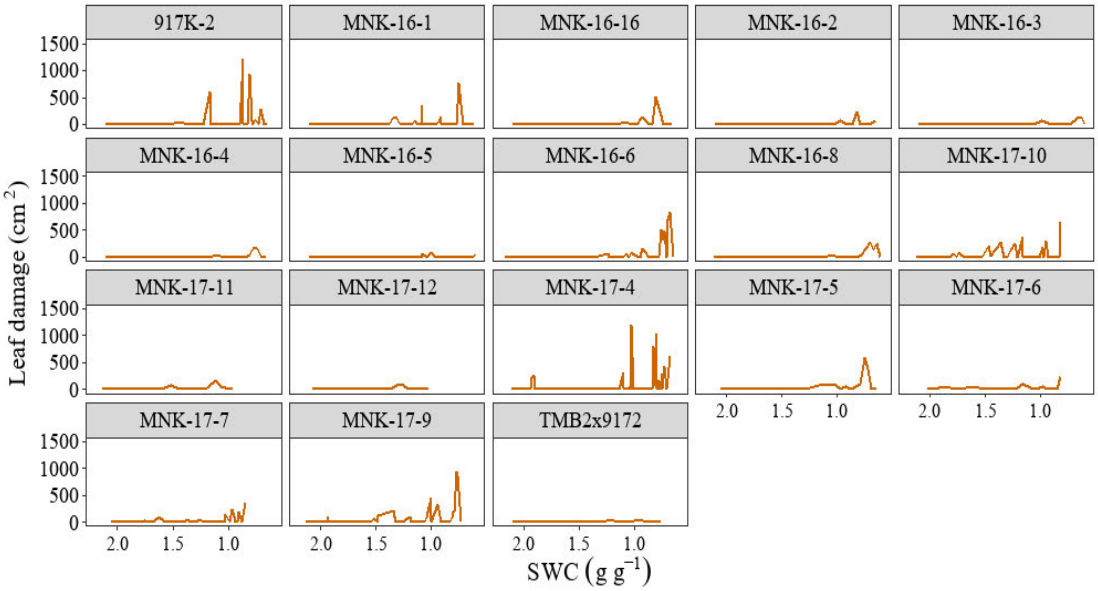
Whitley, R., B. Medlyn, M. Zeppel, C. Macinnis-Ng, and D. Eamus. 2009. Comparing the Penman-Monteith equation and a modified Jarvis-Stewart model with an artificial neural network to estimate stand-scale transpiration and canopy conductance. J. Hydrol. 373:256–266.

Wortmann, C.S., and C.A. Eledu. 1999. Uganda's Agroecological Zones: A guide for planners and policy makers. Centro Internacional de Agricultura Tropical, Kampala, Uganda.

7.6 Appendices



Appendix 7.1 Environmental conditions measured in the greenhouse hosted at the Bioversity International phenotyping facility in Leuven, Belgium. VPD values are the mean of eight environmental sensors evenly distributed across the greenhouse. Light intensity (PAR) was recorded in the centre of the greenhouse.



Appendix 7.2 Leaf area damage of 18 banana genotypes (two parents [917K-2 and TMB2x9172] and their offsprings) increased with progressive declining soil water content.

Appendix 7.3 Classification of 18 banana genotypes (two parents [917K-2 and TMB2x9172] and their offsprings) based on the transpiration behaviour and associated leaf damage.

Genotype	Transpiration behaviour	Leaf damage (cm^2)	Classification based on water deficit (i.e. is not able to keep E_{max})	Classification based on E_{rate} reduction	General phenotype
MNK-17-4	2.7	1827	is not responsive to water deficit and is able to keep E_{max} until a low soil water content (risk taker)	has a strong reduction in Erate	acceptable damage in relation to transpiration behaviour
MNK-17-5	2.2	1492	is not responsive to water deficit and is able to keep E_{max} until a low soil water content (risk taker)	has a strong reduction in Erate	acceptable damage in relation to transpiration behaviour
MNK-17-9	1.1	2087	no deviating transpiration behaviour	no deviating transpiration behaviour	drought sensitive, has more damage as anticipated by transpiration behaviour
917K-2	0.8	1270	is not responsive to water deficit and is able to keep E_{max} until a low soil water content (risk taker)	has a strong reduction in Erate	acceptable damage in relation to transpiration behaviour
MNK-16-16	0.6	970	no deviating transpiration behaviour	no deviating transpiration behaviour	acceptable damage in relation to transpiration behaviour
MNK-17-7	0.5	667	no deviating transpiration behaviour	no deviating transpiration behaviour	has less damage as anticipated by transpiration behaviour
MNK-16-8	0.5	1237	no deviating transpiration behaviour	no deviating transpiration behaviour	acceptable damage in relation to transpiration behaviour
MNK-16-3	0.4	600	is not responsive to water deficit and is able to keep E_{max} until a low soil water content (risk taker)	no deviating transpiration behaviour	has less damage as anticipated by transpiration behaviour
MNK-17-11	-0.5	404	is very responsive to water deficit reducing E_{max} at a high soil water content	no deviating transpiration behaviour	has less damage as anticipated by transpiration behaviour
MNK-17-12	-0.5	281	is very responsive to water deficit reducing E_{max} at a high soil water content	has a low reduction in Erate	has less damage as anticipated by transpiration behaviour
MNK-16-5	-0.6	125	no deviating transpiration behaviour	no deviating transpiration behaviour	has less damage as anticipated by transpiration behaviour

Genotype	Transpiration behaviour	Leaf damage (cm^2)	Classification based on water deficit (i.e. is not able to keep E_{max})	Classification based on E_{rate} reduction	General phenotype
MNK-16-4	-0.6	475	no deviating transpiration behaviour	no deviating transpiration behaviour	acceptable damage in relation to transpiration behaviour
TMB2x9172	-0.7	158	no deviating transpiration behaviour	no deviating transpiration behaviour	has less damage as anticipated by transpiration behaviour
MNK-16-6	-0.7	1641	is not responsive to water deficit and is able to keep E_{max} until a low soil water content (risk taker)	has a low reduction in Erate	drought sensitive, has more damage as anticipated by transpiration behaviour
MNK-16-2	-0.7	444	no deviating transpiration behaviour	no deviating transpiration behaviour	acceptable damage in relation to transpiration behaviour
MNK-16-1	-0.7	868	no deviating transpiration behaviour	no deviating transpiration behaviour	acceptable damage in relation to transpiration behaviour
MNK-17-10	-0.8	1497	is very responsive to water deficit reducing E_{max} at a high soil water content	no deviating transpiration behaviour	drought sensitive, has more damage as anticipated by transpiration behaviour
MNK-17-6	-2.8	606	is very responsive to water deficit reducing Emax at a high soil water content	no deviating transpiration behaviour	acceptable damage in relation to transpiration behaviour

E_{rate} = Transpiration rate; E_{max} = maximum transpiration rate.

Chapter 8: General overview and implications of the study

8.1 Introduction

Banana (*Musa* spp.) is one of the major food crops in the world and a key income source for smallholder resource-limited rural farmers, particularly in central and east African regions. However, the potential of the banana crop has not been fully realized due to several production challenges, including biotic and abiotic constraints. Drought is the primary limiting abiotic constraint, causing large banana yield losses due to significant reductions in bunch weight and loss of productive mats. Sustainable banana production in the East African region, where East African highland bananas (EAHB) dominate the cropping system, is greatly threatened by recurrent droughts caused by climate change and variability. The high vulnerability of EAHBs to moisture stress necessitates developing drought-tolerant cultivars that can sustain growth during periods of limited soil water. The present study, therefore, specifically aimed to (i) assess the effects of drought on banana production and identify management strategies deployed by farmers (ii) determine the response of 14 *Musa* genotypes to water stress based on phenotypic and physiological traits and to select promising genotypes for use in banana drought tolerance breeding (iii) to analyse the hybridisation success of the two selected drought-tolerant candidate male parental lines based on pollination success, embryo recovery and embryo germination rates and to generate genotypes for use in subsequent genetic drought tolerance analyses (iv) to determine the variability of water usage and assess the growth behaviour and transpiration responses of secondary banana hybrids and their parental lines to declining soil water content in function of light intensity and vapour pressure deficit and (v) determine the genetic relationships and genetic diversity among 55 banana genotypes using DArT-based SNP markers.

The study identified several (15) effects of drought on banana growth, bunch yield and yield-related attributes that should be considered during the screening and assessment of germplasm for drought tolerance, especially under field conditions. Twelve drought mitigation measures were deployed by banana farmers in the study region at different times (i.e., before or during drought period) and for different purposes. Three drought-tolerant

candidate genotypes were also identified, and two of them were used as male parents in subsequent banana crosses. Given its high hybridization efficiency and success, TMB2x9172 appeared to be the ideal drought-tolerant candidate male parent for generating progeny populations for subsequent drought tolerance studies compared to TMB2x9722-1.

The study also reported for the first time the performance of banana hybrids developed purposely for enhanced drought tolerance and avoidance. Great variability in water usage among test F1 *Musa* spp. (with an EAHB background), growth and transpiration responses to declining soil water content in conjunction with fluctuating environmental conditions caused by a meiotic event were detected. Principal component (PC) analysis classified the F1 progenies into two heterotic groups based on the genetic background of their parental genotypes. Further, genetic prediction based on PC analysis revealed that NM101F1, MNK-17-11 and MNK-17-12 were the most genetically similar to the drought-tolerant candidate male parent (TMB2x9172), while the rest of the F1s had a higher genetic resemblance with their female parent (917K-2). NM101F1, MNK-17-11 and MNK-17-12 have the potential to be used in future gene discovery and or advanced field testing for drought tolerance.

8.2 Implications and recommendations of research findings to banana drought tolerance breeding

8.2.1 Assessing drought effects on banana production and on-farm coping strategies by farmers – a study in the cattle corridor of Uganda

Knowledge of the impact of water stress on the growth and yield of East African highland bananas (EAHBs), as well as the genetic diversity maintained on-farm, would largely support the successful breeding and deployment of cultivars with improved drought tolerance in the East African Great Lakes region (EAGL). This study's findings indicate that drought poses a major threat to sustainable banana production and food- and income security in Uganda's cattle corridor region or the EAGL region in general. Therefore, there is an urgent need for banana breeders to improve the existing EAHBs for drought tolerance through classical or modern breeding approaches. Further, researchers should consider

farmers' traits or indicators of drought tolerance or sensitivity during screening of germplasm for drought tolerance. This will ensure that the traits measured by researchers are translated back to the farmers and only then will farmers be convinced that the recommended varieties or hybrids are drought-tolerant. Particularly, for field experiments, scientists should prioritise floral and yield-related traits such as flowering time, bunch weight, number and size of hands and fingers. These traits are the most important indicators of the impact of water stress / drought on banana yield. Still on-farm, farmers need to prioritise preventive coping strategies and ensure timely deployment of mitigation practices. Lastly, there is a need to strengthen data collection and climate information systems to generate more reliable data for better climate predictions.

8.2.2 Response of banana (*Musa* spp.) to water stress based on phenotypic and physiological traits

The three promising drought-tolerant genotypes, including 'ITC.0987', 'TMB2x9722-1' and 'TMB2x9172', are valuable genetic resources for banana drought tolerance and are recommended for further field testing to confirm whether the observed WUE increase under stress will translate into reasonable yield, particularly during the drought seasons. Very importantly, breeders could utilise these valuable superior candidates as male parents to cross with other cultivars (having desirable agronomic and taste attributes) to improve banana drought tolerance anytime soon. Moreover, these candidates could be used to generate *Musa* populations segregating for drought tolerance that are required in several genetic investigations, such as quantitative trait loci analysis, marker-trait associations, etc., under well-watered and water stress conditions. This study also confirmed the high sensitivity of 'Mpologoma' to water stress, as previously reported by farmers in the cattle corridor of Uganda (Chapter 3). Therefore, 'Mpologoma' is recommended as a drought-sensitive check in routine drought tolerance screening experiments. Furthermore, this study recommends plant height, total leaf area, total dry matter and WUE as promising traits to detect the significant impact of water stress on the early growth stage. Therefore, researchers should consider these traits in future screening initiatives.

8.2.3 Evaluation of hybridisation success of two improved drought-tolerant candidate diploid bananas (*Musa* spp.)

In this study, two promising drought-tolerant candidates, including 'TMB2x9722-1' and 'TMB2x9172', were each crossed with four tetraploid breeding lines ('660K-1', '917K-2', '1201K-1' and '222K-1') having enhanced pest and disease resistance and bunch yield. 'TMB2x9172' had relatively higher pollination success, embryo recovery and germination compared to 'TMB2x9722-1'. Therefore, 'TMB2x9172' is not only a valuable genetic resource for generating progeny populations for subsequent drought tolerance investigations but also for integrating drought tolerance in banana through crossbreeding. On the other hand, the poor hybridization success observed in crosses with 'TMB2x9722-1' could greatly limit its utilization in banana drought tolerance crossbreeding programs. Nonetheless, the putative drought tolerance genes in 'TMB2x9722-1' could be identified, extracted, stacked and incorporated into commercial cultivars using genetic engineering techniques. Given that 'TMB2x9722-1' is a potential host of drought tolerance genes/ alleles, it might be worthwhile to cross it with 'TMB2x9172' to generate and select offspring that are even more drought tolerant. Lastly, further investigation of the possible causes for the low male fertility levels (e.g., pollen and embryo studies) in 'TMB2x9722-1' is required as this knowledge will be essential in improving its usability in banana drought tolerance improvement through crossbreeding.

8.2.4 Genetic relationships and diversity analysis of banana germplasm using Diversity Array Technology (DArT)-based SNP markers: implications for banana drought tolerance breeding

Neighbour-joining (NJ) clustering provided important insight into the genetic relationships among 54 *Musa* genotypes. The study's genotypes were grouped into two major clusters. Cluster I comprised genotypes solely containing the '*acuminata*' genome (except for 'TMB2x9722-1' whose genomic constitution is not yet known) while all the genotypes with a '*balbisiana'* genome clustered together in cluster II. The study further zoomed into the genetic relationships among 16 selected *Musa* progenies. The unique sub-clustering of these progenies is indicative of greater genetic similarity among progenies within a given sub-cluster. Principal component analysis (PCA) was consistent with the NJ tree and

partially grouped the progenies into two heterotic groups based on the genetic distances from their parental genotypes. PCA predicted that three of the *Musa* progenies, including 'NM101F1', 'MNK-17-11' and 'MNK-17-12', were the most genetically identical to the drought-tolerant candidate male parent ('TMB2x9172') while the 35 remaining progenies had closer genetic proximity to the female parent ('917K-2'). The genetic diversity of the study population was affected by the presence of common ancestors. For instance, 'Calcultta-4' is either a male parent or grandparent of many of the study's genotypes, particularly the breeding lines and progenies. Nonetheless, the DArT-based SNP markers used in this study distinguished the germplasm. This study lays a foundation for further exploration (e.g. gene discovery) and validation of drought tolerance, particularly within the progenies.

8.2.5 Differential growth and transpiration responses among banana hybrids - hope for banana drought tolerance breeding?

Improving drought tolerance of EAHBs predominantly grown in the EAGL region without compromising their great agronomic and taste qualities would potentially impact the livelihoods of millions of banana farmers in the region. The water-conservative and relatively drought-tolerant hybrids, including 'MNK-17-11' and 'MNK-17-12', should be further tested for their growth and yield potential under both optimum and water stress field conditions. In this field testing, the agronomic traits reported by farmers in Chapter 3 should be given priority to ensure that the traits measured by scientists reflect farmers' perceptions of drought tolerance and /or sensitivity. The contrasting phenotypic response of genetically similar progenies could be either due to the significant impact of the environment on the plant's phenotype or the underlying genetic differences between these progenies. The consistent genetic clustering (Chapter 6) and similar phenotypic behaviour of 'MNK-17-11' and 'MNK-17-12' with the drought-tolerant male parent ('TMB2x9172') implies that 'TMB2x9172' is a suitable 'transferer' of tolerance genes into its offsprings. The great variation in the water usage, growth and transpiration behaviour in relation to the fluctuating environment of the *Musa* progenies implied that the suitability of a given genotype would depend on the prevailing drought scenario. Therefore, the studied progenies could be deployed in environments that best suit them. The great genotypic variation in response to water stress conditions also implied that the tested *Musa* progenies

were segregating for drought tolerance at some loci. Therefore, further research (with a larger progeny segregating population) into the mode of inheritance of tolerance to water stress is recommended. Progeny studies normally utilise hundreds of progenies, but in this study, it was challenging to generate several progenies due to insufficient funds (huge research budget cut), inability to travel to the banana fields where crosses were done (due to the national travel ban during the recent Covid-19 pandemic), numerous administrative challenges and time limitations. Still, because of the Covid-19 lockdown and travel ban, monitoring the banana embryos and plantlets developing on growth media in the tissue culture laboratory was impossible. As such, a considerable number of tissue culture plantlets were lost to contamination during the lockdown. Despite the above challenges, the preliminary results from the few *Musa* progenies give hope that banana drought tolerance improvement through crossbreeding is possible.