UNIVERSIDADE ESTADUAL DE SANTA CRUZ

PROGRAMA DE PÓS-GRADUAÇÃO EM GENÉTICA E BIOLOGIA MOLECULAR

RESPOSTAS DE GENÓTIPOS DE BANANEIRA AO DÉFICIT HÍDRICO

ADRIADNA SOUZA SANTOS

ILHÉUS – BAHIA – BRASIL

Fevereiro de 2019

ADRIADNA SOUZA SANTOS

RESPOSTAS DE GENÓTIPOS DE BANANEIRA AO DÉFICIT HÍDRICO

Tese apresentada à Universidade Estadual de Santa Cruz como parte das exigências para obtenção do título de Doutora em Genética e Biologia Molecular.

Área de concentração: Genética e Biologia Molecular.

ILHÉUS – BAHIA – BRASIL

Fevereiro de 2019

S237 Santos, Adriadna Souza.
Resposta de genótipos de bananeira ao déficit hídrico / Adriadna Souza Santos. – Ilhéus, BA: UESC, 2019.
46f. : il.

Orientador: Carlos Priminho Pirovani.
Tese (Doutorado) – Universidade Estadual de Santa Cruz. Programa de Pós-Graduação em Genética e Biologia Molecular.
Inclui referências.

1. Bananeira – Melhoramento genético. 2. Plantas – Efeito da seca. 3. Genótipos (Análise). I. Título.

CDD 634.772

ADRIADNA SOUZA SANTOS

RESPOSTAS DE GENÓTIPOS DE BANANEIRA AO DÉFICIT HÍDRICO

Tese apresentada à Universidade Estadual de Santa Cruz, como parte das exigências para obtenção do título de Doutora em Genética e Biologia Molecular.

Área de concentração: Genética e Biologia Molecular.

APROVADA: 25 de fevereiro de 2019

Prof. Fabio Pinto Gomes	Profª. Fabienne Micheli	Profª. Fabiana Zanelato Bertolde
UESC - Membro 1	UESC - Membro 2	IFBA - Membro 3

Prof. Laís Freire Santos	Prof. Carlos Priminho Pirovani
Escola Adventista - Membro 4	UESC - Orientador

DEDICATÓRIA

Dedico a Deus, autor de minha existência.

À mamis e papis, os melhores pais do mundo.

Ao Elivaldo, esposo e companheiro nessa jornada.

EPÍGRAFE

"Tenho-vos dito isto, para que em mim tenhais paz; no mundo tereis aflições, mas tende bom ânimo, eu venci o mundo".

João 16:33

AGRADECIMENTOS

A Deus, meu alicerce, minha força e proteção.

Aos meus pais, Renilda Menezes de Souza e José Alfredo dos Santos, que deram todo o apoio espiritual e psicológico e, por muito tempo, financeiro, para que eu pudesse cumprir essa longa jornada de estudos e conhecimento.

À toda minha família, especialmente tia Railda Menezes de Souza que foi instrumento de Deus em muitas decisões que precisei tomar e que culminaram em minha felicidade.

Ao meu esposo, Elivaldo Lozer Fracalossi Ribeiro, que se juntou a mim no início de minha vida acadêmica. Nesses anos teve que ser paciente, interceder, corrigir e amparar infinitas vezes.

Aos meus sogros Sr. Liu e D. Beth que cuidam de mim como filha.

Às amigas de graduação Milena, Regina, Ana Paula e Laíse, que levarei para a vida. E às pessoas maravilhosas que conheci por meio delas, Guto, Edu, Héber e nossa princesa mais linda Lulu. Aos amigos da pós-graduação, os SATianos e as CBGet's, especialmente Luana, Diana, Tainã e Maria que se fizeram presentes nos últimos 4 anos. E mais recentemente, a Monique, Giovano e Christian, sempre parceiros.

Ao meu orientador, Prof. Carlos Priminho Pirovani, que me acolheu em uma nova área, ensinando, apoiando e desafiando, para que eu pudesse crescer e expressar o melhor de mim. Obrigada, pelo exemplo de humildade, perseverança e fé em Deus. Agradeço, pelas orações direcionadas a mim. Foi recíproco.

Aos professores e parceiros, Drª Claudia Fortes Ferreira, Dr. Maurício Antônio Coelho Filho e Dr. Edson Perito Amorim, pela atenção e auxílio no desenvolvimento da pesquisa.

A Embrapa Mandioca e Fruticultura, pela estrutura e suporte. Aos amigos que encontrei por lá, especialmente, Andressa, Elaine e Matheus pela acolhida.

À UESC e ao CBG também, pela estrutura e aos funcionários que ajudaram direta e indiretamente na realização desse trabalho, especialmente Horlei.

À CAPES, pelo apoio financeiro.

ÍNDICE

RESUMO

SANTOS, Adriadna Souza. Universidade Estadual de Santa Cruz, Ilhéus, 25 de fevereiro de 2019. **Respostas de genótipos de bananeira ao déficit hídrico**. Orientador: Carlos Priminho Pirovani. Coorientadores: Drª. Claudia Fortes Ferreira e Alex-Alan Furtado de Almeida. Colaboradores: Dr. Mauricio Antônio Coelho Filho e Dr. Edson Perito Amorim.

Fatores abióticos, como, temperatura, precipitação, luminosidade, salinidade e umidade do solo, têm um alto impacto nas relações hídricas das plantas. As bananeiras, apesar de serem originadas em regiões de clima tropical e subtropical, onde são afetadas por condições climáticas adversas, apresentam efeitos negativos do estresse hídrico sobre seu crescimento, produtividade e desenvolvimento. Para compreender os mecanismos fisiológicos, bioquímicos e moleculares que conferem tolerância ao déficit hídrico em bananeiras, esse trabalho apresenta no Capítulo 1, a primeira revisão sistemática de literatura sobre o tema, no Capítulo 2, um método inédito de seleção de diploides tolerantes à seca e no Capítulo 3, o estudo do proteoma das raízes de genótipos contrastantes quanto à deficiência hídrica, em condições controle e submetidos ao déficit hídrico. No primeiro capítulo, um protocolo previamente estabelecido e objetivos bem definidos foram utilizados para selecionar 47 artigos em portais acadêmicos de livre acesso. Os resultados indicam que existem lacunas nos estudos publicados sobre estresse hídrico em bananeira, especialmente sobre as proteínas expressas em diferentes tecidos, mecanismos de regulação pós-traducionais, herança epigenética e estresse por inundação. No segundo capítulo, um sistema hidropônico foi testado em 14 genótipos de bananeira, sendo 12 diploides e duas variedades comerciais de resposta conhecida quanta a tolerância seca, Grand Naine (altamente susceptível ao déficit hídrico) e o híbrido BRS Princesa (tolerante ao déficit hídrico). Os resultados demostram que esse sistema pode ser de grande utilidade para seleção de genótipos de bananeira tolerantes a ambientes secos. No terceiro capítulo foi realizada a análise do proteoma radicular, por eletroforese bidimensional, do genótipos PMGB043 e PMGB099, considerados suscetível e tolerante ao déficit hídrico, respectivamente, com base nas respostas fisiológicas dos genótipos apresentadas no Capítulo 2. Os resultados evidenciam que as extrações de proteínas utilizando TCA em Acetona, seguido de Extração com fenol/SDS-acetato de amônio são eficientes para isolar proteína em alta qualidade de tecidos recalcitrantes de bananeira, como raízes. O genótipo susceptível apresentou mais *spots* proteicos no tratamento controle e o genótipo tolerante apresentou mais *spots* proteicos no tratamento estresse severo. Esse trabalho fornece um estudo completo dos diferentes níveis de regulação ativados em bananeiras submetidos ao déficit hídrico. Além disso, apresenta lacunas que precisam ser investigadas em trabalhos futuros.

Palavras-chave: genes candidatos, tolerância a seca, *Musa* spp., s*pots* proteicos, melhoramento genético.

ABSTRACT

SANTOS, Adriadna Souza. Universidade Estadual de Santa Cruz, Ilhéus, February 25, 2019. **Responses of banana genotypes to water deficit**. Advisor: Carlos Priminho Pirovani. Co-advisors: Drª. Claudia Fortes Ferreira e Alex-Alan Furtado de Almeida. Contributors: Dr. Mauricio Antônio Coelho Filho e Dr. Edson Perito Amorim.

Abiotic factors, such as temperature, precipitation, luminosity, salinity and soil moisture, have a high impact on the water relations of plants. Although they originate in regions of tropical and subtropical climate, where they are affected by adverse climatic conditions, banana plants present negative effects of water stress on their growth, productivity and development. In order to understand the physiological, biochemical and molecular mechanisms that confer tolerance to water deficit in bananas, this work presents in Chapter 1, the first systematic review of the literature on the subject, in Chapter 2, an unprecedented method of selection of diploid tolerant to drought and in Chapter 3, the study of the proteome of roots of contrasting genotypes for water deficiency under control conditions and submitted to water deficit. In the first chapter, a pre-established protocol and well-defined objectives were used to select 47 articles in open-access academic portals. The results indicate that there are gaps in published studies on water stress in banana, especially on proteins expressed in different tissues, post-translational regulatory mechanisms, epigenetic inheritance and flood stress. In the second chapter, a hydroponic system was tested on 14 banana genotypes, 12 diploids and two commercial varieties with a known response, such as dry tolerance, Grand Naine (highly susceptible to water deficit) and hybrid BRS Princesa (tolerant to water deficit). The results show that this system can be very useful for selection of banana genotypes tolerant to dry environments. In the third chapter the analysis of the root proteome by two-dimensional electrophoresis of the genotypes PMGB043 and PMGB099, considered susceptible and tolerant to the water deficit, respectively, was performed. The results show that extractions of proteins using TCA in Acetone, followed by Extraction with phenol/SDS-ammonium acetate are efficient to isolate high-quality protein from recalcitrant banana tissues, such as roots. The susceptible genotype showed more protein spots in the control treatment and the tolerant genotype presented more protein spots in the treatment of severe stress. This work provides a complete study of the different levels of regulation activated in banana plants submitted to water deficit. Also, some gaps need to be investigated in future work**.**

Key words: candidate genes, drought tolerance, Musa spp., protein spots, genetical enhancement.

1. INTRODUÇÃO GERAL

O aquecimento global pode causar eventos de seca extrema em diferentes partes do mundo, promovendo a mortalidade de populações de planta e a modificação da distribuição espacial das espécies dominantes (ALIZADEH et al. 2014). Algumas plantas são naturalmente capazes de adaptar-se ás condições de seca episódica, através de processos complexos em nível molecular, bioquímico e fisiológico. O estudo dessas plantas pode servir de base para o conhecimento de mecanismos de resposta de tolerância à seca, que serão necessários à produção de variedades de rendimento econômico satisfatório sob estresse hídrico (FAROOQ et al. 2009).

As bananas são monocotiledôneas de grande porte, de ciclo de vida anual e consideradas extremamente sensíveis ao déficit hídrico (ROBINSON; BOWER 1986). Essa característica era atribuída ao elevado índice de área foliar, as raízes rasas e à baixa capacidade de absorção de água do solo. Entretanto, sugere-se que a rápida sinalização das raízes até as folhas confere essa sensibilidade, pois permite que a planta desacelere seu metabolismo, mantendo-se um estado hidratado por um longo período, embora haja perdas na produção (TURNER et al. 2007).

Para atualização dos conhecimentos disponíveis sobre os mecanismos de tolerância ao déficit hídrico das bananeiras, esse trabalho apresenta a primeira revisão sistemática de

literatura sobre os efeitos do estresse hídrico em diferentes variedades de *Musa* spp, além disso, indica um método inédito de *screening* de diploides tolerantes à seca e o estudo do proteoma de genótipos contrastantes quanto deficiência hídrica.

Na revisão sistemática foram selecionados artigos de qualidade, publicados em um determinado período, com base em critérios previamente estabelecidos e um objetivo geral, respondido através de conclusões confiáveis. Assim, a revisão aqui apresentada visou reconhecer componentes fisiológicos, bioquímicos e moleculares indicados nos últimos 10 anos, para conferir tolerância à seca em *Musa* spp.

Devido à importância das variedades comestíveis para a fruticultura mundial, observa-se que os genótipos mais estudados são triploides e tetraploides (VANHOVE et al., 2012; XU et al. 2014; MIAO et al. 2017), originados da hibridação de espécies diploides selvagens de *M. acuminata* Colla (genoma A) e *M. balbisiana* Colla (genoma B) (VON LOESECKE, 1949). Esse trabalho propõe a seleção prévia de diploides nunca caracterizados quanto a tolerância à seca, antes de utilizá-los em cruzamentos tradicionais e produção de variedades comerciais. Os diploides foram desenvolvidos pela Embrapa e são melhorados para resistência genética à 'Sigatoka-amarela' e murcha de Fusarium, raça 1 e alguns à 'Sigatoka-negra.

O uso de sistema de hidroponia adaptado, associado às análises fisiológicas, fenológicas e enzimáticas pode ser uma nova estratégia para seleção de diploides tolerantes a estresse hídrico. Muitos estudos utilizam ensaios *in vitro*, devido as condições controladas e rapidez dos resultados (BIDABADI et al. 2011; BIDABADI et al. 2012; VANHOVE et al. 2012; RUKUNDO et al. 2012), contudo, a criação de condições experimentais de déficit hídrico para uma cultura como banana que é de grande porte e longa duração, ainda apresenta muitas dificuldades práticas (RAVI et al. 2013).

O perfil proteico de raízes de bananeira pode fornecer informações inéditas sobre as mudanças bioquímicas em diferentes condições experimentais (VAGANAN et al. 2015), mas esses tecidos são recalcitrantes para extração de proteínas. Para obter perfis proteômicos de qualidade, alguns autores sugerem utilização de protocolos baseados em TCA em acetona, seguido de extração fenólica (BERTOLDE et al. 2014; PIROVANI et al. 2008). Por se tratar de um ácido forte, o TCA em Acetona, é eficaz na precipitação de proteínas, cessando a atividade de enzimas proteolíticas durante a extração. A Extração Fenólica subsequente elimina interferentes, como, polissacarídeos e outros contaminantes solúveis em água (SURABHI et al. 2016).

Portanto, este trabalho apresenta um estudo integrado dos efeitos do estresse hídrico sob aspectos fisiológicos, bioquímicos e moleculares em *Musa* ssp., abordagem ainda escassa para esse gênero (DAVEY et al. 2009), se comparado a outros com similar importância comercial, como, *Citrus* (NEVES et al. 2017; SOUSA et al. 2018), *Theobroma* (BERTOLDE et al. 2012; BERTOLDE et al. 2014; SANTOS et al. 2014) e *Vitis* (CASTELLARIN et al. 2007; CHAVES et al. 2010).

2. HIPÓTESE:

- Genótipos de bananeira apresentam respostas contrastantes de tolerância ao estresse hídrico com modificações fisiológicas, bioquímicas e moleculares.

3. OBJETIVOS:

3.1 Geral

- Reconhecer componentes fisiológicos, bioquímicos e moleculares que conferem tolerância à seca em *Musa* spp e identificar genótipos diploides contrastantes quanto ao déficit hídrico.

3.2 Específicos

- Apresentar uma atualização sobre as respostas de genótipos de bananeira ao déficit hídrico à luz da literatura científica de qualidade.

- Identificar genótipos diploides de *Musa acuminata* susceptíveis e tolerantes ao estresse hídrico através da análise de aspectos fisiológicos, atividade enzimática e *western blot*.
- Elucidar respostas bioquímicas diferenciais de genótipos de bananeira a partir da caracterização do perfil proteico e de redes de interação proteína-proteína.

Capítulo 1

PLOS ONE

RESEARCH ARTICLE

Water stress in *Musa* spp.: A systematic review

Adriadna Souza Santos[1☯], **Edson Perito Amorim**[2], **Claudia Fortes Ferreira**[2*], **Carlos Priminho Pirovani**[1☯]

1 Department of Biological Sciences, State University of Santa Cruz (UESC), Ilhéus, Bahia, Brazil,
2 Embrapa Mandioca e Fruticultura, Cruz das Almas, Bahia, Brazil

☯ These authors contributed equally to this work.
* claudia.ferreira@embrapa.br

OPEN ACCESS

Citation: Santos AS, Amorim EP, Ferreira CF, Pirovani CP (2018) Water stress in *Musa* spp.: A systematic review. PLoS ONE 13(12): e0208052. https://doi.org/10.1371/journal.pone.0208052

Editor: Anil Kumar Singh, ICAR-Indian Institute of Agricultural Biotechnology, INDIA

Received: August 14, 2018

Accepted: November 9, 2018

Published: December 3, 2018

Data Availability Statement: The minimal underlying data set of the study is available at the link: http://doi.org/10.5281/zenodo.1454052.

Funding: This work was supported by a CAPES PhD scholarship to ASS.

Competing interests: The authors have declared that no competing interests exist.

Abstract

Background

The cultivation of bananas and other plants is limited by environmental stresses caused by climate change. In order to recognize physiological, biochemical and molecular components indicated to confer tolerance to water stress in *Musa* spp. we present the first systematic review on the topic.

Methods

A systematic literature review was conducted using four databases for academic research (Google Academic, Springer, CAPES Journal Portal and PubMed Central). In order to avoid publication bias, a previously established protocol and inclusion and exclusion criteria were used.

Results

The drought tolerance response is genotype-dependent, therefore the most studied varieties are constituted by the "B" genome. Tolerant plants are capable of super-expressing genes related to reisistance and defense response, maintaining the osmotic equilibrium and elimination of free radicals. Furthermore, they have higher amounts of water content, chlorophyll levels, stomatic conductance and dry root matter, when compared to susceptible plants.

Conclusions

In recent years, few integrated studies on the effects of water stress on bananas have been carried out and none related to flood stress. Therefore, we highlight the need for new studies on the mechanisms of differentially expressed proteins in response to stress regulation, post-translational mechanisms and epigenetic inheritance in bananas.

Introduction

Bananas are among the oldest cultivated plants to be domesticated [1]. They are classified botanically as monocotyledons of the Musaceae family which include the genus Ensete of Asian and African origin, the genus *Musella*, genetically close to Asian origin, and the *Musa* of East Asia, which is divided into sections with 22 (*Eumusa*, Rhodochlamys) and 20 chromosomes (*Australimusa*, Callimusa) [2]. Most edible bananas belong to the *Eumusa* section and are hybrids from *Musa acuminata* (genome "A") or from crosses with *Musa balbisiana* (genome "B"). A smaller group, including "Fe'i" or "Fehi" bananas, is limited to the Pacific region and is derived from *Australimusa* species [3].

The cross between species and subspecies of *M. acuminata* and *M. balbisiana* contributed to plant sterility and the appearance of parthenocarpy in the triploid and tetraploid genotypes [4].

It is known that the varieties constituted with the genome "A" produce fruits of high yield and quality, with long fingers and durability in the green and mature phases [5]. Molecular studies indicate that the "A" genome harbors more genes that are important for banana production and quality and can be used as candidates in breeding programs [6, 5]. However, bananas constituted with the "B" genome were domesticated under more severe climatic conditions, such as wide temperature variation and soil water scarcity, supporting better environmental stresses [7]

Plants undergo successive abiotic stress events during its life cycle. When water is not available to roots or when the transpiration becomes intense it is said that the plant is under water stress. Water stress may be caused by water deficit or high salinity in the soil. In the case of high salinity in the soil, periods of inundation and low soil temperature, there is still water in the solution of the soil, however, the plants are not capable of absorbing it, leading to a phenomenon called "physiological drought"[8].

Water deficit is one of the main limiting factors for the cultivation of *Musa* spp. In the highlands of East Africa, when annual rainfall is less than 1,100 mm, there may be losses of 20–60% in yield compared to other, more humid areas of the region. The weight of the bunch is affected due to the effect of water deficit during the flowering period, which reduces the number of fingers produced [9].

The response of banana varieties during periods of drought can be genotype-dependent [1]. Studies indicate that the presence of the "B" genome contributes to a better drought tolerance [1, 10]. However, in the absence of a conclusive definition of drought, it is a challenge to identify correct parameters and stress intensities for assessing water deficit tolerance [11].

This paper contributes to this challenge, since through a systematic review of the studies carried out over the past 10 years on the effects of water deficit on bananas and with the genetic sequencing of the species already carried out [12], it is possible to recognize, gather, classify and identify relevant new knowledge produced by other researchers.

A systematic review of the literature is a formalized and repetitive process where the state of the art of a given subject is documented through systematized searches in specific databases. This type of review is very common in Medical Sciences, since it is able to gather in a single document detailed and high level conclusions about diseases that one wishes to study [13, 14].

This paper proposes the first systematic review on water deficit in bananas. To guarantee its efficiency, the search process was conducted around a general objective, which in this review, was to recognize physiological, biochemical and molecular components indicated to confer drought tolerance on *Musa* spp.

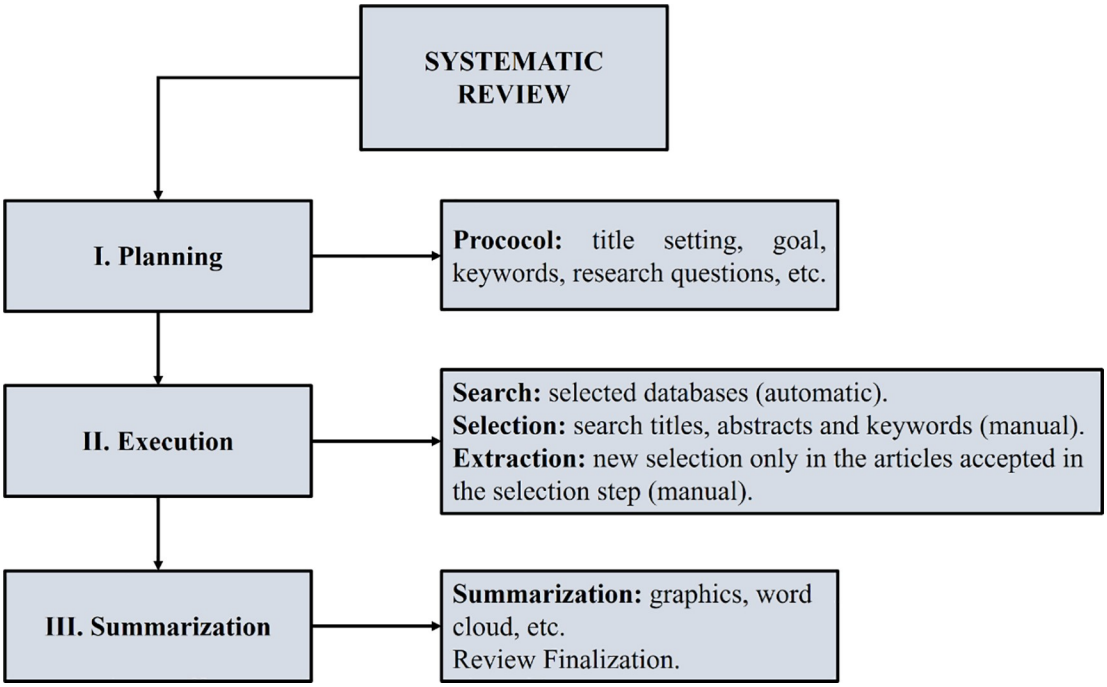


Fig 1. General systematic literature review flowchart.

https://doi.org/10.1371/journal.pone.0208052.g001

Materials and methods

The free software StArt (State of the Art through Systematic Review) v.3.3 Beta 03 was used to perform the systematic review. Developed by the Federal University of São Carlos (UFSCar), the tool provides computational support to researchers who seek answers to research questions using the systematic review technique. The software is available for download at the link: <http://lapes.dc.ufscar.br/tools/start_tool>.

The review process was run on StArt in three steps: Planning, Execution and Summarization (Fig 1).

Each step performed is described below:

I. Planning: A protocol was developed to be followed throughout the review process (http://doi.org/10.5281/zenodo.1465055). Title, objective, keywords, research questions, research sources, inclusion/exclusion criteria and the quality of the collected files were defined. The issues underlying this review are given in Table 1.

Table 1. List of review questions.

Research Questions
Q1. In what countries has more knowledge been produced about water stress in bananas?
Q2. What are the main institutions and/or groups involved in the study of water deficit tolerance?
Q3. What are the main genotypes and varieties studied?
Q4. What types of trials are proposed for studies of water stress?
Q5. What are the types of stresses addressed in papers on water stress?
Q6. Has there been any mention of using the banana genome?
Q7. Which components confer drought tolerance on *Musa* spp.?
Q8. What are the stressors used in the drought studies?

https://doi.org/10.1371/journal.pone.0208052.t001

II. Execution: the searches were carried out in databases previously selected: Google Academic, Springer, CAPES Journal Portal and PubMed Central. The results were imported into BIBTEX, MEDILINE, RIS or Cochrane formats, compatible with StArt. The automatic search performed in the databases searches the central themes in the titles, abstracts and keywords. Relevant papers not found by searches were added later. To make the query expressive, the OR logical connector was used to group the synonymous keywords and AND to group the main parts. Thus, the search string used in all databases is represented in the following Box 1:

Box 1: Search string

("Musa" ***OR*** *"banana")* ***AND*** *("drought stress"* ***OR*** *"water deficit"* ***OR*** *"water stress")*

In order to guarantee the international spectrum of the papers, only works written in English and available in academic channels were selected.

After automatic sorting, the manual selection and extraction phases were performed. In these phases, the inclusion and exclusion criteria are presented in the Table 2.

In the Selection, all papers imported to the software were classified as inclusion criteria: accepting, rejecting or excluding because they were duplicated. In Extraction, a new choice was made, considering only the papers accepted in the selection stage. In extraction, in addition to accepting, we can also reject and/or delete duplicate papers. PRISMA Checklist is presented in S2 Table.

III. Summarization: Graphs, Table and Word Cloud were generated to make up the systematic review.

Results

Search in databases

The StArt software selected 1,410 papers related to the search string. No papers were found on water stress due to flooding in bananas. Google Academic contributed with the largest number of papers for this review, 46% of the total (Fig 2A). The majority of the papers in this database not related to the theme, gray literature (theses, dissertations, reports, annals . . .) and/or duplication, due to small differences in titles, such as capital letters, italicized formatting and accentuation. The Springer, PubMed Central and CAPES journals represented, respectively, 25%, 20% and 9% of the papers found, and presented lower rejection criteria (Fig 2).

Table 2. Criteria used to include or exclude papers in the review process.

Inclusion Criteria	Exclusion Criteria
Papers that contain in the title, abstract or keywords, the terms banana (or *Musa*) and drought stress (or water deficit/stress), as detailed below in the search string.	Theses, dissertations, manuals and reports.
	Review Papers.
	Papers published in journals with no impact factor (individually checked on the sites: https://sucupira.capes.gov.br andhttps://www.scimagojr.com)*
	Papers without clear contribution.
	Papers published before 2008.

* Only one exception for the only article found on epigenetic studies in banana.

https://doi.org/10.1371/journal.pone.0208052.t002

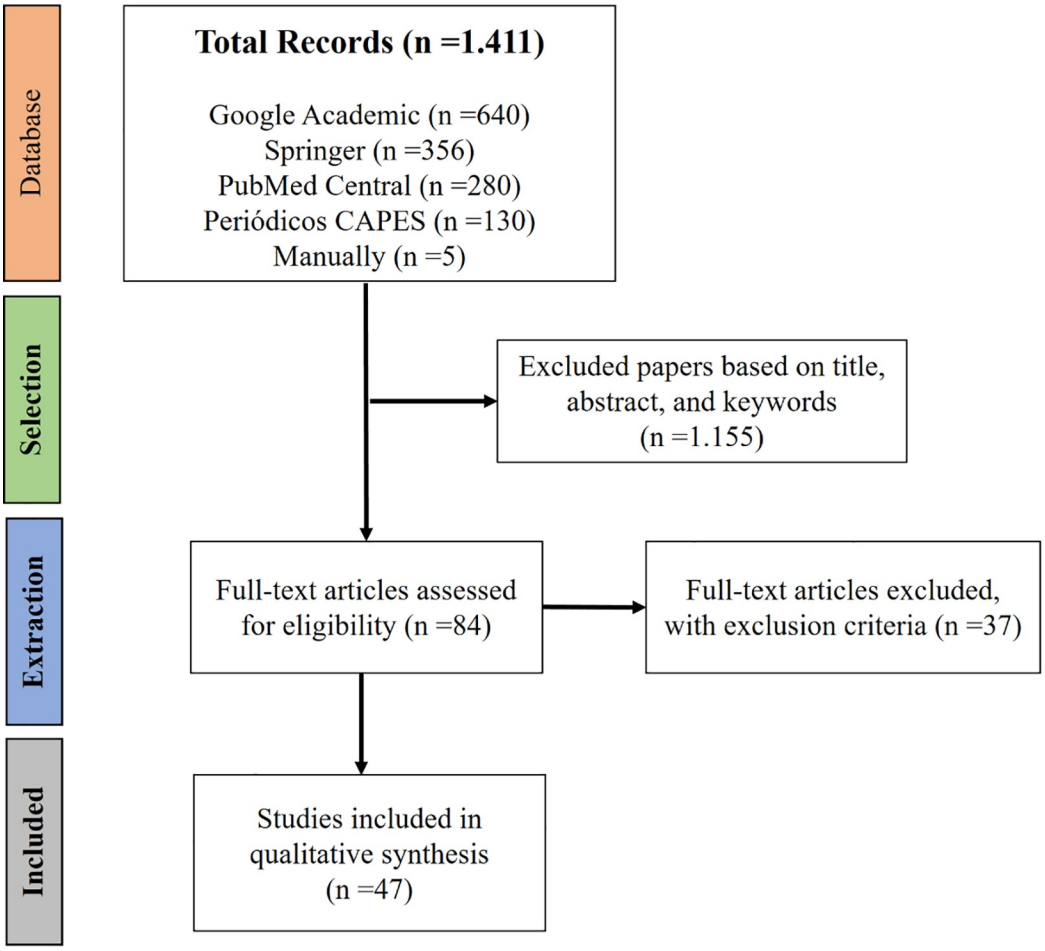


Fig 2. PRISMA flow diagram. Papers collected considering the search string in the databases.

https://doi.org/10.1371/journal.pone.0208052.g002

Other databases could have been used, including Musalit (http://www.musalit.org/), which is specific to bananas. However, when using the search string, the results were restricted or insufficient, so we opted for databases with broader search spectrum such as Google Academic.

After Selection phase, only 6% or 84 papers out of a total of 1, 411 were accepted. In the Extraction phase, of the 84 previously selected papers, 55% were accepted, 45% were rejected by the exclusion criteria. Thus, we followed the review with 47 papers. For consultation purposes, the manuscripts were stored in a free access digital library at the following link: http://doi.org/10.5281/zenodo.1454052.

A cloud of words was generated considering the frequency with which they appeared in papers of the Extraction phase (Fig 3). All the keywords used in database searches are represented and the other words are related to the research on water stress in banana plants.

Research questions

Most of the research works were developed in Asian countries, approximately 80% of the total of 47 (Fig 4). In Asia are also the main institutes and/or research institutions that are dedicated to the studies of Musa spp. under water deficit (S1 Table). In Europe, Africa and South America, 13%, 8% and 3% of the works were performed, respectively.

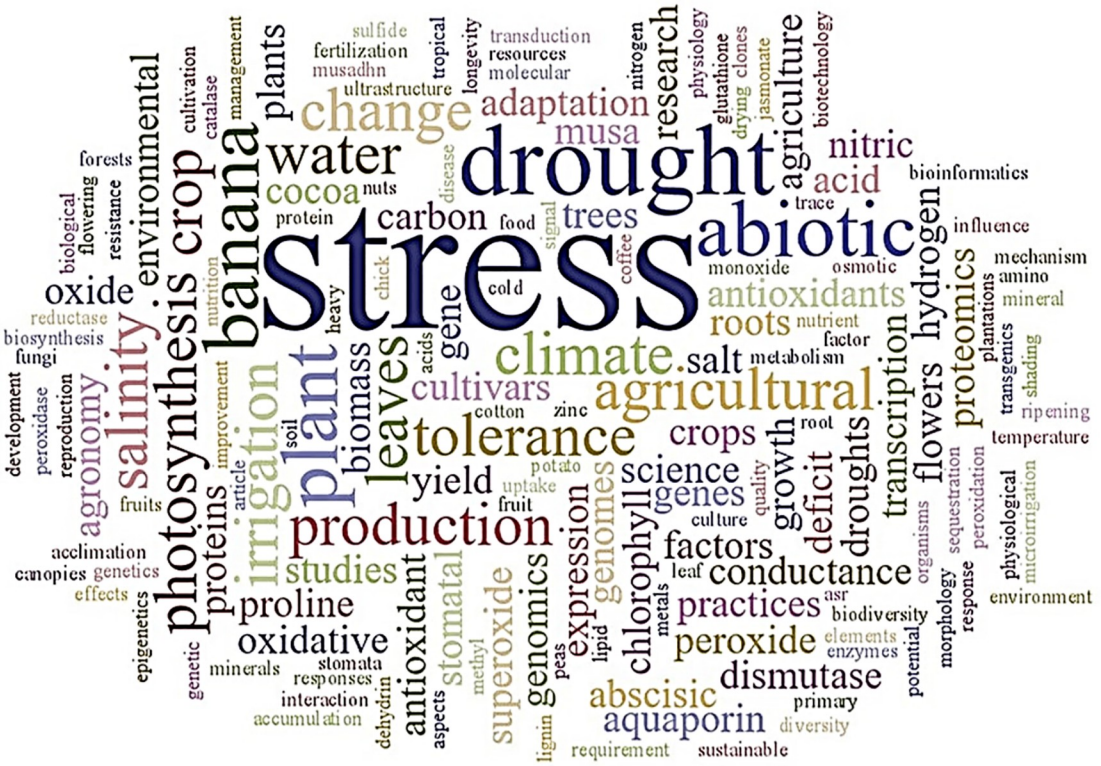


Fig 3. Relevant search word cloud.

https://doi.org/10.1371/journal.pone.0208052.g003

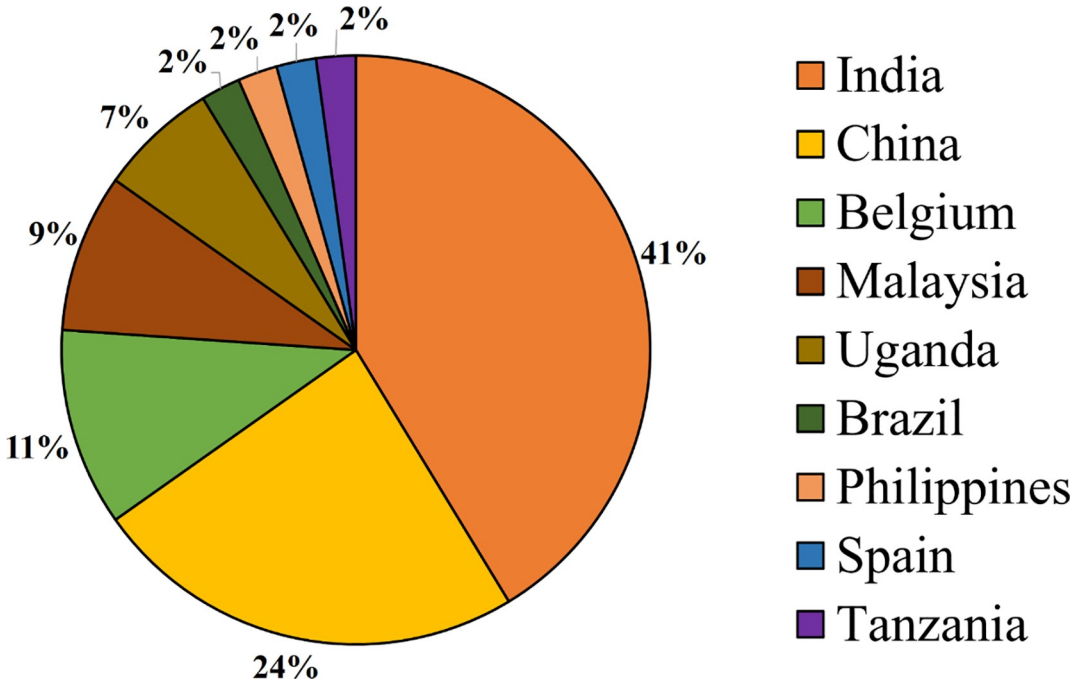


Fig 4. Main producing knowledge countries on water stress in banana plantations.

https://doi.org/10.1371/journal.pone.0208052.g004

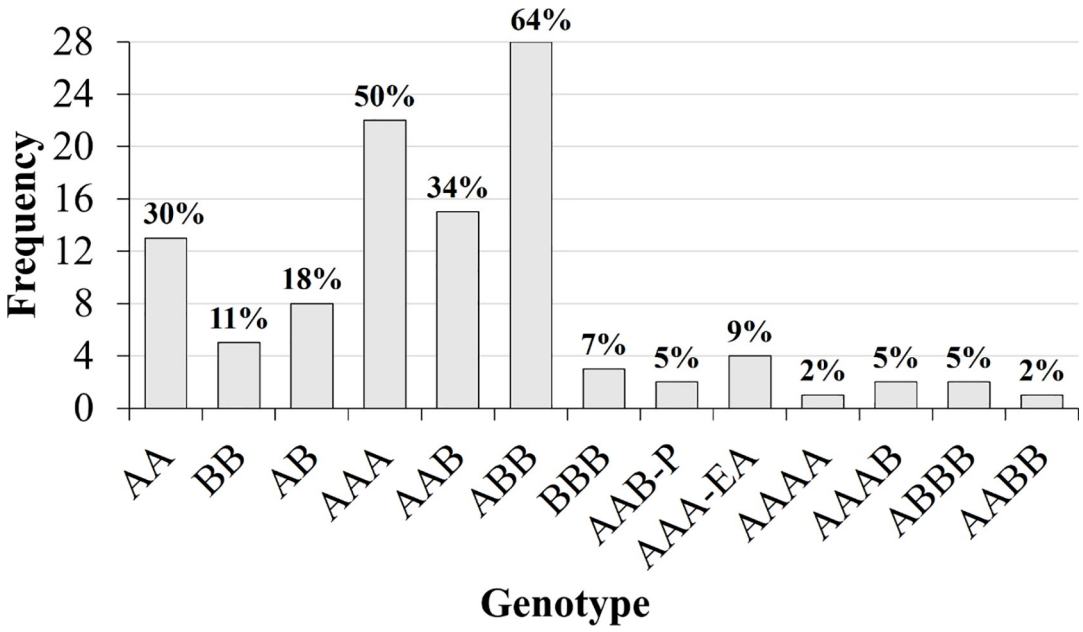


Fig 5. Frequency of genotypes of *Musa* spp. used in papers published in the last 10 years.

https://doi.org/10.1371/journal.pone.0208052.g005

In the studies on water deficit, the main varieties studied are triploids AAA, AAB, ABB present in 50%, 34% and 64% of the papers, respectively (Fig 5). The diploids were studied in 59% of the works and the tetraploids in 14%. The total frequency of genotypes does not reflect the total number of papers in Fig 5, since some authors considered more than one genotype.

A list of cultivars and species used in the 47 papers is presented in Table 3. Among the most widely used varieties are triploids 'Saba', 'BaXi Jiao', 'Fen Jiao', 'Poovan', 'Karibale Monthan', 'Berangan', 'Cachaco' and 'Grande Naine', and the diploid 'Ney Poovan'.

Most of the studies were performed in the field, in vitro, greenhouse and growth chamber, corresponding to 30%, 30%, 25% and 14%, respectively. The remaining studies accounted for 19% (Fig 6A). The main stresses observed were drought, osmotic, salinity, cold and hormonal

Table 3. *Musa* spp. most used in studies on water deficit in the last 10 years.

Genomic Group	Genotypes (varieties/types)
AA	*M. acuminata*, *M. paradisiaca*, Matti, Sanna Chenkathali, Anaikomban, Calcutta-4.
BB	Bee hee kela, Bhimaithia.
AB	Ney Poovan.
BBB	Gubao, Cardaba, Saba Puti.
AAA	Berangan, Berangan Intan, Tianbaojiao, BaXi Jiao, Grande Naine, Brazilian, Yangambi Km5, Mpologoma, Mbwazirume, Williams, Uganda.
AAA-EA	Kisansa, Mbwazirume.
AAB	Rasthali, Latundan, PK Malaccacina, Pokpok, Sukali Ndizi, Popoulou, Nendran, Poovan, Karpuravalli.
AAB-P	Obino l'Ewai
ABB	Cachaco, Fen Jiao, Prata anã, Saba, Karibale Monthan, K. Namwa, Maduranga, Matavia, Paa Dalaga, Pelipia, Tindok, Kayinja, Karpooravalli.
AAAB	BRS Tropical.
ABBB	Tiparot.

https://doi.org/10.1371/journal.pone.0208052.t003

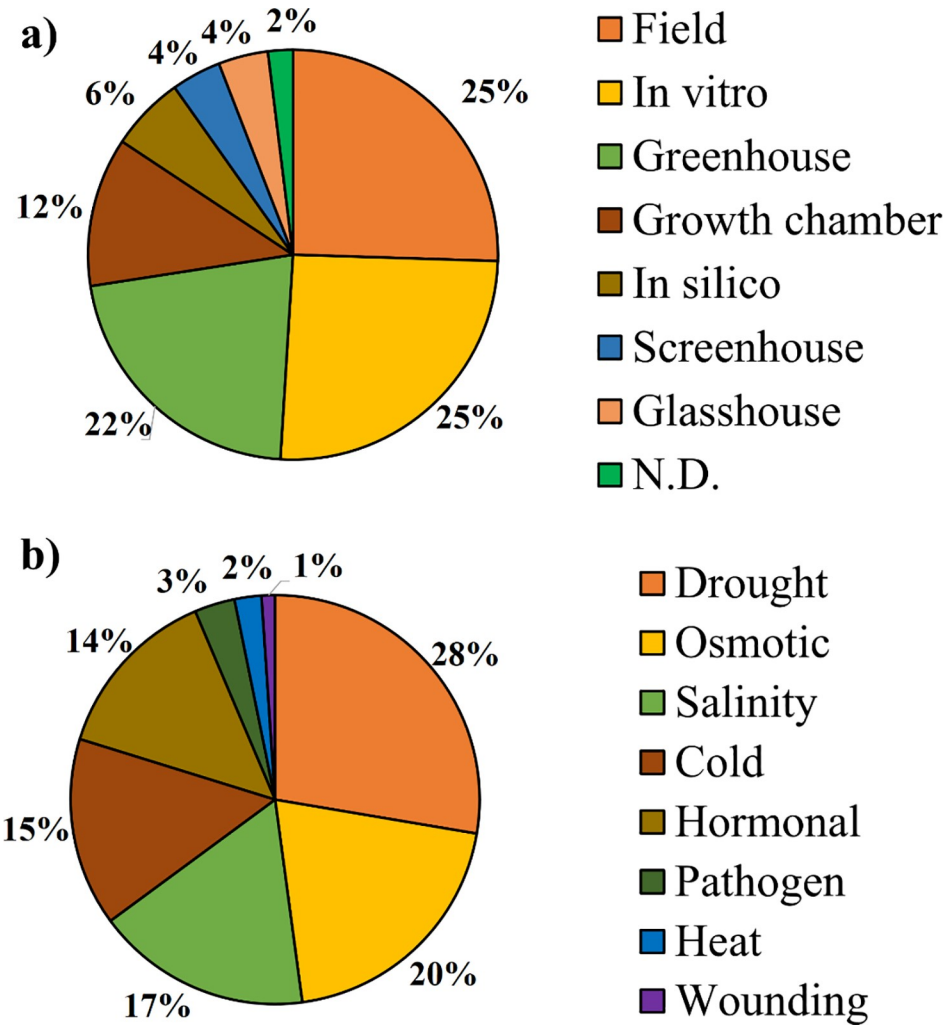


Fig 6. a) Frequency of study environments for application of water stress in banana plants. b) Types of stress applied on *Musa* spp.

https://doi.org/10.1371/journal.pone.0208052.g006

stress (Fig 6B). The following discussions considered only osmotic stress and water deficit, although some papers addressed multiple stresses.

Stress applied on Musa spp. were grouped into physical, chemical and biotic (Table 4). The agents causing water stress in selected papers were water deficit, water deficit progressive, PEG6000, mannitol, mannitol+PEG, sucrose+sorbitol and methyl viologen (Table 4).

Some markers have been successfully used in drought tolerance research in banana plantations with different applications (Fig 7). There are 21 candidate genes, 6 hormones, 3 proteins, 2 nutrients, 2 RNAs and 1 mutagenic agent.

Table 4. Classification of stresses applied on *Musa* spp.

Type of stress	Stressors
Physical	Water deficit, water deficit progressive, low temperature, high temperature, injury, NaCl.
Chemical	PEG6000, mannitol, mannitol+PEG, sucrose+sorbitol, methyl viologen, ethephon, ABA, AIA, MeJA, GI_3, SA, $CuSO_4$.
Biotic	*Fusarium oxysporum* f. sp. cubense, *Xanthomonas campestris* pv. musacearum (Xcm).

https://doi.org/10.1371/journal.pone.0208052.t004

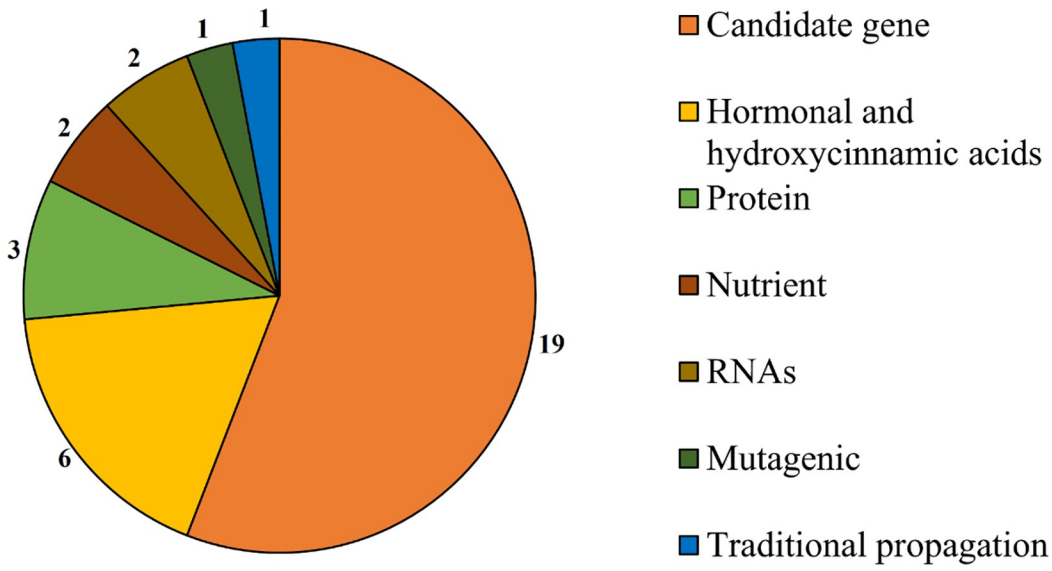


Fig 7. A summary of markers used in banana research.

https://doi.org/10.1371/journal.pone.0208052.g007

Molecular markers and their effects under drought tolerance in *Musa* spp. are addressed in 21 papers (Table 5).

Some papers have addressed the existence of ARNs regulating the response to water deficit in banana (Table 6).

Only two papers of this review presented the proteomic study of *Musa* spp. The ATPase, Heat shock and Dehydrogenases, are among the main proteins observed in contrasting banana genotypes for tolerance to water deficit (Table 7).

Three papers deal with the Hormonal and Hydroxycinnamic Acids study to confer drought tolerance on banana varieties (Table 8).

Relative water content, chlorophyll levels, photosynthetic efficiency and leaf water loss are among the main physiological analyzes used to measure of the effects of water stress on banana genotypes (Fig 8A). The content of Malondialdehyde, Proline, Hydrogen peroxide and Soluble protein are among the main biochemical analysis (Fig 8B)

Discussion

This systematic review presentes a compilation of quality articles and main institutions involved in the study of tolerance to water stress in *Musa* spp. The great interest of Asian countries in the study of the water deficit in *Musa* sp. probably stems from the fact that these countries are at the center of origin and world banana trade. Molecular evidence confirmed that edible banana genotypes originated in the islands of Southeast Asia and developed through complex pathways of geodomestication [39]. India is by far the world's largest banana producer, and China is the fourth largest importer. Malaysia is also among the domestic market consumers in Asia. The Philippines was the world's second largest banana exporter by 2015, when they lost their position due to the long period of drought caused by El Niño [40]. Brazil is among the five largest producers in the world and the European Union and the United States are among the largest importers of this fruit, approximately 60% of the global distribution [40].

The triploid cultivars are the most studied. In natural germplasm, there are few ABB triploid varieties that have good palatability and high productivity, and usually the most consumed are the Cavendish (AAA) subgroup, such as Grande Naine and Williams [39, 41]. Therefore,

Table 5. Candidate gene used in research with *Musa* spp. and applications.

Candidate Gene	Applications	Authors
AhSIPR10	Improvement of photosynthetic efficiency and reduction of plasma membrane damage in the presence of NaCl and mannitol.	Rustagi et al., 2014 [15]
MaAGPase	Regulates the signaling pathway of biotic and abiotic stress and is involved in the development and maturation of fruits.	Miao et al., 2017 [6]
MaAQP	Promotes the early development of fruits, accelerating post-harvest banana maturation processes and plant resistance to saline and osmotic stress.	Hu et al., 2015 [16]
MaARFs	Involved in banana growth, fruit development, post-harvest maturation and responses to osmotic, saline and cold stress.	Hu et al., 2015 [17]
MabZIP	Involved in stages of organ development, fruit maturation and responses to abiotic stresses, including dry, cold and salt.	Hu et al., 2016 [5]
MaCCS	Induced in response to light, heat, drought stress, abscisic acid and indole-3-acetic acid.	Feng et al., 2016 [18]
MaHsfs	Involved in the growth of specific tissues or stages of development, such as fruit maturation and biotic and abiotic stress.	Wei et al., 2016 [19]
MaPIP1;1	It imparts tolerance to saline and water stress, reducing membrane damage, improving ionic distribution (K^+/Na^+ ratio) and maintaining the osmotic balance.	Xu et al., 2014 [20]
MaSODs	Plays an important role in the elimination of reactive oxygen species caused by abiotic and hormonal stresses in banana.	Feng et al., 2015 [21]
MpASR	Demonstrates positive activity to *F. oxysporum* f. sp. cubense and cold stress, dehydration, ABA and high salt concentration.	Liu et al., 2010 [22]
MusaDHN-1	Induced in leaves by drought, salinity, cold, oxidation, heavy metals, as well as by treatment with signaling molecules such as abscisic acid, ethylene and methyl jasmonate.	Shekhawat et al., 2011 [23]
MusaNAC042	Modulates the response to abiotic stress in banana preserving high levels of total chlorophyll and maintaining lower MDA content (malondialdehyde).	Tak et al., 2017 [24]
MusaNAC68	Regulates stress tolerance induced by NaCl and mannitol and root development.	Negi et al., 2015[25]
MusaPIP1;2	It improves survival characteristics under abiotic stress by maintaining low levels of malondialdehyde and high concentrations of proline, relative water content and photosynthetic efficiency.	Sreedharan et al., 2013 [26]
MusaSAP1	Involved in reducing malondialdehyde levels and regulating polyphenoloxidases (PPOs) that play important roles in multiple defense pathways.	Sreedharan et al., 2012 [27]
MusaWRKY71	An important constituent in the transcriptional reprogramming involved in several responses to stress in bananas, such as improved photosynthetic efficiency and reduction in leaf membrane damage.	Shekhawat et al., 2013 [28]
Non-redundant DEGs	Involved mainly in protein modifications, lipid metabolism, alkaloid biosynthesis, carbohydrate degradation, glycan metabolism, amino acid biosynthesis, cofactor, sugar-nucleotide, hormone, terpenoids and other secondary metabolites.	Muthusamy et at., 2016 [29]
PYL-PP2C-SnRK2	Regulates maturation and tolerance of banana fruits to cold, saline and osmotic stresses.	Hu et al., 2017 [8]
MaSWEETs	Increases sugar transport during initial fruit development and under abiotic and biotic stresses.	Miao et al., 2017 [30]
WRKY	Regulated in multiple stresses, involved in the growth, development and process of ripening fruits	Goel et al., 2016 [31]

https://doi.org/10.1371/journal.pone.0208052.t005

an improvement strategy is to combine edible AB cultivars with wild *M. balbisiana* to create new ABB varieties that are productive, palatable and tolerant to drought and cold [1]. In nature, *M. acuminata* and its subspecies are typically delicate and thin, and require favorable environmental conditions for good development, since they originated in the wetlands of Asia. *M. balbisiana* was diversified and domesticated in regions of drought-prone monsoons in Southeast Asia, which probably contributed to tolerance to abiotic stresses [9].

The most common growth environment were field and in vitro and the main stresses applied were water deficit and osmotic stresses. Drought stresses are given by the suspension of irrigation in plants grown in the greenhouse [39] or in typically dry seasons with

Table 6. ARNs involved in drought tolerance in *Musa* spp.

RNAs	Applications	Authors
microRNAs	The *miR169*, *miR156* and *miR2118* were upregulated during stress due to water deficiency of the soil. MiR169 can control the expression of *NFY* and eventually the expression of *AQN* and *DHN* by reducing transcription, or by a mechanism of post-transcriptional degradation.	Muthusamy et al., 2014 [32]
LncRNAs	They are crucial regulators of gene transcription in plants in response to biotic and abiotic stress.	Muthusamy et al., 2015 [33]

https://doi.org/10.1371/journal.pone.0208052.t006

Table 7. Important proteins in the response of banana plants to water stress.

Protein	Applications	Authors
ATPase	Provides the main driving force for many cellular processes such as osmoregulation, signal transduction and reaction metabolism.	Mattos-Moreira et al., 2018 [34]
Heat shock	Promotes balance between antioxidants (AOX) and ROS during water stress.	
Dehydrogenases involved in NAD/ NADH homeostasis	Limitation of ROS production by the NADPH matrix produced by NADP-isocitrate dehydrogenase and the non-proton-pumping transhydrogenase activities	Vanhove et al., 2012 [35]

https://doi.org/10.1371/journal.pone.0208052.t007

commercial plantations [3]. Field and greenhouse studies generally approach the conditions of agricultural cultivation [4]. Mannitol, polyethylene glycol and methyl viologen are organic compounds with osmotic action. As liquid solution, they are commonly used to simulate water deficit conditions [5]. In the selected works, mannitol was used, for example, in a water stress test with five leaves of the cultivars 'BaXi Jiao' and 'Fen Jiao' [6, 10], in different leaf discs *Musa* spp. [5, 15, 25, 42] and in vitro in the variety 'Karibale Monthan' [27]. Polyethyleneglycol (PEG) was used in vitro as it has a high molecular weight and reduces the water potential of the medium, thus inhibiting the growth of bananas, which can not absorb water and nutrients [7, 9, 38]. The methyl viologen was used in leaves of transgenic banana plants to simulate osmotic stress, as it acts by receiving electrons from the primary acceptors of the photosystem-I and transferring them to the oxygen, generating ROS [43]. Sorbitol was considered a neutral inducer of osmotic stress in banana when compared to culture media that use sucrose as the only source of carbon [2]. Therefore, cultures initially acclimated with sucrose in vitro respond better to osmotic stress, but over time, sugar metabolism consumes ATP and boosts respiration, resulting in limiting levels of oxygen, which can eventually lead to anoxia. [10].

In all studies on osmotic stress, the efficiency of at least one of the compounds was determined in the selection of water stress tolerant genotypes. In vitro studies are considered the first step in characterizing the biodiversity of tolerant *Musa* spp. in a germplasm bank, since they allow a high yield and control of the experiment, however, the artificial conditions are pointed out as disadvantages [18].

More than 20 candidate genes were indicated as potential markers for banana drought tolerance. The *MaAGPase*, *MaAQP*, MabZIP, non-redundant DEGs, MaSWEETs and *PYL-PP2C-SnRK2* genes showed genome-dependent response in genome "B" compared to genome "A" [26, 30, 38, 44, 45]. The expression of cuticular wax biosynthesis genes (*FATB* and *KCS11*) is also a dependent genotype, influencing the water retention capacity of leaves and

Table 8. Hormonal and Hydroxycinnamic Acids in bananas under water déficit and its applications.

Hormonal and Hydroxycinnamic Acids	Applications	Authors
Methyl jasmonate	Improves drought tolerance, since it moderates the effects of oxidative stress, leading to better plant performance, fresh weight and shoot proliferation rate.	Mahmood et al., 2012 [36]
Abscisic Acid	Trigger adaptation of the plant to drought, as well as reduction of stomatal conductance, photosynthetic rate, plant growth in height, circumference, number of leaves and area.	Mahouachi et a., 2014 [37]
Indole-3-acetic acid	It can alleviate leaf senescence, improve survival levels and maintain cell stretching.	
Cinnamic acids	They are photoprotectors, since they are involved in the folding mechanism of the leaves, which reduces the area of irradiation and loss of water.	
Ferulic acids		
Salicylic acid	They may be limited to a rapid and transient increase at the beginning of the first stress period, as they did not respond to consecutive stresses.	
Jasmonic acid		
Salicylic acid	Promotes increase in the rate of proliferation, fresh weight gain, maintenance of the relative water content and reduction of H_2O_2.	Bidabadi et al., 2012 [38]

https://doi.org/10.1371/journal.pone.0208052.t008

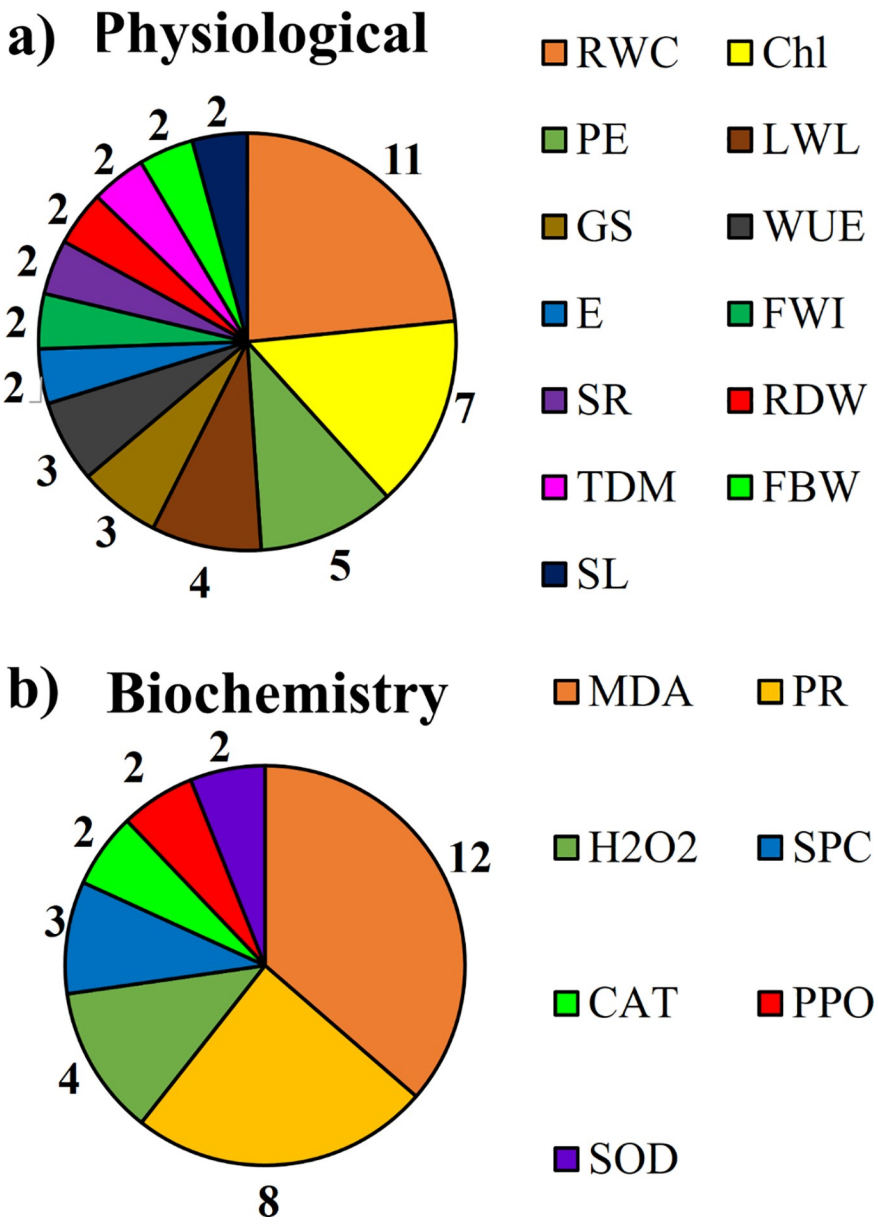


Fig 8. Main biochemical and physiological parameters measured. a) RWC—Relative water content, Chl—chlorophyll levels, PE—Photosynthetic efficiency, LWL—leaf water loss, GS—Stomatal conductance, WUE—Water Use Efficiency, E—Transpiration rate, FWI- fresh weight increase, SR- survival rates, RDW- Root dry weight, TDM- Total dry matter yield, FBW- fresh bunch weight, SL- Shoot Length. b) MDA—Malondialdehyde, PR- Proline, H_2O_2—Hydrogen peroxide, SPC—Soluble protein content, AN—Acid ninhydrin, CAT—Catalase, PPO—Polyphenol oxidase, SOD- Superoxide dismutase.

https://doi.org/10.1371/journal.pone.0208052.g008

mitigating the effects of water stress on varieties constituted by the "B" genome, a promising line for future studies [46]. [38] suggests that the degradation of mRNA driven by the enzyme exoribonuclease 5'-3' during abiotic stress may be responsible for sensitivity to drought in cv. 'Grande Naine' (AAA).

In one study, the heterologous expression of the banana aquaporin gene, *MaPIP1;1*, in Arabidopsis was presented as an alternative to improve the response of species, independent of the genotype, since in Arabidopsis it showed an increase in the primary root length, number of

root hairs and survival rates [47]. The same stress acclimation behavior was observed in transgenic plants overexpressing *MusaNAC68* [25].

The banana transcriptome and proteome are also altered in response to water stress. The expression of RNAs involved in drought tolerance of the the 'Saba' cultivar ("ABB" genome-tolerant) and Grande Naine ("AAA" genome-susceptible) depends on the genetic constitution of the cultivars, whereas the genome "B" is the most efficient [46, 48]. The proteome analysis of banana plants submitted to water stress shows that there is a new equilibrium in stressed plants and that respiration, ROS metabolism, growth and development, especially of plants with the "B" genome, are altered in order to adjust to tolerate stress [16, 18].

The exogenous use of Hormone and Hydroxycinnamic Acids to confer tolerance to *Musa* spp. triploids (AAA) seems promising. With the exception of Salicylic acid and Jasmonic acid that did not present a significant response in progressive water stress [20], all other acids contributed to the acclimatization of banana varieties to water deficit [21, 33].

In addition to exposure to hormones, water stress tolerant lineages can be obtained from variations induced by ethyl methanesulphonate (EMI) [34] or by maintaining the nitrogen and potassium ratio in the soil, since potassium mitigates the production of dry matter and loss of bunches resulting from water stress in the early stages of development [36].

Of the 47 articles in this review, only 13 cited the use of the banana genome. Until 2009, few complete studies on abiotic stress in bananas had been published, and none on drought [7]. The sequencing of the *Musa acuminata* genome, whose DNA is in the composition of all edible varieties [13], is an important step for the knowledge of genes with specific functions and of interest for the genetic improvement of the species.

The first epigenetic study relating to the response of in vitro micropropagated banana varieties to mechanisms underlying abiotic stresses was published in 2012. Cytosine hypomethylation of DNA is related to increased leaf stomata density, resulting in excessive water loss and increased foliar senescence [49]. This work was published in a journal with no impact factor. Their results are presented due to the importance of this knowledge for the production of varieties tolerant to water stress.

A series of physiological and biochemical changes were observed in different banana varieties submitted to water stress. In superior plants, a small part of the water absorbed by the roots is used in photosynthesis and biomass production and most of it is returned to the environment in the process and transpiration [49]. The stomatal closure is one of the main strategies used by plants to control the amount of water evaporated into the medium [11]. Under conditions of water deficit, the relative water content in the leaves decreases, consequently leading to the reduction of stomatal conductance, photosynthetic rate [50,51,52] and transpiration rate [53]. Within the cells, the formation of reactive oxygen species (ROS) occurs, leading to the formation of harmful free radicals, lipid peroxidation [47] and denaturing proteins [30]. Species susceptible to water stress are more vulnerable to increased polyphenol oxidase, which is an enzyme which catalyzes the darkening reaction that occurs in many fruits and vegetables [54]. Varieties of bananas tolerant to water deficit are capable of increasing the activity of osmoprotective components, such as Proline, which eliminate free radicals in the cytoplasm and increase the solubility of poorly soluble proteins, free amino acids and total sugars [55, 56]. The activity of antioxidant enzymes, such as Catalase, Superoxide dismutase and Peroxidase of ascorbate [57] is also higher in tolerant species when compared to those susceptible to water stress.

Conclusions

Bananas are a product of export and subsistence in several countries. They are susceptible to a wide range of biotic and abiotic stresses where cultivars with the "B" genome show some

superiority over those presenting the "A" genomes, such as increased expression of genes related to plant defense, higher relative content of water, length of primary roots and fresh bunch weight as well as better rates of survival and photosynthesis. Many efforts have been made in studies on the effect of water stress on the expression of genes that are candidates for drought tolerance, but few studies in the last 10 years have addressed the expression of proteins and post-translational mechanisms. We also highlight the need to study the epigenetic inheritance in bananas, elucidating the effects of environmental stresses on DNA and chromatin sequences and possible regulatory mechanisms that are maintained and passed on to the next generations, making them more tolerant through genetic "*imprinting*".

Supporting information

S1 Table. Main institutions responsible for the production of worldwide agriculture knowledge.
(DOCX)

S2 Table. PRISMA checklist.
(DOC)

Author Contributions

Conceptualization: Adriadna Souza Santos, Carlos Priminho Pirovani.

Data curation: Adriadna Souza Santos.

Formal analysis: Adriadna Souza Santos, Claudia Fortes Ferreira.

Funding acquisition: Edson Perito Amorim.

Investigation: Edson Perito Amorim.

Methodology: Adriadna Souza Santos, Carlos Priminho Pirovani.

Project administration: Edson Perito Amorim.

Supervision: Claudia Fortes Ferreira, Carlos Priminho Pirovani.

Validation: Carlos Priminho Pirovani.

Writing – original draft: Adriadna Souza Santos, Claudia Fortes Ferreira, Carlos Priminho Pirovani.

Writing – review & editing: Adriadna Souza Santos, Claudia Fortes Ferreira, Carlos Priminho Pirovani.

References

1. Ravi I, Uma S, Vaganan MM, Mustaffa MM. Phenotyping bananas for drought resistance. Frontiers in Physiology. 2013; 4: 1–15. https://doi.org/10.3389/fphys.2013.00009
2. Häkkinen M. Reappraisal of sectional taxonomy in *Musa* (Musaceae). TAXON. 2013; 62: 809–813. https://doi.org/10.12705/624.3
3. Perrier X, De Langhe E, Donohue M, Lentfer C, Vrydaghs L, Bakryet F, et al. Multidisciplinary perspectives on banana (*Musa* spp.) domestication. Proceedings of the National Academy of Sciences. 2011; 108: 11311–11318. https://doi.org/10.1073/pnas.1102001108 PMID: 21730145
4. Von Loesecke H. W. Bananas, chemistry, physiology and technology. New York: Interscience Publishers, 1949. 189pp.
5. Hu W, Wang L, Tie W, Yan Y, Ding Z, Liu J, et al. Genome-wide analyses of the bZIP family reveal their involvement in the development, ripening and abiotic stress response in banana. Scientific Reports. 2016; 6: 1–15. https://doi.org/10.1038/s41598-016-0001-8

6. Miao H, Sun P, Liu Q, Miao Y, Liu J, Xu B, et al. The AGPase Family Proteins in Banana: Genome-Wide Identification, Phylogeny, and Expression Analyses Reveal Their Involvement in the Development, Ripening, and Abiotic/Biotic Stress Responses. International Journal of Molecular Sciences. 2017; 18: 1–17. https://doi.org/10.3390/ijms18081581 PMID: 28757545
7. Davey MW, Graham NS, Vanholme B, Swennen R, May ST, Keulemans J. Heterologous oligonucleotide microarrays for transcriptomics in a non-model species; a proof-of-concept study of drought stress in Musa. BMC Genomics. 2009; 10: 1–19. https://doi.org/10.1186/1471-2164-10-1
8. Lisar SYS, Motafakkerazad R, Hossain MM, Rahman IMM. Water Stress in Plants: Causes, Effects and Responses. In: Rahman IMM. (Ed.). Water Stress. INTECH Open Access Publisher. ISBN 978-953-307-963-9; 2012. pp 1–14. https://doi.org/10.5772/39363
9. Van Asten PJA, Fermont AM, Taulya G. Drought is a major yield loss factor for rainfed East African highland banana. Agricultural Water Management. 2011; 98: 541–552. https://doi.org/10.1016/j.agwat.2010.10.005
10. Kissel E, Van Asten P, Swennena R, Lorenzenb J, Carpentiera SC Transpiration efficiency versus growth: Exploring the banana biodiversity for drought tolerance. Scientia Horticulturae. 2015; 185: 175–182. http://dx.doi.org/10.1016/j.scienta.2015.01.035
11. Miao H, Sun P, Liu Q, Miao Y, Liu J, Zhang K. et al. Genome-wide analyses of SWEET family proteins reveal involvement in fruit development and abiotic/biotic stress responses in banana. Scientific Reports. Scientific Reports. 2017; 7: 1–15. https://doi.org/10.1038/s41598-016-0028-x
12. D'Hont A, Denoeud F, Aury J-M, Baurens F-C., Carreel F, Garsmeur O, et al. The banana (*Musa acuminata*) genome and the evolution of monocotyledonous plants. Nature. 2012; 488: 213–217. https://doi.org/10.1038/nature11241 PMID: 22801500
13. Gensous N, Aurélie M, Thomas B, Patrick B, Estibaliz L, Julien S,et al. Predictive biological markers of systemic lupus erythematosus flares: a systematic literature review. Arthritis Research & Therapy 2017; 19: 1–12. https://doi.org/10.1186/s13075-017-1442-6 PMID: 29065901
14. Wang Y, Xiaoling W, Xun D, Yanjiao M, Siy H, Yong W. Association of CD14-260 (-159) C/T and Alzheimer's disease: systematic review and trial sequential analyses. 2018; 125: 1313–1318. Journal of Neural Transmission. https://doi.org/10.1007/s00702-018-1896-y
15. Rustagi A, Jain S, Kumar D, Shekhar S, Jain M, Bhat V, et al. High Efficiency Transformation of Banana [*Musa acuminata* L. cv. Matti (AA)] for Enhanced Tolerance to Salt and Drought Stress Through Overexpression of a Peanut Salinity-Induced Pathogenesis-Related Class 10 Protein. Molecular Biotechnology. 2015; 57: 27–35. https://doi.org/10.1007/s12033-014-9798-1 PMID: 25173686
16. Hu W, Hiu X, Huang C, Yan Y, Tie W, Ding Z, et al. Genome-Wide Identification and Expression Analyses of Aquaporin Gene Family during Development and Abiotic Stress in Banana. International Journal of Molecular Sciences. 2015; 16: 19728–19751. https://doi.org/10.3390/ijms160819728 PMID: 26307965
17. Hu W, Zuo J, Hou X, Yan Y, Wei Y, Liu J, et al. The auxin response factor gene family in banana: genome-wide identification and expression analyses during development, ripening, and abiotic stress. Frontiers in Plant Science. 2015; 6: 1–16. https://doi.org/10.3389/fpls.2015.00742
18. Feng X, Chen F, Liu W, Thu MK, Zhang Z, Chen Y, et al. Molecular Characterization of *MaCCS*, a Novel Copper Chaperone Gene Involved in Abiotic and Hormonal Stress Responses in *Musa acuminata* cv. Tianbaojiao. International Journal of Molecular Sciences. 2016; 17: 1–13. https://doi.org/10.3390/ijms17040441 PMID: 27023517
19. Wei Y, Hu W, Xia F, Zeng H, Li X, Yan Y, et al Heat shock transcription factors in banana: genome-wide characterization and expression profile analysis during development and stress response. Scientific Reports. 2016; 6: 1–11. https://doi.org/10.1038/s41598-016-0001-8
20. Xu Y, Hu W, Liu J, Zhang J, Jia C, Hu H, et al. A banana aquaporin gene, *MaPIP1;1*, is involved in tolerance to drought and salt stresses. BMC Plant Biology. 2014; 14: 1–14. https://doi.org/10.1186/1471-2229-14-1
21. Feng X, Lai Z, Lin Y, Lai G, Lian C. Genome-wide identification and characterization of the superoxide dismutase gene family in *Musa acuminata* cv. Tianbaojiao (AAA group). BMC Genomics. 2015; 16: 1–16. https://doi.org/10.1186/1471-2164-16-1
22. Liu H-Y., Dai J-R., Feng D-R., Liu B., Wang H-B., Wang J-F Characterization of a Novel Plantain Asr Gene, *MpAsr*, that is Regulated in Response to Infection of *Fusarium oxysporum* f. sp. cubense and Abiotic Stresses. Journal of Integrative Plant Biology. 2010; 52: 315–323. https://doi.org/10.1111/j.1744-7909.2010.00912.x PMID: 20377692
23. Shekhawat UKS, Srinivas L, Ganapathi TR. *MusaDHN-1*, a novel multiple stress-inducible SK3-type dehydrin gene, contributes affirmatively to drought- and salt-stress tolerance in banana. Planta. 2011; 234: 915–932. https://doi.org/10.1007/s00425-011-1455-3 PMID: 21671068

24. Tak H, Negi S, Ganapathi TR. Banana NAC transcription factor *MusaNAC042* is positively associated with drought and salinity tolerance. Protoplasma. 2017; 254: 803–816. https://doi.org/10.1007/s00709-016-0991-x PMID: 27352311

25. Negi S, Tak H, Ganapathi TR. Expression analysis of *MusaNAC68* transcription factor and its functional analysis by overexpression in transgenic banana plants. Plant Cell, Tissue and Organ Culture. 2015; 125: 59–70. https://doi.org/10.1007/s11240-015-0929-6

26. Sreedharan S, Shekhawat UKS, Ganapathi TR. Transgenic banana plants overexpressing a native plasma membrane aquaporin *MusaPIP1;2* display high tolerance levels to different abiotic stresses. Plant Biotechnology Journal. 2013; 11: 942–952. https://doi.org/10.1111/pbi.12086 PMID: 23745761

27. Sreedharan S, Shekhawat UK.S, Ganapathi TR. *MusaSAP1*, a A20/AN1 zinc finger gene from banana functions as a positive regulator in different stress responses. Plant Molecular Biology. 2012; 80: 1–15. https://doi.org/10.1007/s11103-012-9947-5

28. Shekhawat UK, Ganapathi TR. *MusaWRKY71* overexpression in banana plants leads to altered abiotic and biotic stress responses. PLoS One. 2013; 8: 1–7. https://doi.org/10.1371/journal.pone.0075506 PMID: 24116051

29. Muthusamy M, Uma S, Backiyarani S, Saraswathi MS, Chandrasekar A. Transcriptomic Changes of Drought-Tolerant and Sensitive Banana Cultivars Exposed to Drought Stress. Frontiers in Plant Science. 2016; 7: 1–14. https://doi.org/10.3389/fpls.2016.00001

30. Miao H, Sun P, Liu Q Miao Y, Liu J., Zhang K, et al. Genome-wide analyses of SWEET family proteins reveal involvement in fruit development and abiotic/biotic stress responses in banana. Scientific Reports. Scientific Reports. 2017; 7: 1–15. https://doi.org/10.1038/s41598-016-0028-x

31. Goel R, Pandey A, Trivedi PK, Asif MH. Genome-Wide Analysis of the Musa WRKY Gene Family: Evolution and Differential Expression during Development and Stress. Frontiers in Plant Science. 2016; 7: 1–13. https://doi.org/10.3389/fpls.2016.00001

32. Muthusamy M, Uma S, Backiyarani S, Saraswathi MS. Computational prediction, identification, and expression profiling of microRNAs in banana (Musa spp.) during soil moisture deficit stress. Journal of Horticultural Science & Biotechnology. 2014; 89: 208–214. https://doi.org/10.1080/14620316.2014.11513070

33. Muthusamy M, Uma S, Backiyarani S, Saraswathi MS. Genome-wide screening for novel, drought stress-responsive long non-coding RNAs in drought-stressed leaf transcriptome of drought-tolerant and -susceptible banana (*Musa* spp) cultivars using Illumina high-throughput sequencing. Plant Biotechnology Reports. 2015; 9: 279–286. https://doi.org/10.1007/s11816-015-0363-6

34. Mattos-Moreira LA, Ferreira CF, Amorim EP, Pirovani CP, Andrade EM, Coelho Filho MA et al. Differentially expressed proteins associated with drought tolerance in bananas (*Musa* spp.). Acta Physiologiae Plantarum. 2018; 40: 1–15. https://doi.org/10.1007/s11738-018-2638-3

35. Vanhove A-C, Vermaelen W, Panis B, Swennen R, Carpentier S. 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. Frontiers in Plant Science. 2012; 3: 1–10. https://doi.org/10.3389/fpls.2012.00001

36. Mahmood M, Bidabadi SS, Ghobadi C, Gray DJ. Effect of methyl jasmonate treatments on alleviation of polyethylene glycol -mediated water stress in banana (*Musa acuminata* cv. 'Berangan', AAA) shoot tip cultures. Plant Growth Regulation. 2012; 68: 161–169. https://doi.org/10.1007/s10725-012-9702-6

37. Mahouachi J, Lopez-Climent MF, Gomez-Cadenas A. Hormonal and Hydroxycinnamic Acids Profiles in Banana Leaves in Response to Various Periods of Water Stress. The Scientific World Journal. 2014; 2014: 1–9. http://dx.doi.org/10.1155/2014/540962

38. Bidabadi SS, Mahmood M, Baninasab B, Ghobadi C. Influence of salicylic acid on morphological and physiological responses of banana (*Musa acuminata* cv. 'Berangan', AAA) shoot tips to in vitro water stress induced by polyethylene glycol. Plant Omics Journal. 2012; 5: 33–39.

39. Li L-F, Wang H-Y, Zhang C, Wang X-F, Shi F-X, Chen W-N, et al. Origins and Domestication of Cultivated Banana Inferred from Chloroplast and Nuclear Genes. PLoS ONE. 2013; 8: 1–11. https://doi.org/10.1371/journal.pone.0080502 PMID: 24260405

40. FAO (2017). Banana market review. Food and Agriculture Organization of the United Nations. 2015–2016. Disponível em: http://www.fao.org/economic/est/est-commodities/bananas/en/

41. Thill DC, Schimman RD, Appleby AP. Osmotic stability of mannitol and polyethyleneglycol 20000 solutions used as seed germination media. Agronomy Journal. 1979; 71:105–108. https://doi.org/10.2134/agronj1979.00021962007100010027x

42. Hu W, Yan Y, Shi H, Liu J, Miao H, Tie W, et al. The core regulatory network of the abscisic acid pathway in banana: genome-wide identification and expression analyses during development, ripening, and abiotic stress. BMC Plant Biology 2017; 17: 1–16. https://doi.org/10.1186/s12870-016-0951-9

43. Bidabadi SS, Mahmood M, Meon S, Wahab Z, Ghobadi C. Evaluation of in vitro Water Stress Tolerance among EMS–Induced Variants of Banana (*Musa* spp., AAA), Using "Morphological, Physiological and Molecular" Traits. Journal of Crop Science and Biotechnology. 2011; 14: 255–263. https://doi.org/10.1007/s12892-011-0073-8

44. Bidabadi SS, Meon S, Wahab Z, Subramaniam S, Mahmood M, In'vitro'selection and characterization of water stress tolerant lines among ethyl methanesulphonate (ems) induced variants of banana ('Musa'spp., with AAA genome). Australian Journal of Crop Science. 2012; 6: 567–575.

45. Placide R, Christian CS, Rony S. Development of in vitro technique to screen for drought tolerant banana varieties by sorbitol induced osmotic stress. African Journal of Plant Science. 2012; 6: 416–425. https://doi.org/10.5897/AJPS12.101

46. Sampangi-Ramaiah MH, Ravishankar KV, Seetharamaiah SK, Roy TK, Hunashikatti LR, et al. Barrier against water loss: relationship between epicuticular wax composition, gene expression and leaf water retention capacity in banana. Functional Plant Biology. 2016; 43: 492–501. http://dx.doi.org/10.1071/FP15296

47. Ochola D, Ocimati W, Tinzaara W, Blomme G, Karamura EB. Effects of water stress on the development of banana xanthomonas wilt disease. Plant Pathology. 2105; 64: 552–558. https://doi.org/10.1111/ppa.12281

48. Carpentier SC, Vertommen A, Swennen R, Witters E, Fortes C, Souza MT, et al. Sugar-Mediated Acclimation: The Importance of Sucrose Metabolism in Meristems. Journal of Proteome Research. 2010; 9; 5038–5046. https://doi.org/10.1021/pr100321v PMID: 20738108

49. Msogoya TJ, Grout BW Cytosine DNA methylation changes drought stress responses in tissue culture derived banana (Musa AAA–East Africa) plants. Journal of Applied Biosciences. 2012; 49: 3383–3387.

50. Torres-Ruiz JM, Diaz-Espejo A, Perez-Martin A, Hernandez-Santana V. Role of hydraulic and chemical signals in leaves, stems and roots in the stomatal behaviour of olive trees under water stress and recovery conditions. Tree Physiology. 2014; 35: 415–424. https://doi.org/10.1093/treephys/tpu055 PMID: 25030936

51. Surendar KK, Devi DD, Ravi I, Jeyakuma R P, Velayudham K. Effect of Water Stress on Leaf Temperature, Transpiration Rate, Stomatal Diffusive Resistance and Yield of Banana. Plant Gene and Trait. 2013; 8: 43–47. https://doi.org/10.5376/pgt.2013.04.0008

52. Surendar KK, Devi DD, Ravi I, Krishnakumar S, Kumar SR, Velayudham K. Impact of Water Deficit on Photosynthetic Pigments and Yield of Banana Cultivars and Hybrids. Plant Gene and Trait. 2013; 4: 17–24. https://doi.org/10.5376/pgt.2013.04.0004

53. Surendar KK, Devi DD, Ravi I, Jeyakumar P, Velayudham K. Water Stress Affects Plant Relative Water Content, Soluble Protein, Total Chlorophyll Content and Yield of Ratoon Banana. International Journal of Horticulture. 2013; 3: 96–103. https://doi.org/10.5376/ijh.2013.03.0017

54. Surendar KK, Devi DD, Jeyakumar P, Velayudham K, Ravi I. Changes in Proline and Polyphenol oxidase enzyme activity in some Banana Cultivars and Hybrids under water stress. Genomics and Applied Biology. 2015; 6: 1–6. https://doi.org/10.5376/gab.2015.06.0004

55. Chandrashekar N, Ravishankar KV, Laxman RH, Rekha A, Swarupa V. Physiological and biochemical changes during moisture stress in banana. Asian Journal of Bio Science. 2012; 7: 1–4.

56. Surendar KK, Devi DD, Ravi I, Jeyakumar P, Velayudham K. Physiological and biochemical behavior in banana cultivars and hybrids under water deficit. African Journal of Agricultural Research. 2013; 8: 4198–4208. https://doi.org/10.5897/AJAR2013.7464

57. Surendar K.K, Devi DD, Ravi I, Jeyakumar P, Velayudham K. Influence of water stress on antioxidative enzymes and yield of banana cultivars and hybrids. Pakistan Journal of Biological Sciences. 2013; 6; 1997–2002. https://doi.org/10.3923/pjbs.2013.1997.2002

Capítulo 2

Running title: Effects of water stress on *Musa* sp.[A]

Manuscript category: Genetics and Plant Breeding

Screening of banana genotypes for drought tolerance based on hydroponics system

Adriadna Souza Santos[1]; Edson Perito Amorim[2], Maurício Antônio Coelho Filho[2*], Claudia Fortes Ferreira[2], Alex-Alan Furtado[2], Carlos Priminho Pirovani[1]

[1] Department of Biological Sciences, State University of Santa Cruz (UESC), 45662-900, Ilhéus, Bahia, Brazil.

[2] Embrapa Cassava and Fruits, Rua Embrapa s/n CP 007, 44380-000 Cruz das Almas, Bahia, Brazil.

*Corresponding author <mauricio-antonio.coelho@embrapa.br>

[A] Instructions to authors available in: < http://www.scielo.br/revistas/sa/iinstruc.htm>

ABSTRACT

SCREENING OF BANANA GENOTYPES FOR DROUGHT TOLERANCE BASED ON HYDROPONICS SYSTEM

Rationale _____ Bananas are typical of regions of tropical and subtropical climate, where they perform better due to the high humidity of the air and low depletion of the available soil water. However, these conditions are also favorable to the onset of fungal diseases. Thus, the production of drought tolerant varieties is an alternative to expand cultivation sites to rainfed areas and reduce rates of infection by pathogenic fungi.

Objective _____ Selecting genotypes of Musa sp. (AA), through a hydroponic system, to be used in crosses for the development of cultivars resistant to water stress.

Methods _____ A hydroponic system was tested on 14 banana genotypes, 12 diploids and two commercial varieties with a known response, such as dry tolerance, Grand Naine (highly susceptible to water deficit) and hybrid BRS Princesa (tolerant to water deficit)

Results _____ The results show that the improved diploids presenting contrasting responses regarding tolerance to water deficit.

Conclusion ___ In conditions of water deficit, most of the banana diploids maintain high relative water content, however there is a reduction of biometric characteristics and elongationof roots.

Keywords: phenotyping, root system, plant growth, leaf resistance, water content

1. Introduction

Edible bananas (*Musa* spp. *Eumusa* section) are parthenocarpic, triploid or tetraploid fruits and are generally sterile due to crosses between species and subspecies of *M. acuminata* (genome "A") or hybridization of *M. acuminata* with *M. balbisiana* (genome "B") (VON LOESECKE, 1949).

Bananas are considered the fourth most important food in the world, behind rice, wheat and corn (PERRIER et al., 2011). In world fruit production, it occupies fourth place, after grapes, citrus fruits and apples (SURENDAR et al., 2013).

This production is threatened by biotic factors, such as yellow and black sigatokas, Fusarium wilt race 1 and Tropical race 4 (TR4) and nematodes, and abiotic factors, such as water deficit, salinity and temperature (CARVALHO et al., 2012).

Dry season regimes may cause reduction of relative water content in leaves, plant growth, total leaf area, transpiration, photosynthetic capacity, stomatal conductance and internal CO_2 concentration (BANANUKA et al., 1999), which influence yield (VAN-ASTEN et al., 2011) and fruit production (RAVI et al., 2013).

Bananas are typical of regions of tropical to subtropical climate, presenting optimal the production between 1200 mm and 2690 mm (ROBINSON and ALBERTS, 1986) depending on weather, plant and soil water managment (ALLEN et al., 1998) principally regarding the referency evapotranspiration (ETo), crop phenological phase and rainfall sazonality all togheter affecting the soil water balancy on plant rootzone. The plant is sensitivy of water deficit spscially during the fruit production, and the yield tend to increase with irrigation level (FIGUEREDO et al., 2006; COELHO et al., 2006) meaning reduction in water use efficiency (WUE). Nevertheless, even in semiarid climate, is possible the reduction irrigation level maintaining yield with high WUA by

using appropriated strategy of water irrigation management (SANTOS et al., 2016) and use of varieties (COELHO et al., 2013).

Thus, this study used an adapted hydroponics system, associated with physiological and phenological, as a method for the screening of improved diploids (developed by Embrapa) for tolerance to water deficit. The selected genotypes (AA) could be used in crosses aiming to develop cultivars resistant to water deficit.

2. Materials and Methods

2.1 Plant material

Micropropagated seedlings of 12 improved diploids developed by Embrapa, all with genetic resistance to 'Sigatoka-amarela' and Fusarium wilt race 1 and some to 'Sigatoka-negra' (Appendice 1, Table 1.1), were used. The cultivars Grand Nain (low tolerance to water deficit) and the BRS Princesa hybrid developed by Embrapa (tolerant to the water deficit), were used as controls (MUTHUSAMY et al., 2016; SANTOS et al., 2017).

2.2 Growing conditions

The experiment was conducted under greenhouse conditions at Embrapa Mandioca e Fruticultura (12°40’S and 39°06’WO). A hydroponic system adapted to perform the screening allowed the experimental control, ensuring that the treatments were applied evenly to all the plants. Air temperature and relative humidity were monitored using a portable thermohigrometer.

The system was mounted in aluminum boxes measuring 35 cm high, 70 cm long and 55 cm wide (Appendice 2, Fig. 2.1.). A total of 10 boxes were covered with plastic canvas and filled with a nutrient solution in order to avoid any nutritional deficiency, composed of 115 L of distilled water and 325 g of Forth Soluble fertilizer (10% N; 42% de P_2O_5; 10% K_2O; 0.03% B; 1.4% S; 0.2% Fe; 1% Mg) of the brand Forth Jardim. In order to avoid any stress related to oxygenation, the aeration of the system was controlled by using five aquarium pumps (Dimensions: 6.5 x 4 x 3.8 cm) with a flow rate of 1.600cm³ / min, one pump for each two boxes, running throughout the experimental period.

Two hundred and eighty plants measuring 8 to 10 cm tall were transplanted and cultured individually for a period of acclimatization of 21 days in PVC tubes filled with sterile, washed coarse sand immersed in nutrient solution in the aluminum boxes. Each box contained 28 tubes with 50 cm in height, 75 mm in diameter and the bottom lined with shading screen of 30%. The organization of the plants followed the randomized block design (DBC), with 5 replicates and 1 plant per experimental unit. (Appendice 2, Fig. 2.2.).

The experiment was set up with 14 genotypes, with 5 plants per treatment, adding a total of 70 plants under control condition, 70 in moderate stress and 70 in severe stress (Fig. 1). After acclimatization, the control plants remained in nutrient solution throughout the experiment (field capacity 0.3 cm^3 cm^{-3}), with constant volume replacement in the boxes, being collected one per day, from the beginning of the collections. On the other hand, the water deficit treatment plants were removed from the nutrient solution and their collections were conditioned to the following specifications: i) moderate deficit: when the stomatal resistance (SR) of the stressed genotype was superior to its respective control and the water content of the substrate maintained was between 0.10 and 0.5 cm^3 cm^{-3}; ii)

severe deficit: when SR of the stressed genotype was higher than its respective control, the water content remained between 0.5 and 0.05 cm^3 cm^{-3} and occurred wilting in the morning (two consecutive days). The water stress experiment lasted 11 days at most.

During the experiment, the water content of the substrate in each experimental unit (vessel with a plant) was monitored by time domain reflectometry (TDR), one TDR probe per tube, and correlated with stomatal resistance (SR) to confirm water deficit treatments as described above. SR ($s.cm^{-1}$) was measured with an AP4 digital model (Delta T Devices, Cambridge, England).

The relative water content in the leaf (TRA) was determined by the Barrs and Weatherley method (1969) and calculated according to the following equation:

Eq. 1

$$TRA = [(Fresh\ mass - dry\ mass) / (turgid\ mass - dry\ mass) \times 100].$$

2.3 Biometric Data

For comparison of the initial and final biometrics, three control and three treatment plants of the severe stress treatment of each genotype were measured on the first and last day of the experiment. These plant calculations of the percentage difference between the initial and final measurements were made with the following formula:

Eq. 2

$$x = \frac{(f - i)}{i} * 100$$

Whereas: x is percentage variation (in %); f is the final value; and i is the initial value. In the equation, x can assume three values: $x > 0$, when $f > i$; $x < 0$, when $f < i$; e $x = 0$, when $f = i$. In all cases, it is assumed that $i \neq 0$.

On the day of collection, height, diameter of the pseudostem, leaf area (RAVI et al., 2013), number of leaves, number and maximum length of the main roots in centimeter, and photographic record of the whole plant were measured. In order to visualize the difference of biometry between the plants on the day of collection and their respective controls, the following calculations were performed:

Eq. 3

$$x = \frac{(c - ds)}{ds} * 100$$

Where: x is percent change (%); c is the biomass value of the control; and ds is the value of biomass in the treatment of drought stress (moderate or severe). In the equation, x can assume three values: $x > 0$, biomass smaller than the control; $x < 0$, biomass greater than control; and $x = 0$, when $c = ds$. In all cases, it is assumed that $i \neq 0$.

2.4 Measures of fresh and lyophilized mass

On the day of assessment, the plants were separated into leaves, pseudostem, rhizome and root and the fresh and freeze-dried masses of the plants were determined. Later, it was possible to analyze the ratio between dry aerial matter (DAM) and dry mass root (DMR), calculated by equation:

Eq. 4

$$\text{RDMAR} = \frac{DAM}{DMR}$$

Where: DAM corresponds to the dry aerial matter, in grams, and DMR corresponds to the dry mass root, also in grams.

2.5 Statstical analysis

Multivariate analyzis (ANOVA) was performed with the aid of the statistical program R (R DEVELOPMENT CORE TEAM, 2017).

3. Results

3.1 Air humidity and ambient temperature

The relative humidity varied around 2% during the experiment, with a minimum of 84% and a maximum of 86%. The temperature varied approximately 2 °C in the experiment period, with a minimum of 22.9° C and a maximum of 25°C.

3.2 Substrate humidity and number of days

Within each treatment, there was no significant difference between genotypes regarding the number of days for harvest (test of Scott-Knott $p<0.05$). The water content of ssubstrate of control plants was maintained above 0.28 $cm^3\ cm^{-3}$ in each experimental unit (Fig. 2).

The first plants were collected from the PMGB044 and PMGB052 genotypes after seven days without replacement of the nutrient solution, presenting moderate stress characteristics (SR>C and SWC<0.10 and 0.5 $cm^3\ cm^{-3}$) (Fig. 3a). Only one plant of the genotypes Grand Nain, PMGB043, PMGB048, PMGB052, PMGB060, PMGB070 and PMGB099 reached severe stress (SR>C, SWC<0,10$cm^3.cm^{-3}$ e) on the eleventh day (Fig. 3b); the other replicates were collected before the tenth day of the same treatment. Two

plants of the PMGB075 genotype, three of the PMGB044 and PMGB065 genotypes and four of the PMGB050, PMGB069 and PMGB098 genotypes, reached severe stress condition after 11 days of experiment. The BRS Princesa cultivar and the genotypes PMGB043, PMGB070, PMGB075 and PMGB099, showed a significant difference in the number of days between treatments (test of Tukey ($p<0.05$).

3.3 Stomatal resistance (SR)

The plants in the control condition maintained stomatal resistance (SR) below 1.27 s cm^{-1}, on average (Fig.4). On the day they were collected, the plants in moderate stress had greater stomatal regulation than the control. In water deficit treatments, plants with severe stress presented higher SR, particularly G. Nain, PMGB043, PMGB044, PMGB050, PMGB075 and PMGB099.

3.4 Relative water content (RWC)

The banana genotypes presented RWC higher than 70%. The lowest mean, 72.39%, was observed in the severe stress treatment of the PMGB044 genotype. The highest mean, 93.72%, is presented in moderate treatment of genotype PMGB043. Only Grand Nain cultivars and genotypes PMGB043, PMGB044, PMGB048 and PMGB098 showed significant interaction between treatments and genotypes, especially for severe stress (Table 1).

3.5 Biometric data

223 *3.5.1 Control vs severe stress*

224

225 O diameter of pseudo-stem (D), total leaf area (TLA) and number of leaves (NL)
226 did not present significant interaction between genotypes and treatments, while the height
227 (H) of the pseudostem showed significant variation. Most genotypes showed increased
228 measurements at the end of the experiment (Appendice 3, Table 3.1 and 3.2). The
229 percentage difference in plant height in the control condition was higher than in plants
230 with severe stress, except for PMGB069 (Fig. 5a). Between the initial and final period,
231 the control plants showed a larger diameter increase than the plants with severe deficit,
232 except for PMGB099 genotype (Fig. 5b). The Grand Nain variety showed reduction of
233 the pseudostem diameter between the periods of the severe deficit experiment. The total
234 leaf area growth between periods of the plants in the control condition was higher in
235 comparison with the plants with severe deficit, except for genotypes BRS Princesa,
236 PMGB048 and PMGB099. Genotypes G. Nain, PMGB043, PMGB044, PMGB069,
237 PMGB070 and PMGB075 reduced leaf area under stress (Fig. 5c). The number of leaves
238 of the plants under control condition was higher than in the plants under severe stress,
239 except for genotype PMGB044 and PMGB065. Under severe deficit, the Grand Nain
240 variety presented a reduction in the number of leaves in comparison to the initial period
241 (Fig. 5d).

242

243 *3.5.2 Control vs moderate and severe*

244

245 Biometric variables measured at the end of the experiment, except pseudostem
246 height, showed no significant interaction between genotypes and treatments (Appendice
247 4, Tables 4.1 and 4.2). The positive differences (Fig. 6) indicate that the control plants

were significantly larger than the plants in water deficit, especially in terms of height of pseudostem, total leaf area and number of leaves. The exception was for pseudostem height (Fig. 6a) of genotypes PMGB070 and PMGB098, where the plants in severe stress were larger when compared to the respective controls. Plants of the genotype PMGB050, PMGB069, and PMGB098, presented larger diameter under severe stress (Fig. 6b). The total leaf area of the PMGB060 and PMGB098 genotypes was higher than the control (Fig. 6c). The number of leaves of G. Nain and PMGB070 in the severe stress treatment was relatively higher than that of the control (Fig. 6d). The percentage difference in root length shows that under moderate and severe stress, most plants lengthened their main roots (Fig. 6e). Plants with moderate deficit presented lower number of main roots in comparison to their respective controls, except BRS Princesa and PMGB098. Under severe water deficit, most plants presented a higher length of main roots (Fig. 6f).

3.6 Lyophilized dry mass

The growth of the aerial part in relation to the growth of the root system was superior in the control, when the treatments of water deficit were compared (Fig. 7). Of the plants in water deficit, those of the severe stress treatment accumulated more dry mass of the aerial part in relation to the moderate stress, except for the genotypes PMGB048, PMGB060, PMGB065, PMGB070 and PMGB075.

Plants with moderate and severe water deficit differed significantly in terms of dry mass of the root system (Fig. 8). The genotypes PMGB048, PMGB052 and PMGB099 presented the highest averages in water deficit.

3.7 Different water deficit treatments

The majority of the plants presented well developed aerial part in the control, in comparison with their respective treatments under water deficit (moderate and severe). In general, the main roots were larger in plants with water deficit (Appendice 5, Figure 5.1). Genotypes such as PMGB050 and PMGB099, maintained the large size in all treatments.

4. Discussion

The 12 improved diploids developed by Embrapa with resistance to the main diseases of the banana plant had not yet been characterized as to the tolerance to water deficit and for that reason the genotypes Grand Nain (AAA), susceptible (MUTHUSAMY et al., 2016) and BRS Princesa (AAAB), tolerant to drought (SANTOS et al., 2017) for purposes of comparison in the different variables. The BRS Princesa cultivar is a product of Embrapa's banana genetic breeding program; and is classified as a genotype of the “Silk” type and resistant to the yellow and black sigatokas and Fusarium wilt race 1 (REBOUÇAS et al., 2018).

In addition, the banana species that have the "B" genome presented greater tolerance to drought (VANHOVE et al., 2012), since they were domesticated under more severe climatic conditions, such as wide variation in temperature and soil water status (DAVEY et al., 2013). The use of plants of the “A” genome in traditional crosses, aims at the aggregation of characteristics desirable to the crop, such as high fruit quality, high yield, green and yellow life and taste (HU et al., 2015).

In order to identify banana genotypes tolerant to water deficit, an adapted hydroponic system was tested using washed sand as a substrate and nutrient solution to

provide macro and micronutrients essential for plant growth. Therefore, this aimed to minimize the costs with the assembly of an experiment in the field, with reduction also of the physical space. The 5L plastic vessels, which are usually used in experiments in each of the plants, were replaced by PVC pipes with washed sand, considered low cost compared to commercial substrates. With this strategy, it was possible to reduce the planted space needed to grow banana plantlets to 385 m^2, as well as the time to impose the water deficit treatment for 11 days.

The environmental variations of this study were minimal. The use of the greenhouse provides reliable results for phenotype studies, since it allows the reduction of errors from environmental variations and also allows a greater accuracy in the physiological responses of the plants when compared to in vitro tests (VANHOVE et al., 2012). However, in vitro assays have been more used to answer specific questions related to water deficit in banana (BIDABADI et al., 2011; MAHMOOD and BIDABADI et al., 2012; RUKUNDO et al., 2012; SHEKHAWAT et al., 2011; VANHOVE et al., 2012) leaving a gap in studies that bring plants closer to field conditions.

In the hydroponic system, the improved diploids PMGB044, PMGB050, PMGB065, PMGB069 and PMGB098 were more tolerant to the water deficit, since they arrived at the last day of stress with more than half of the replicates (when they reached the wilting point of the leaves and the low water content of the substrate). Most plants of the Grand Nain genotype, selected as a pattern of susceptibility to water deficit (MUTHUSAMY et at. 2016), were also susceptible in this experiment, reaching severe stress more rapidly.

Water deficit is one of the abiotic factors that most limit agriculture, especially in the case of banana plantations, which are highly sensitive to environmental changes (TURNER et al., 2007). Plants require abundant water supply, indispensable during

development processes due to their permanent green coverage, short roots and rapid growth rate (VAN ASTEN et al., 2011).

According to the results found for the genotypes BRS Princesa, PMGB043. PMGB44, PMGB50, PMGB75 and PMGB99 under water deficit conditions, these plants increase the stomatal resistance to minimize water loss.. Stomatal closure is a recognized strategy used by plants to avoid loss of water by transpiration (DE SENA et al., 2007). The genotypes PMGB050 and PMGB099, also added a root growth superior to the standard genotype of susceptibility, Grand Nain, in the conditions of water deficit, which makes them promising when raising the climatic risks related to the water deficit.

Despite the significant response of some genotypes to soil water deficit, all were able to maintain high RWC, corroborating the behavior shown for commercial banana genotypes, such as the 'Grand Nain' (AAA genome) (MAHOUACHI et al., 2014) and 'Rasthali' (AAB genome) (SREEDHARAN et al., 2013). Among genotypes affected in the RWC, PMGB044 is one of the most susceptible, however, even with this predisposition, most plants tolerated water stress and reached the wilting point of leaves in the last day of the experiment.

Regarding the biometric data there was higher growth rate of plants under control conditions compared with plants stressed.This suggests that the hydroponic system can be used for plantlet cultivation, reducing the time of acclimatization and availability for field planting. Plants under water deficit also grew, but at a lower rate, because continue to accumulate dry mass even during stress. When the stomata close, the CO_2 exchange is affected and the plants start to mobilize the CO_2 reserves of the ^{13}C isotopes for the construction of the carbon skeleton. Under ideal conditions, during the Calvin cycle, Rubisco prefers the CO_2 consumption of the ^{12}C isotope rather than the ^{13}C isotope as substrate (KISSEL et al., 2015).

The PMGB043, PMGB044, PMGB069, PMGB070 and PMGB075 diploids had a greater negative effect of water deficit on their growth, as observed for the hybrid (Sukalindizi, genome AB) and commercial genotypes ('Nfuuka', genome AAA-EA, 'French Plantain', genome AAB, 'Gros Michel', genome AAA, 'Lep Chang Kut', genome BBB and 'FHIA-02', genome AAAA) (BANANUKA et al., 1999).

5. Conclusions

To the best of our knowledge, this is the first hydroponic-based sorting system for selection of drought-tolerant Musa ssp. Cultures such as banana, which are large and long-lasting (12 to 20 months) present many practical difficulties in the reproducibility of experimental conditions (RAVI et al., 2013). In this work, conditions were created for banana phenotype tests for drought tolerance, with low cost and large scale. The parameters such as water content, stomatal regulation, biometric data and biomass indicate that genotype PMGB043 and PMGB099 are more susceptible and tolerant to water deficit. Thus, the parents of Musa acuminata, developed by Embrapa, present contrasting responses related to water deficit.

6. References

ALLEN, RG; PEREIRA, LS; RAES, D; SMITH, M. 1998. Crop evapotranspiration-Guidelines for computing crop water requirements - FAO Irrigation and drainage paper 56. Fao. 300: 1-15.

BANANUKA, JA; RUBAIHAYO, PR; TENYWA, MM. 1999. Reactions of *Musa* Genotypes to Drought Stress. African Crop Science Journal. 7: 333–339.

BARRS, HD; WEATHERLEY, PE. 1962. A re-examination of the relative turgidity technique for estimating water deficits in leaves. Australian Journal of Biological Sciences. 24: 519-570.

BIDABADI, S. S. 2011. Evaluation of *in vitro* water stress tolerance among EMS – induced variants of banana (*Musa* spp., AAA), using "morphological, physiological and molecular" Traits. Journal of Crop Science and Biotechnology. 14: 255-263.

CARVALHO, ACPP; RODRIGUES, AAJ; SANTOS, EO. 2012. Production of micropropagated banana seedlings. Brasília: Embrapa, 12p. (Technical Circular 34). (in Portuguese). Available in: <http://ainfo.cnptia.embrapa.br/digital/bitstream/item/72455/1/CIT12001.pdf>.

COELHO, EF; COSTA, ED; LEDO, CDS; SILVA, SOE. 2006. Productivity and efficiency of water use of dwarf silver banana prata anã egrand naine under irrigation in the third cycle in northern Minas Gerais. Irriga, 11: 460-468. (in Portuguese)

COELHO, E. F., DE OLIVEIRA, R. C., PAMPONET, A. J. M. 2013. Water requirements type Terra banana in coastal conditions. Pesquisa Agropecuária Brasileira, 48: 1260-1268. (in Portuguese)

DAVEY, MW; GUDIMELLA, R; HARIKRISHNA, JÁ; SIN, LW; KHALID, N; KEULEMANS, J. 2013. A draft *Musa balbisiana* genome sequence for molecular genetics in polyploid, inter- and intra-specific *Musa* hybrids. BMC Genomics 14, 1-20.

DE SENA, JOA; ZAIDAN, HA; CAMARGO, E; CASTRO, PR. 2007. Transpiration and stomatal resistance variations of perennial tropical crops under soil water availability conditions and water deficit. Brazilian Archives of Biology and Technology. 50: 225-230.

FIGUEIREDO, FD; MANTOVANI, EC; SOARES, AA; COSTA, LC; RAMOS, MM; OLIVEIRA, FG. 2006. Produtividade e qualidade da banana prata anã, influenciada por lâminas de água, cultivada no Norte de Minas Gerais. Revista Brasileira de Engenharia Agrícola e Ambiental. 10: 798-803.

HU, W; ZUO J; HOU, X; YAN, Y; WEI, Y; LIU, J; LI, M; XU, B; JIN, Z. 2015. The auxin response factor gene family in banana: genome-wide identification and expression analyses during development, ripening, and abiotic stress. Frontiers in Plant Science. 6: 1-16.

KISSEL, E; VAN-ASTEN, P; SWENNEN, R; LORENZEN, J; CARPENTIER, SC. 2015. Transpiration efficiency versus growth: Exploring the banana biodiversity for drought tolerance. Scientia Horticulturae. 185: 175-182.

MAHMOOD, M; BIDABADI, SS. 2012. Effect of methyl jasmonate treatments on alleviation of polyethylene glycol -mediated water stress in banana (*Musa acuminata* cv. 'Berangan', AAA) shoot tip cultures. Plant Growth Regulation, 68:161-169.

MAHOUACHI, J; LÓPEZ-CLIMENT, MF; GÓMEZ-CADENAS, A. 2014. Hormonal and hydroxycinnamic acids profiles in banana leaves in response to various periods of water stress. The Scientific World Journal. 2014: 1-9.

MUTHUSAMY, M; UMA, S; BACKIYARANI, S; SARASWATHI MS; CHANDRASEKAR, A. 2016. Transcriptomic Changes of Drought-Tolerant and Sensitive Banana Cultivars Exposed to Drought Stress. Frontiers in Plant Science. 7: 1-14.

PERRIER, X; LANGHEB, E; DONOHUEC, M; LENTFERD, C; VRYDAGHSE, L; BAKRYA, F; CARREEL, F. 2011. Multidisciplinary perspectives on banana (*Musa* spp.) domestication. Proceedings of the National Academy of Sciences. 108: 11311-11318.

RAVI, I; UMA, S; VAGANAN, MM; MUSTAFFA, MM. 2013. Phenotyping bananas for drought resistance. Frontiers in Physiology. 4: 1-15. Doi: 10.3389/fphys.2013.00009

R CORE TEAM. 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vianna, Austria. Disponível em: URL https://R-project.org/. Acesso em nov. 2017.

REBOUÇAS, TA; HADDAD, F; FERREIRA CF; OLIVEIRA, SAS; LEDO, CAS; AMORIM, EP. 2018. Identification of banana genotypes resistant to Fusarium wilt race 1 under field and greenhouse conditions. Scientia Horticulturae. 239: 308-313.

ROBINSON, JC; ALBERTS, AJ. 1986. Growth and yield responses of banana (cultivar 'Williams') to drip irrigation under drought and normal rainfall conditions in the subtropics. Scientia Horticulturae, 30: 187-202.

RUKUNDO, P; CARPENTIER, SC; SWENNEN, R. 2012. Development of in vitro technique to screen for drought tolerant banana varieties by sorbitol induced osmotic stress. African Journal of Plant Science Vol. 6: 416-425.

441 SANTOS, MRD; DONATO, SL; LOURENÇO, LL; SILVA, TS; COELHO FILHO, MA.
442 2016. Irrigation management strategy for Prata-type banana. Revista Brasileira de
443 Engenharia Agrícola e Ambiental, 20: 817-822.

444 SANTOS, MR; DONATO, SLR; ARANTES, AM; COELHO, EF; OLIVEIRA, PM.
445 2017. Gas exchange in 'BRS Princesa' banana (*Musa* spp.) under partial rootzone drying
446 irrigation in the north of Minas Gerais, Brazil. Acta Agronómica. 66: 378-384.

447 SHEKHAWAT, UKS; GANAPATHI, TR; LINGAM, S. 2011. Cloning and
448 characterization of a novel stress-responsive WRKY transcription factor gene
449 (*MusaWRKY71*) from *Musa* spp. cv. *Karibale Monthan* (ABB group) using transformed
450 banana cells. Molecular Biology Reports. 38:4023–4035

451 SREEDHARAN, S; SHEKHAWAT, UK; GANAPATHI, TR. 2013. Transgenic banana
452 plants overexpressing a native plasma membrane aquaporin *MusaPIP1;2* display high
453 tolerance levels to different abiotic stresses. Plant Biotechnology Journal. 11: 942-952.

454 SURENDAR, KK; RAJENDRAN, V; DURGA, DD; JEYAKUMAR, P; RAVI, I;
455 VELAYUDHAM, K. 2013. Impact of water deficit on growth attributes and yields of
456 banana cultivars and hybrids. African Journal of Agricultural. 8: 6116-6125.

457 TURNER, DW; FORTESCUE, J. A; THOMAS, DS. 2007. Environmental physiology of
458 the bananas (*Musa* spp.). Brazilian Journal of Plant Physiology. 19, 463-484.

459 VAN-ASTEN, PJA; FERMONT, AM; TAULYA, G. 2011. Drought is a major yield loss
460 factor for rained East African high land banana. Agricultural Water Management. 98:
461 541-552.

462 VANHOVE, AC; VERMAELEN, W; PANIS,B; SWENNEN, R; CARPENTIER, SC.
463 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. Frontiers in Plant Science. 2: 1-10.

VON LOESECKE, H. W. 1949. Bananas, chemistry, physiology and technology. New York: Interscience Publishers. 1-189.

Figures and Tables

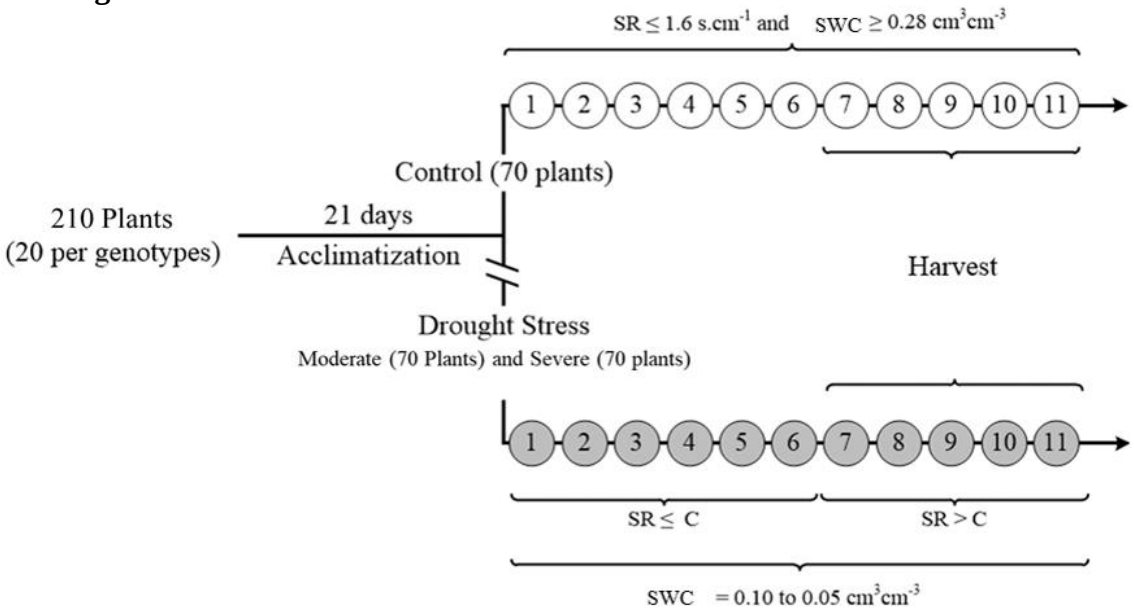


Fig. 1. Schematic overview of the experimental dynamics. SR and SWC correspond to stomatal resistance and substrate water content, respectively. The numbers in the circles indicate the time-period in days during the experiment; white circles correspond to control plants (C) and gray circles, plants under water deficit.

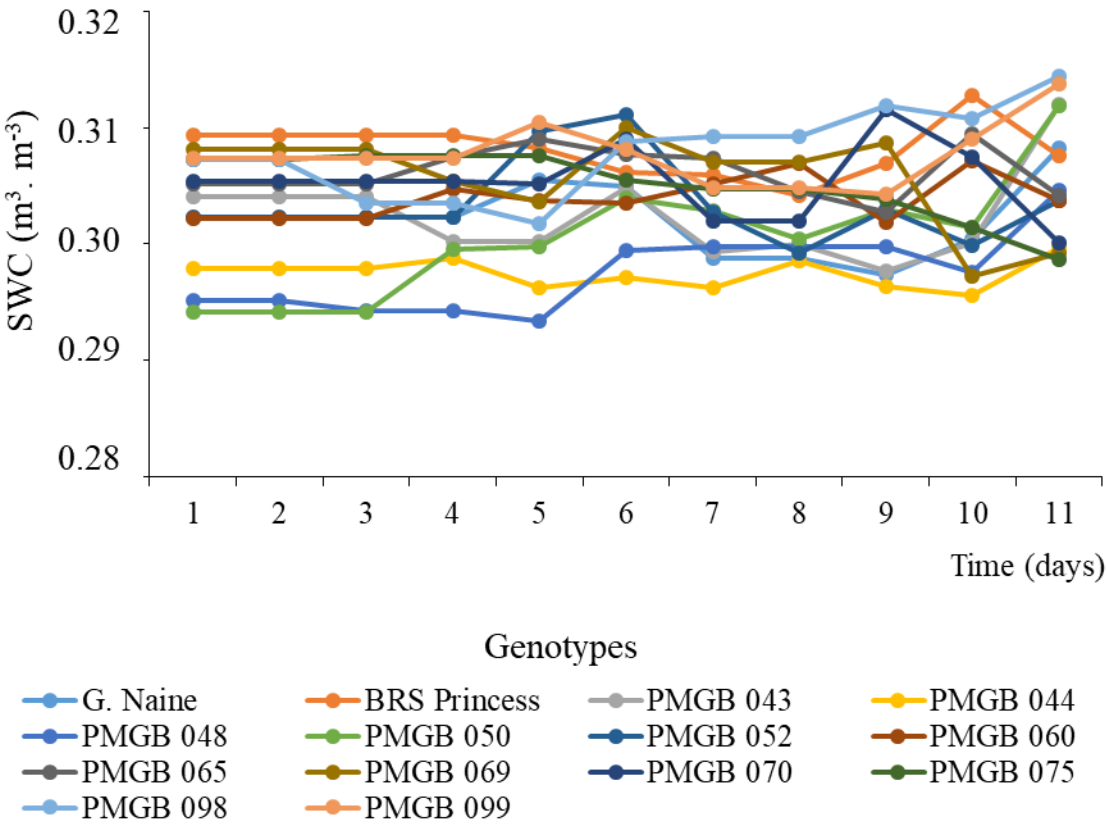


Fig. 2. Mean of substrate water content (SWC) determined with TDR probes in the control treatment.

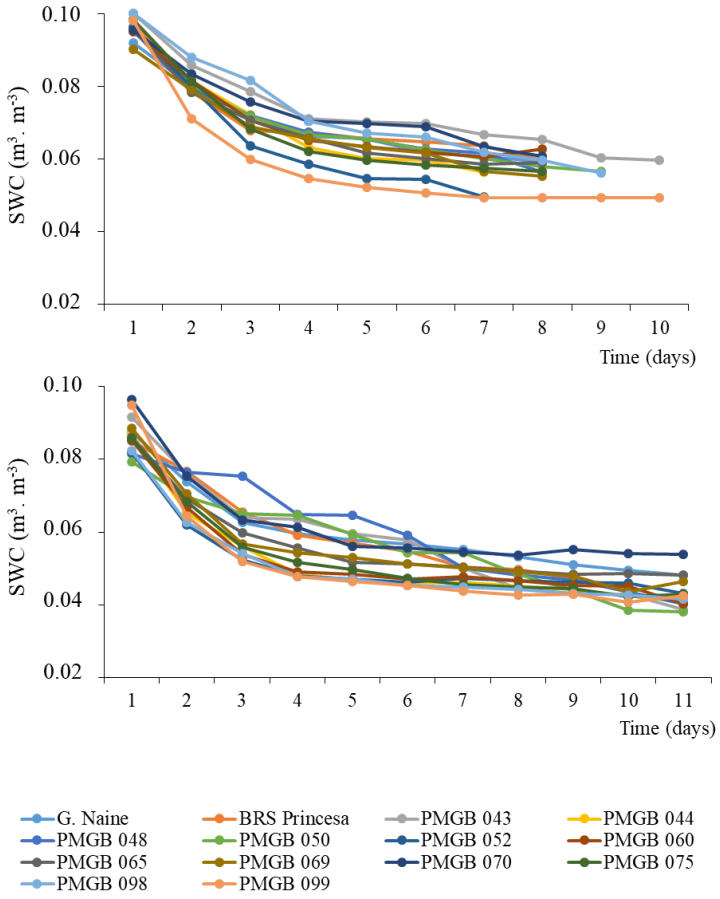


Fig. 3. Mean of substrate water content (SWC) determined with TDR probes. Each plot corresponds to a banana genotype submitted to moderate and severe water deficits. The discontinuity of the line indicates that all plants in the treatment were collected.

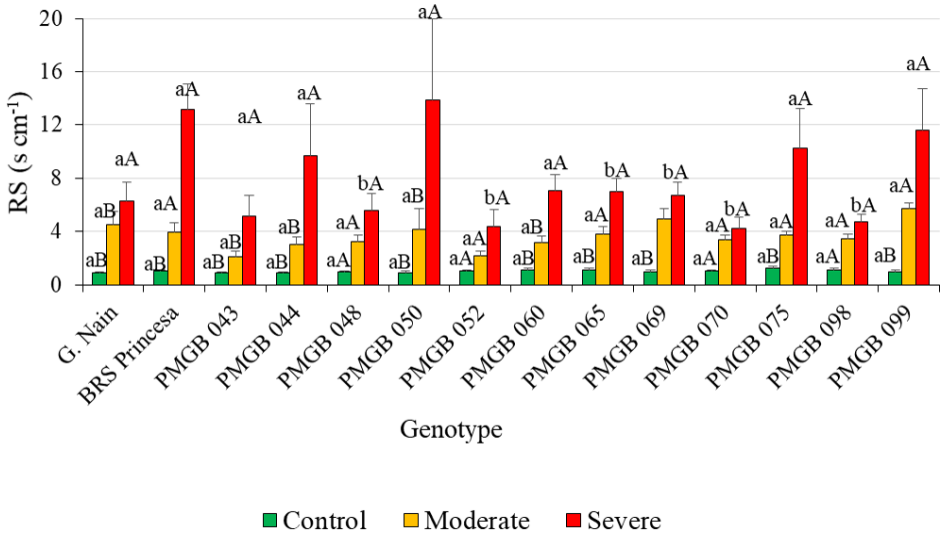
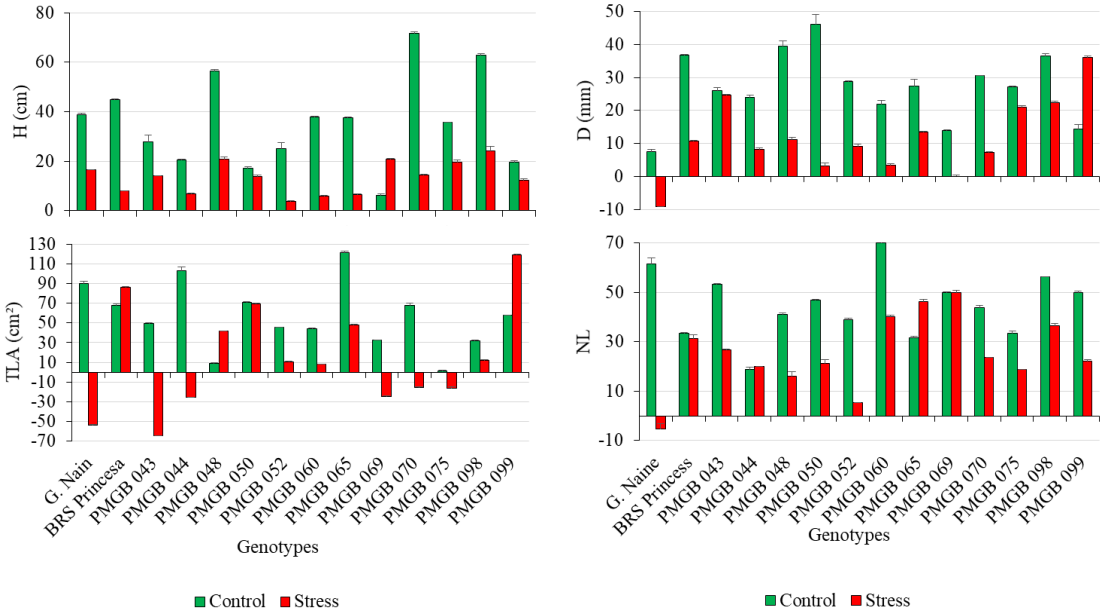


Fig. 4. Mean of stomatal resistance (SR) of banana genotypes submitted to hydrological regimes in a modified hydroponic system. Average values measured on the day of collection. Lower case letters represent the analysis of genotypes within each treatment by the Scott-Knott test ($p < 0.05$, $n = 5$) and upper case letters correspond to analysis of treatments in each genotype by Tukey's test ($p < 0.05$).

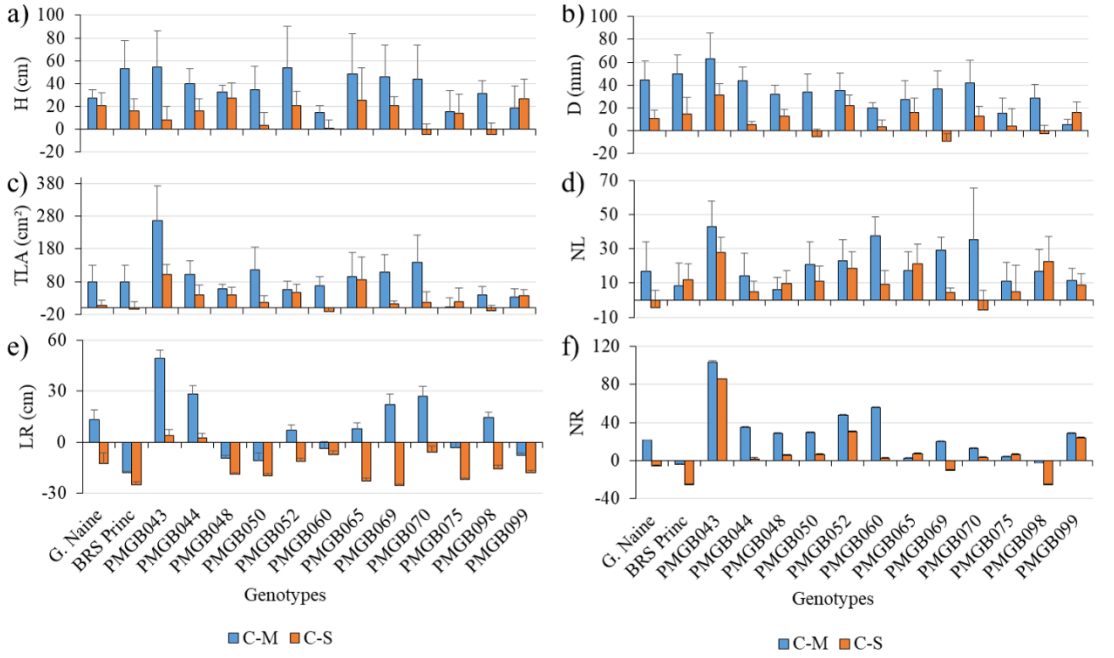
Table 1. Mean values of the relative water content of banana genotypes submitted to water regimes. Analysis of the interaction between banana genotypes and treatments (control, moderate and severe water deficit). X - corresponds to the mean, SD - corresponds to the standard deviation, Min, corresponds to the minimum value, Max - corresponds to the maximum value, G - corresponds to genotype and T - corresponds to the treatment (n = 5).

Genotypes	**Treatments**		
	Control	**Moderate**	**Severe**
Grand Nain	90.63 aA	90.34 aA	82.59 bAB
BRS Princesa	89.52 aA	93.72 aA	89.16 aA
PMGB 043	86.93 aAB	84.22 aAB	78.88 bB
PMGB 044	87.33 aA	88.12 aA	72.39 bB
PMGB 048	90.06 aA	87.15 aA	89.30 aA
PMGB 050	90.58 aA	87.88 aA	88.64 aA
PMGB 052	90.47 aA	87.55 aA	90.27 aA
PMGB 060	85.41 aA	88.22 aA	86.71 aA
PMGB 065	82.44 aA	90.67 aA	90.56 aA
PMGB 069	86.54 aA	88.26 aA	88.77 aA
PMGB 070	88.39 aA	92.55 aA	90.45 aA
PMGB 075	88.83 aA	91.64 aA	91.92 aA
PMGB 098	91.17 aA	89.98 aA	75.58 bB
PMGB 099	89.56 aA	88.96 aA	89.45 aA
X	88.42	89.23	86.05
SD	2.37	2.36	5.96
Min	82.44	82.22	72.39
Max	91.17	93.72	91.92
G	*	*	*
T	NS	NS	*
T x G	*	*	*

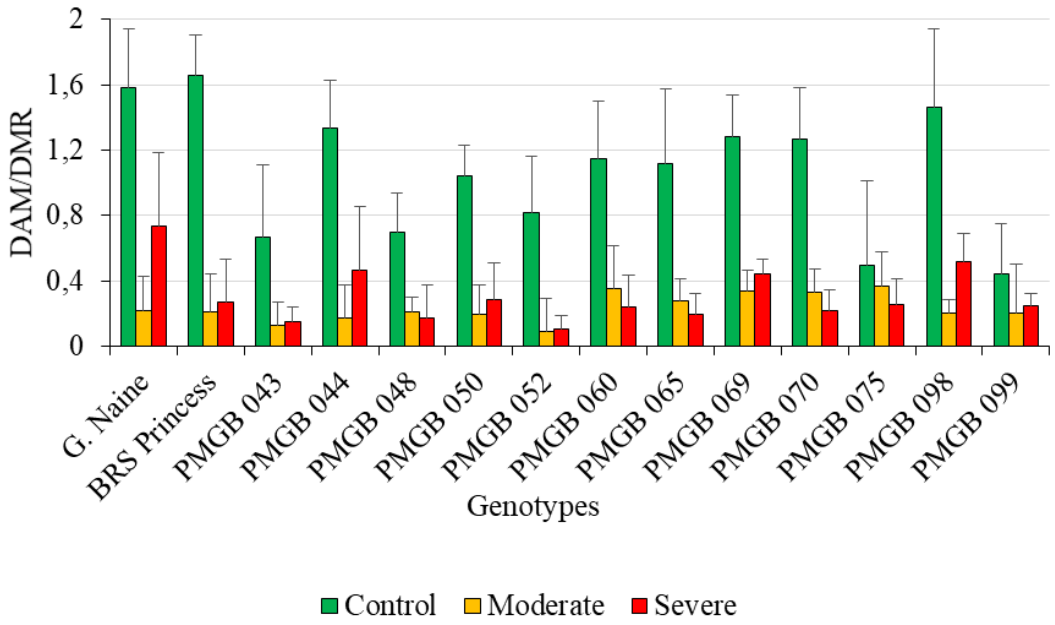
Means followed by the same lowercase letters in the columns belong to the same group by the Scott-Knott test at ($p < 0.05$, n = 70) and averages followed by the same uppercase letters do not differ statistically from each other by Tukey's test ($p < 0.05$, n = 20).



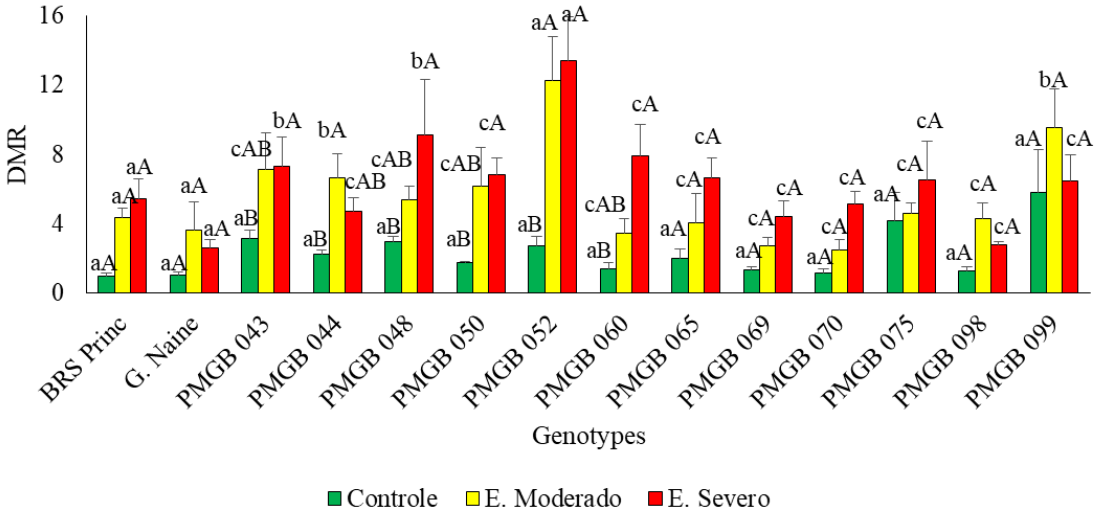
548
549
550 **Fig. 5.** Mean of percentage difference (%) of biometry of banana genotypes submitted to
551 control and severe stress treatments (n = 3). a - height of pseudostem (H); b - diameter of
552 pseudostem (D); c - total leaf area (TLA) and d - number of leaves (NL). Data collected
553 at the beginning and end of the experiment (n = 5).
554
555



556
557
558 **Fig. 6.** Mean of percentage difference (%) of biometry of banana genotypes submitted to
559 water deficit in comparison to their respective controls (n = 5). a - height of pseudo-stem
560 (H); b - diameter of pseudo-stem (D); c - total leaf area (TLA); d - number of leaves (NL);
561 e - root length (LR); and f - number of main roots (NR). Data collected at the beginning
562 and end of the experiment (n = 5).
563
564



565
566
567 **Fig. 7.** Mean of ratio between dry aerial matter (DAM) and dry matter of roots (DMR) of
568 banana genotypes submitted to control and water deficits (moderate and severe) (n = 5).
569
570



571
572
573 **Fig. 8**. Dry matter of roots (DMR) of banana genotypes in control and water deficit
574 treatments (moderate and severe). Mean of values in grams (n = 5).
575
576
577
578
579
580
581
582
583
584

Appendice 1.

Table 1.1. Characterization of improved banana diploids used in the experiment.

Improved diploids	Genealogy	B. S*	Y. S**	M. P ***
PMGB043	Borneo *x* Guyod	R	R	R
PMGB044	Calcutta 4 *x* Madang	R	R	R
PMGB048	Malaccensis *x* Madang	R	R	R
PMGB050	Tuu Gia *x* Calcutta 4	R	R	R
PMGB052	003004-01 (Calcutta 4 *x* Madang) *x* 001004-01 (Borneo *x* Madang)	-	R	R
PMGB060	M53 *x* 028003-01 (Tuu Gia *x* Calcutta 4)	-	R	R
PMGB065	Khai *x* 003004–01 (Calcutta 4 *x* Madang)	-	R	R
PMGB069	03037-02 (Calcutta 4 *x* Galeo) *x* SH3263	-	R	R
PMGB070	013018-01 (Malaccensis *x* Sinwobogi) *x* 003038-01 (Calcutta 4 *x* Heva)	-	R	R
PMGB075	Terrinha *x* Calcutta 4	-	R	R
PMGB098	091087-01 [010116 (Boeneo x Guyod) x 003038-01 (Calcuta 4 x Heva)]	-	R	
PMGB099	Jari Buaya *x* 003004–01 (Calcutta 4 *x* Madang)	-		R
Grand Nain		S	S	R
BRS Princesa	Yaganbi nº2 x M53	R	R	R

* black Sigatoka; ** yellow Sigatoka; *** Fusarium wilt, R: resistant; S: susceptible

Appendice 2.

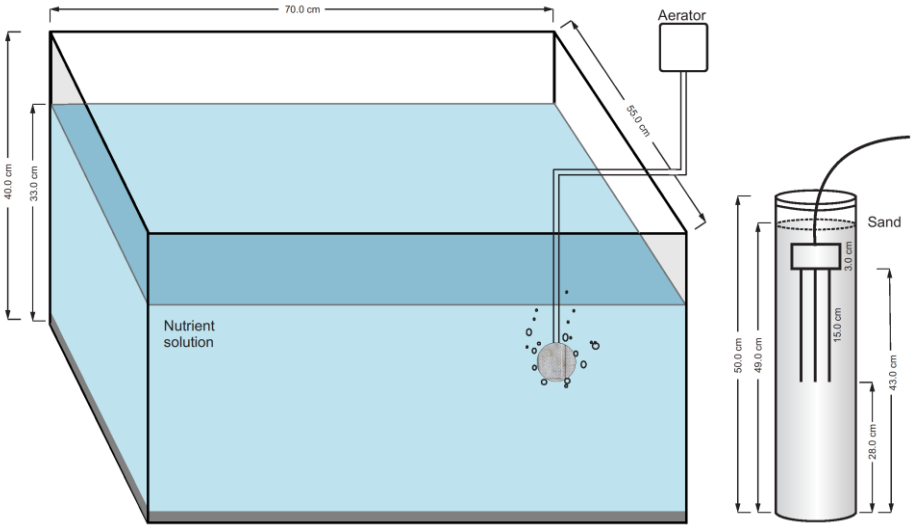


Fig.2.1. Schematic design of the aluminum box and PVC pipe used in the experiment. The blue color indicates the volume of water held per refill in the box.

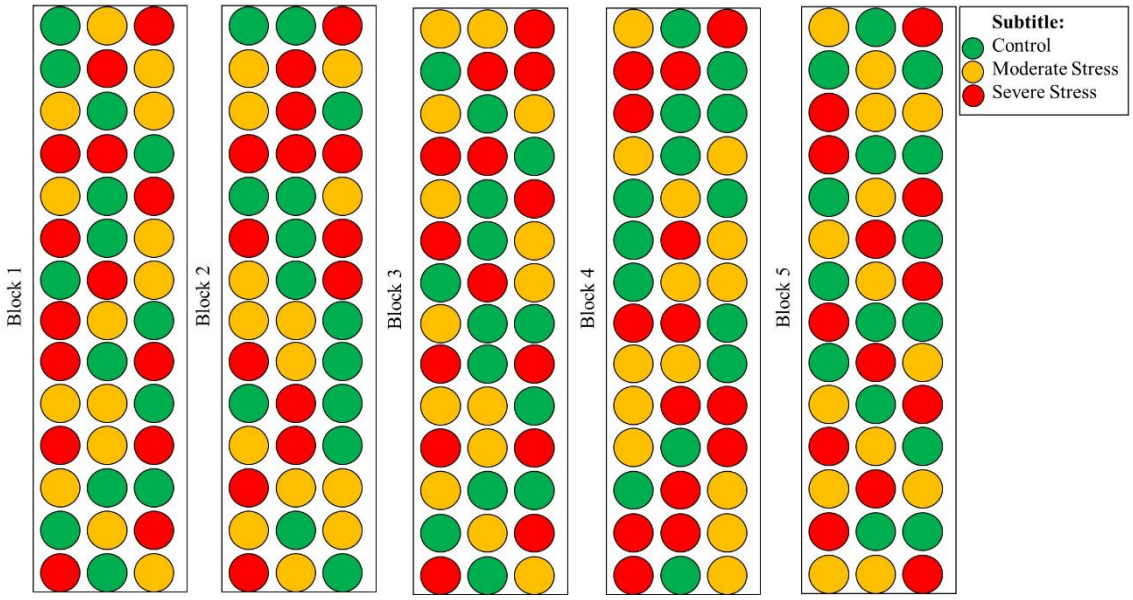


Fig.2.2. Schematic design of randomized blocks in the experiment. Circles indicate each experimental unit composed of a plant and the numbers indicate the genotypes. 1 - Grand Nain; 2 - BRS Princesa; 3 - PMGB043; 4 - PMGB044; 5 - PMGB048; 6 - PMGB050; 7 - PMGB052; 8 - PMGB060; 9 - PMGB065; 10 - PMGB069; 11 - PMGB070; 12 - PMGB075; 13 - PMGB098 and 14 - PMGB099.

Appendice 3. Statistical analysis of biometric data in the beginning and final stages of the experiment

Table 3.1: Averages of heights (cm) of the Musa genotypes submitted to control and severe stress treatments. HP: - Height of the Pseudostem, DP-Diamter of Pseudostem, TLA - Total leaf área and NL – number of leaves.

Genotypes	Control		Severe stress	
	Periods			
	Beginning	**Final**	**Beginning**	**Final**
Grand Nain	9.00aB	12.50aA	8.67aA	10.10bA
BRS Princesa	11.00aA	11.93aA	12.67aA	18.33aA
PMGB 043	11.00aB	14.07aA	10.67aA	12.17bA
PMGB 044	16.33aB	19.67aA	14.50aA	15.50aA
PMGB 048	11.03aB	20.83aA	16.17aA	20.13aA
PMGB 050	11.67aA	13.67aA	11.57aA	13.20bA
PMGB 052	12.33aB	15.43aA	11.83aA	12.27bA
PMGB 060	10.33aB	14.83aA	12.33aA	13.07bA
PMGB 065	12.50aB	17.20aA	11.90aA	12.67bA
PMGB 069	14.83aA	16.67aA	8.33aA	10.30bA
PMGB 070	7.17aB	12.30aA	9.33aA	10.67bA
PMGB 075	12.17aB	16.27aA	11.00aA	13.17bA
PMGB 098	9.50aB	14.83aA	10.67aA	13.23bA
PMGB 099	13.67aA	16.33aA	15.00aA	16.83aA
Average	11.61	15.47	11.76	13.69
Deviation	2.27	2.53	2.22	2.88
Mínimum	7.17	11.93	8.67	10.10
Maximum	16.33	20.83	16.17	20.13
Genotype (G)	*	NS	NS	NS
Period (P)	NS	NS	NS	*
P x G	NS	NS	NS	NS

Means followed by the same lowercase letters in the columns belong to the same group by the Scott-Knott test at 5% probability and averages followed by the same uppercase letters do not differ statistically from each other by Tukey's test at 5% probability. NS = no statistical difference.

Table 3.2. Biometric data of Musa genotypes submitted to control and severe stress treatments. HP: - Height of the Pseudostem, DP-Diamter of Pseudostem, TLA - Total leaf área and NL – number of leaves

Period	Diameter		NF		AFT	
	Control	**Severe Stress**	**Control**	**Severe Stress**	**Control**	**Severe Stress**
Beginning	8.15b	8.09b	5.14b	5.19b	305.56b	331.17b
Final	10.11a	9.22a	7.45a	6.50a	438.55a	339.54a

Means followed by the same lowercase letters in the columns belong to the same group by the Scott-Knott test at 5% probability.

Appendice 4. Statistical analysis of bimetric data at the end of the experimente.

Table 4.1. Biometry of banana genotypes submitted to control and water deficit treatments in hydropic system. HP: - Height of the Pseudostem, DP-Diamter of Pseudostem, TLA -Total leaf área and NL – number of leaves, DAM – Dry aerial matter, FAM- Fresh Aerial Mass, FRM – Fresh Root Mass, NR – number of Main roots and LR, Length of Main roots.

Genotypes	HP	DP	TLA	NL	DAM	FAM	FRM	NR	LR
Grand Nain	10.08b	9.32b	287.50b	5.25e	1.34 b	13.41 b	10.91 c	6.25b	33.93b
BRS Princesa	15.20b	9.06b	308.81b	7.10c	1.24 b	16.75 a	13.56 c	5.90b	39.33a
PMGB043	15.19c	8.34b	337.50b	7.00c	1.27 a	13.40 b	17.51 c	7.10b	33.80a
PMGB044	16.14b	9.45b	432.37a	6.10d	2.04 a	19.05 a	21.01 b	9.15a	38.35a
PMGB048	17.66a	9.66a	398.98a	7.35b	1.65 a	17.59 a	23.97 a	7.55b	42.05a
PMGB050	12.92c	9.42b	400.65a	6.75c	1.68 a	16.71 a	21.48 b	9.10a	41.33a
PMGB052	13.57c	9.15b	378.30a	6.75c	1.51 b	15.93 b	24.90 a	9.50a	42.30a
PMGB060	13.94c	9.32b	336.70b	7.40b	1.46 b	14.81 b	17.03 c	6.95b	41.00a
PMGB065	13.01c	8.71b	410.75a	7.40b	1.51 b	15.37 b	19.12 b	9.35a	38.60a
PMGB069	13.47c	8.87b	378.03a	8.15a	1.41 b	15.34 b	14.44 c	7.70b	37.78a
PMGB070	11.70d	8.83b	271.71b	7.20c	1.16 b	12.48 b	13.11 c	7.25b	39.78a
PMGB075	13.72c	10.58a	393.06a	6.80c	1.88 a	18.66 a	21.28 b	10.05a	41.45a
PMGB098	13.90c	8.37b	305.16b	5.35e	1.33 b	12.79 b	13.83 c	8.70a	38.58a
PMGB099	17.43a	10.27a	456.03a	7.70b	2.00 a	20.83 a	26.01 a	10.75a	42.13a
Mínimo	11.70	8.37	271.71	5.35	1.24	12.79	13.11	5.90	33.93
Máximo	17.43	10.58	456.03	8.15	2.04	20.83	26.01	10.75	42.330

Means followed by the same lowercase letters in the columns belong to the same group by the Scott-Knott test at 5% probability and averages followed by the same uppercase letters do not differ statistically from each other by Tukey's test at 5% probability. NS = no statistical difference.

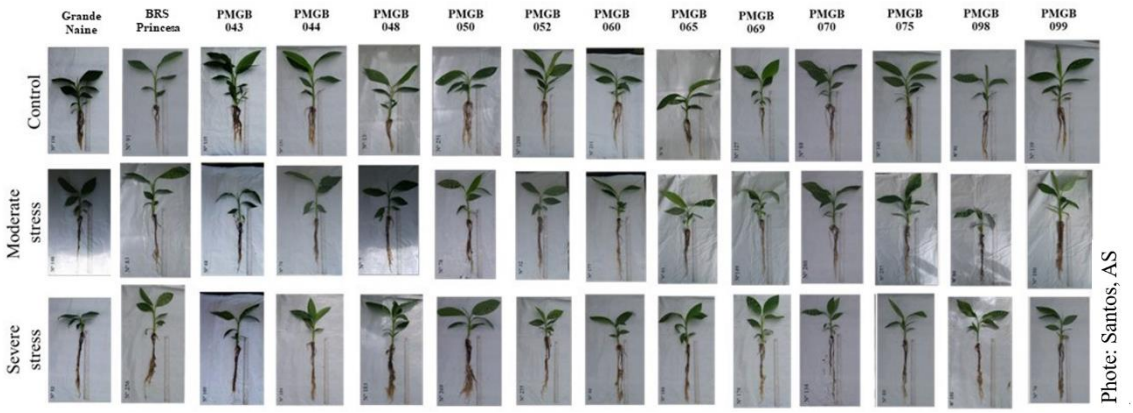
Table 4.2. Biometry of banana genotypes submitted to control and water deficit treatments in hydropic system. HP: - Height of the Pseudostem, DP-Diamter of Pseudostem, TLA -Total leaf área and NL – number of leaves, DAM – Dry aerial matter, FAM- Fresh Aerial Mass, FRM – Fresh Root Mass, NR – number of Main roots and LR, Length of Main roots.

Treatments	HP	DP	TLA	NL	DAM	FAM	FRM	NR	LR
Control	15.86a	10.37a	445.10c	7.51a	1.99 a	20.92 a	16.49 b	8.99a	36.93b
Moderate	12.38c	7.99d	298.09a	6.41cb	1.11 c	11.35 c	16.86 b	7.19b	34.94b
Severe	14.29b	9.60b	374.67b	6.97b	1.61 b	16.40 b	23.06 a	8.47a	43.38a

Averages followed by the same lowercase letter do not differ statistically by the Tukey test at 5% probability.

643 **Appendice 5.1 Photographic record of Musa genotypes.**

644
645



646
647

648 **Figure 5.1** Banana genotypes submitted to different water regimes. A 50 cm ruler was
649 used for comparison among treatments. The plant number is indicated in the lower left-
650 hand corner.

Capítulo 3

EFEITOS DO ESTRESSE HÍDRICO SOBRE O PERFIL PROTEÔMICO E BIOQUÍMICO DE *Musa sp.*[1]

Santos, A. S.[a]; Amorim, E. P. [b], Filho, M. A. C.[b], Ferreira, C. F.[b], ALMEIDA, A-A. F.[a], Pirovani, C. P.[a]

[a] Departamento de Ciências Biológicas, Universidade Estadual de Santa Cruz (UESC), 45662-900, Ilhéus, Bahia, Brasil.

[b] Embrapa Mandioca e Fruticultura, 44380-000, Cruz das Almas, Bahia, Brazil

Autor correspondente: pirovanicp@gmail.com

RESUMO

O déficit hídrico está entre os estresses abióticos que causam maior impacto sobre a agricultura. A percepção do estresse hídrico resulta na regulação do metabolismo e de redes moleculares envolvidas na defesa das plantas. O estudo proteômico de raízes de diploides de *Musa* spp. foi realizado com o objetivo de identificar proteínas diferencialmente expressas relacionadas à tolerância à deficiência hídrica e analisar a interação entre essas proteínas. Com base nas respostas fisiológicas do teor de água, resistência estomática, dados biométricos e biomassa dos híbridos apresentadas no Capítulo 2, foram selecionados os genótipos PMGB043 e PMGB099, susceptível e tolerante à deficiência hídrica, respectivamente. A partir da análise do perfil proteico em 2D-SDS-PAGE do genótipo PMGB043 foram detectados 260 e 188 *spots*, nos géis da condição controle e estresse severo, respectivamente.

[1]Guide for Authors: https://www.elsevier.com/wps/find/journaldescription.cws_home/503316?generatepdf=true

No genótipo PMGB099 foram detectados 373 *spots* nos géis do tratamento controle e 426 *spots* nos géis do tratamento sob estresse severo. Este é o primeiro trabalho de análise proteômica de raízes de bananeiras submetidas ao estresse hídrico em sistema hidropônico. Os resultados permitem uma melhor compreensão da base molecular de resposta ao estresse hídrico em *Musa* sp.

Palavras chave: estresse hídrico, *Musa* ssp., tolerância, eletroforese bidimensional, espectrometria de massas, perfil proteico.

1. INTRODUÇÃO

O déficit hídrico está entre os estresses abióticos mais limitantes à expansão de cultivos agrícolas pelo mundo. A percepção do estresse pelas plantas é o primeiro passo para a regulação da expressão de genes específicos, alterando o transcriptoma, o proteoma e o metaboloma dos organismos (FAROOQ et al. 2009). Todas essas mudanças geram redes de interação moleculares para detectar, sinalizar e mitigar os efeitos prejudiciais do estresse hídrico nas plantas (MISHRA et al. 2018; MIAO et al. 2017; OLIVEIRA et al. 2015).

Uma série de genes que codificam proteínas importantes na resposta de variedades de bananeira ao estresse hídrico foi estudada. Os genes *MaPIP1;1* (XU et al. 2014), *MusaPIP1;2* (SREEDHARAN et al. 2013) e *MaAQP* (HU et al 2015), são da família das aquaporinas, proteínas intrínsecas da membrana plasmática responsáveis pelo fluxo de água e moléculas das células, bem como, manutenção do equilíbrio osmótico. Os genes *MaSWEETs* codificam proteínas SWEETs, envolvidas no transporte de açúcares nas plantas (MIAO et al. 2017). Além de serem vitais na produção de energia, os açúcares participam na eliminação de espécies reativas de oxigênio, sinalização do estresse e balanço hídrico celular (FAROOQ et

al. 2009). Os genes *MaSODs* codificam a enzima Dismutase do Superóxido (SOD) e suas isoformas, primeira na via antioxidativa das plantas, eliminando radicais superóxido para proteger as células do dano oxidativo (FENG et al. 2015). Também foram caracterizados genes que codificam fatores de transcrição, como, *MusaNAC68* (NEGI et al. 2015) e *MusaNAC042* (TAK et al. 2017), das proteínas NAC, *MusaDHN-1* (SHEKHAWAT et al. 2011), que expressam proteínas hidrofílicas específicas conhecidas como abundante embriogênese tardia (LEA) e o gene *MaHsfs* (WEI et al. 2016) que regula a expressão de proteínas de choque térmico. Os fatores de transcrição são proteínas que regulam a expressão de genes de defesa e resistência das plantas, por isso, são alvos potenciais na manipulação gênica (SREEDHARAN et al. 2012; SHEKHAWAT et al. 2013).

A eficácia de todas essas respostas ao estresse hídrico depende do genótipo, da duração e da gravidade do estresse e de modificações pós-traducionais. Como nem todo gene transcrito é expresso, é interessante a realização de estudos para conhecer o conjunto de proteínas codificadas pelo genoma, o chamado proteoma (GRAVES; HAYSTEAD 2002).

Poucos estudos proteômicos foram realizados com bananeiras para inferir sobre os mecanismos de regulação mediante condições de estresse hídrico. A análise proteômica de meristemas de brotos múltiplos de bananeira revelou que as isoformas específicas de fosfoglicerato-quinase, UDP-glicose pirofosforilase (UGPase), fosfoglucomutase, ASR (Aba, Stress and Ripening) podem contribuir para a tolerância à desidratação em ensaios *in vitro* (CARPENTIER et al. 2007). O proteoma de folhas de bananeiras contrastantes quanto ao déficit hídrico mostra que ocorreu um novo equilíbrio nas plantas estressadas cultivadas *in vitro*. A expressão de proteínas relacionadas a respiração, metabolismo de ROS e desidrogenases envolvidas na homeostase de NAD/NADH são fundamentais para a melhora da tolerância das variedades com o genoma B (VANHOVE et al. 2012). Outro estudo, realizado em casa de vegetação, analisou o proteoma do rizoma de plantas contrastantes

quando ao déficit hídrico. A expressão de proteínas relacionadas ao crescimento, desenvolvimento de células vegetais, choque térmico tiveram um papel significativo na tolerância da variedade BRS tropical - AAAB (MATTOS-MOREIRA et al. 2018).

Diante disso, realizamos o primeiro estudo proteômico de raízes de bananeiras submetidas ao déficit hídrico em sistema hidropônico em casa de vegetação. O objetivo desse trabalho foi identificar proteínas diferencialmente expressas relacionadas à tolerância à deficiência hídrica em diploides melhorados (resistência genética à 'Sigatoka-amarela' e murcha de *Fusarium,* raça 1 e alguns à 'Sigatoka-negra') de *Musa* spp. e analisar a interação entre essas proteínas.

2. MATERIAL E MÉTODOS

2.1 Material Vegetal

Os diploides PMGB043 e PMGB099 foram obtidos do Banco Ativo de Germoplasma (BAG) da Embrapa Mandioca e Frutas Tropicais (Cruz das Almas, Bahia, Brasil). As plantas foram selecionadas de acordo com respostas contrastantes ao déficit hídrico em sistema hidropônico adaptado, conforme Capítulo 2. Os genótipos PMGB043, considerado mais susceptível, e PMGB099, considerado mais tolerante, foram utilizados para as análises proteômicas. As plantas foram divididas em dois grupos: (i) controle, no qual o suprimento de solução nutritiva foi mantido próximo à capacidade de campo (0.3 cm^3 cm^{-3}) e (ii) estresse hídrico severo, no qual a umidade do substrato foi mantida entre 0.5 e 0.05 cm^3 cm^-3 e as plantas com resistência estomática superior a do seu respectivo controle. O período máximo de déficit hídrico foi 11 dias. Cada planta em estresse severo foi coletada, após atingir o murchamento no período da manhã por dois dias consecutivos, junto com seu respectivo

controle. Após a coleta, as plantas foram congeladas em nitrogênio líquido e posteriormente armazenados a -80 °C. Antes das análises, as plantas foram liofilizados e armazenados a -20 °C para preservar as características experimentais e minimizar a oxidação durante manipulação das amostras.

2.2 Extração de proteínas

Um pool das raízes de cinco plantas nas condições controle e déficit hídrico severo foi encaminhado ao Laboratório de Proteômica da Universidade Estadual de Santa Cruz (UESC). Uma extração com protocolo original de Pirovani et al. (2008) modificado por Bertolde et al. (2014) foi realizada para testar a eficácia do método em raízes de bananeira (Suplementar A, Figura A.1). Da mesma forma, foi realizada outra extração com o protocolo de Bertolde et al. (2014) com modificações que incluíram mais lavagens às etapas de extração em TCA e acetona.

Após a limpeza inicial do macerado com as lavagens com TCA em acetona e TCA em água, i) foi usado sonicação (3 pulsos de 5 s, com intervalos de 10s e 70% de amplitude) para ressuspender o precipitado em SDS-denso (sacarose 30%, SDS 2%, Tris 0,1 mol.L^{-1} pH 8,0 e 2-mercaptoetanol a 5%); ii) foi adicionado igual volume de fenol (tamponado com Tris, pH 8,0) à amostra ressuspensa em SDS-denso e, iii) a mistura foi homogeneizada em vórtex. Na etapa final de lavagem do precipitado proteico com acetato de amônio em metanol, foi utilizado acetona a 80% em substituição ao etanol a 80 %.

2.3 Eletroforese 1D e 2D

Para eletroforese 1D, 25 microgramas de proteínas foram aplicadas diretamente em gel de poliacrilamida contendo SDS (SDS-PAGE) 12,5%, em minicubas HOEFER SE260 Mighty Small (LAEMMLI 1970). Os géis produzidos mediram 8 x 10 cm.

Para a produção dos géis 2D, as amostras proteicas foram aplicadas em *strips* de 13 cm, com gradiente de pH imobilizado (IPG-immobilized pH gradient) entre 3-10 NL e depois submetidas na unidade de Focalização Isoelétrica EthanIPGphor III. A segunda dimensão foi realizada em gel de poliacrilamida 12.5% no sistema de eletroforese vertical HOEFER SE600 Ruby (Amersham Bioscience). Os *spots* proteicos foram visualizados por impregnação com corante Azul de Coomassie Brilhante 0,08% (NEUHOFF et al. 1988). Para isso, os géis ficaram por 1 hora em tampão de fixação (etanol 40% e ácido acético 10%) e 5 dias em corante azul de Coomassie coloidal (sulfato de amônio 8%, ácido fosfórico 0,08%, azul de coomassie G-250 0.08% e metanol 20%). Após essa etapa, os géis foram mantidos em água destilada sob suave agitação até a remoção do corante. Para cada pool de amostra dos diferentes tratamentos e genótipos foram preparados géis em triplicata.

2.4 Obtenção e análise de imagens dos géis 2-D

As imagens dos géis foram digitalizadas com o LabScanner (Amersham Bioscience) e, em seguida, essas imagens foram analisadas para a identificação e quantificação relativa dos *spot*, usando o ImageMaster 2D Platinum 7.0 (GE Healhcare), considerando área e intensidade dos *spots*. Dados que foram transformados em um valor de volume pelo programa, para cada *spot*. Além disso, para cada tratamento foi estabelecido um gel de referência para a triplicata e o programa detectou os *spots* exclusivos de cada tratamento, bem como o acúmulo relativo das proteínas presentes nos *spots* para cada tratamento.

2.5 Preparação dos *spots* para espectrometria de massas

Os *spots* considerados relevantes, a partir da análise com o Image Master 2D Platinum 7.0, foram excisados do gel com uso de bisturi, cortados em pedaços menores e colocados em microtubos, em seguida foram descorados em 200 µL NH_4HCO_3 contendo acetonitrila 50% e o sobrenadante foi descartado. Os fragmentos de gel foram desidratados em 100 µL de acetonitrila 100% por 5 min e secos a vácuo no Concentrator 5301 (Eppendorf) por 10 min. Foram adicionados 4 µL de tripsina Gold (Promega) 25 ng μL^{-1}, mantidos a 4 º C por 10 min para absorção da solução nos fragmentos de gel. Posteriormente foi adicionado NH_4HCO_3 até cobrir os fragmentos e deixados a 37ºC por 16 horas para ação da tripsina. O sobrenadante foi coletado e transferido para um novo tubo. Os peptídeos foram recuperados dos fragmentos de gel por duas eluições com 50 µL de acetonitrila à 50% contendo ácido fórmico 0.1%, sendo agitados por 15 min no vórtex em cada lavagem. Em seguida, as amostras foram concentradas a vácuo até atingirem um volume entre 10 e 15 µL.

Uma parte dos spots foi analisado no Centro de Biotecnologia e Genética da Universidade Estadual de Santa Cruz (UESC) em Ilhéus, Bahia e a outra parte no Centro Nacional de Pesquisa em Energia e Materiais em Campinas, São Paulo (CNPEM)

2.6 Análise por espectrometria de massas (CBG/UESC)

Os peptídeos resultantes da digestão tríptica foram resolvidos por cromatografia de fase-reversa no nanoAcquity UPLC (WATERS) em duas colunas C18, sendo a primeira uma coluna *trapping* de 5 µm, 180 µm x 20 mm e a segunda de 1,7 µm 100 µm x 100 mm, sob um fluxo de 0,6 $\mu L.min^{-1}$ em uma corrida de 50 min, onde foram coletados 4 µL de cada amostra. Os peptídeos foram separados de acordo com um gradiente de acetonitrila, sendo 1% até 1

min, de 1% a 50% em 40 min, de 50% a 85% em 5 min, mantendo-se nessa concentração por mais 2 min, voltando à concentração de 1 % em 1 min e permanecendo nessa condição por 2 min, totalizando 50 min de corrida. Os peptídeos separados foram ionizados em um capilar sob voltagem de 3000 V (Micromass Q-TOFmicro), fragmentados no modo positivo com seleção da intensidade relativa mínima de 10 counts. Os três íons mais intensos foram analisados por cada varredura de 1 s, com energia de colisão variando entre 20 e 95 V de acordo com a relação massa/carga (m/z) dos peptídeos. Os espectros dos peptídeos trípticos foram detectados e analisados pelo programa ProteinLynx Global Server 4.2 (WATERS), sendo realizado uma comparação contra o banco de dados SWISS-PROT.

2.7 Análise por Espectrometria de Massas (CNPEM):

Os peptídeos foram separados por gradiente de hidrofobicidade em coluna C18 (100 µm x 100 mm) (Waters) por um cromatógrafo tipo nano Acquity Ultra Performance LC (Waters) acoplado a uma interface de ESI nanospray a um espectrômetro de massa do tipo Q-Tof Premier (Waters). Os peptídeos foram injetados em um volume de 4,5µL passando primeiramente por uma coluna de trapping Symmetry C18 (180 µm x 20mm) para dessalinização a um fluxo de 5ul/min durante 2 minutos. Em seguida, os peptídeos foram carregados para a coluna analítica e eluídos em um gradiente de 2-90% de acetonitrila contendo 0,1% de ácido fórmico durante 10 minutos sob fluxo de 0,6 µL/min. A voltagem para o nanoeletrospray foi de 3.5 kV, para o cone de 30 V e a temperatura da fonte 80ºC. O instrumento foi operado em modo DDA (data depend analysis) de forma a adquirir e fragmentar (MS/MS) os três picos mais intensos de cada espectro de MS (top three mode). Após a fragmentação por MS/MS, o íon foi colocado em uma lista de exclusão por 20 segundos. Os espectros foram adquiridos pelo software MassLynx v.4.1 e os arquivos brutos

198 “raw” foram convertidos a uma lista de picos no formato mgf (mascot generic file) pelo
199 programa Mascot Distiller v.2.3.2.0, 2009 (Matrix Science Ldt.). Para a identificação das
200 proteínas foi utilizado o programa Mascot Server v.2.3.01.0 (Matrix Science Ltda.), tendo
201 como parâmetros uma clivagem perdida pela tripsina, modificação fixa de
202 carbamidometilação, modificação variável de oxidação da metionina e 0,1 Da de tolerância de
203 massas para MS e 0,1 Da de tolerância de massas para MSMS. As buscas foram realizadas
204 utilizando o conjunto de dados de *Musa acuminata* disponível na base de conhecimento
205 UniProt (www.uniprot.org).

206 **2.8 Identificação das proteínas**

207

208 Após as proteínas terem sido identificadas, suas ontologias e funções biológica foram
209 verificadas no Uniprot (www.uniprot.org) e BLAST2Go (www.blast2go.com). As proteínas
210 foram categorizadas por processo biológico, função molecular e componente celular. Para fins
211 de discussão, as proteínas relacionadas a processos biológicos foram classificadas em resposta
212 de defesa, energia, metabolismo, metilação, oxirredução, transdução de sinal, translação,
213 transporte e hormônio.

214

215 **2.9 Atividade de guaiacol peroxidase (POX, EC 1.11.1.7)**

216

217 A atividade enzimática foi realizada em pool de cinco réplicas biológicas de cada
218 tratamento. O tecido vegetal liofilizado (raízes ou folhas de bananeira) foi submetido à
219 maceração em nitrogênio líquido e 0.07 g de polivinilpolipirrolidona (PVPP) por grama de
220 tecido para evitar a oxidação do material. A massa de 20 mg do tecido macerado foi
221 ressuspendida em 800 µL de tampão fosfato de potássio e misturado brevemente em vórtex e,
222 em seguida, submetido à sonicação em ultrassonicador de sonda (Gex 130, 130 W) sob

amplitude de 70 %, 8 pulsos de 5 segundos com intervalos de 10 segundos. Após essa etapa o macerado foi submetido à centrifugação por 10 min, a 14000 rpm à 4 °C. O sobrenadante (Extrato bruto) foi coletado para um novo tubo.

A atividade da Peroxidase Guaiacol (POX) foi realizada de acordo com a metodologia descrita por Rehem *et al.* (2011), com algumas modificações. O tampão de atividade utilizado foi composto por fosfato de sódio a 20 mmol L^{-1}, pH 6.0, 40 mmol L^{-1} de guaiacol e peróxido de hidrogênio a 0.06%. Nesta solução foi adicionado 1 µL do extrato. As reações foram realizadas a 25 °C. A atividade da peroxidase foi expressa de acordo com o aumento do consumo de guaiacol em µmol s^{-1} g^{-1} de biomassa liofilizada, a partir de uma curva padrão elaborada por Rehem *et al.* (2011). A leituras de absorbância 470 ηm foram monitoradas no espectrofotômetro de microplacas Spectramax Paradigm (Molecular Devices), com o software SoftMax Pro 6.3.

2.10 Western blotting

Aproximadamente 0.6 g de raízes foram maceradas em almofarizes com nitrogênio líquido na presença de polivinilpolipirrolidona (PVPP) a 0.07 g por g de tecido e as proteínas extraídas conforme o método de extração fenólica (BERTOLDE et al., 2014; PIROVANI et al., 2008). Após a quantificação do extrato proteico com o 2D Quant Kit (GeHealthCare), 20 µg de cada amostra foi resolvida em 1D-SDS-PAGE. A análise do acúmulo da proteína seguiu o método de Sambrook e Russell (1989). As membranas foram sondadas com os anticorpos (Agrisera AB) contra Catalase (57/55 kDa) e ADH (42 kDa). O anticorpo secundário foi Rabbit Anti-IgG Alkaline phosphatase conjuged (AP, ZIMED Laboratories Inc. São Francisco - CA/USA), diluído na concentração de 1:10.000, mantido durante 1 hora sob agitação. A quantificação das bandas, a partir de ensaios em triplicada, foi realizada

utilizando o software GelQuantNET V 1.7.8 e os resultados foram normalizados com base em gel corado com azul de comassie coloidal G 250 0.08 % (NEUHOFF et al., 1988).

3. RESULTADOS

3.1 Perfil proteico das raízes de *Musa acuminata* em 1D e 2D PAGE

O perfil proteico em SDS-PAGE 12,5% mostra bandas com massas moleculares distribuídas nas faixas entre 14 e ~150 kDa (Figura 1). As bandas apresentadas no Gel 1 indicam que as proteínas não foram bem purificadas e separadas de compostos interferentes presentes no tecido. No Gel 2, as bandas estão melhor distribuídas, delimitadas e com menos arrastes.

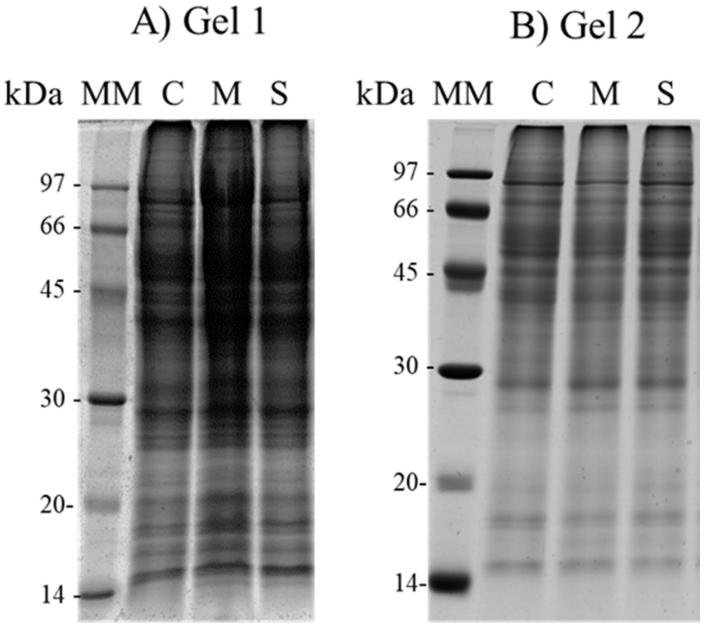


Figura 1: Perfil proteico de raízes de *M. acuminata* em 1D-PAGE. Em A, Extração realizadas com os protocolos de Bertolde et al. 2014 e Pirovani et al. 2008. Em B, Extração realizadas com os protocolos de Bertolde et al. 2014 e Pirovani et al. 2008 com modificações. MM, corresponde a Marcador de Massa Molecular, C, corresponde a controle, M, corresponde a estresse moderado e S, corresponde a estresse severo.

O perfil de *spots* de proteína dos genótipos diploides PMGB043 e PMGB099 em condições controle e estresse severo foi visualizado na revelação dos géis 2-DE (Fig. 2). A análise do perfil de *spots* proteicos do genótipo PMGB043 detectou 260 e 188 *spots* nos géis da condição controle e estresse severo, respectivamente. Para o genótipo PMGB099 foram detectados 373 e 426 *spots*, nos tratamentos controle e estresse severo, respectivamente. Os *spots* estão bem focalizados, com a ausência de arrastes horizontal ou vertical, mesmo para as proteínas mais abundantes.

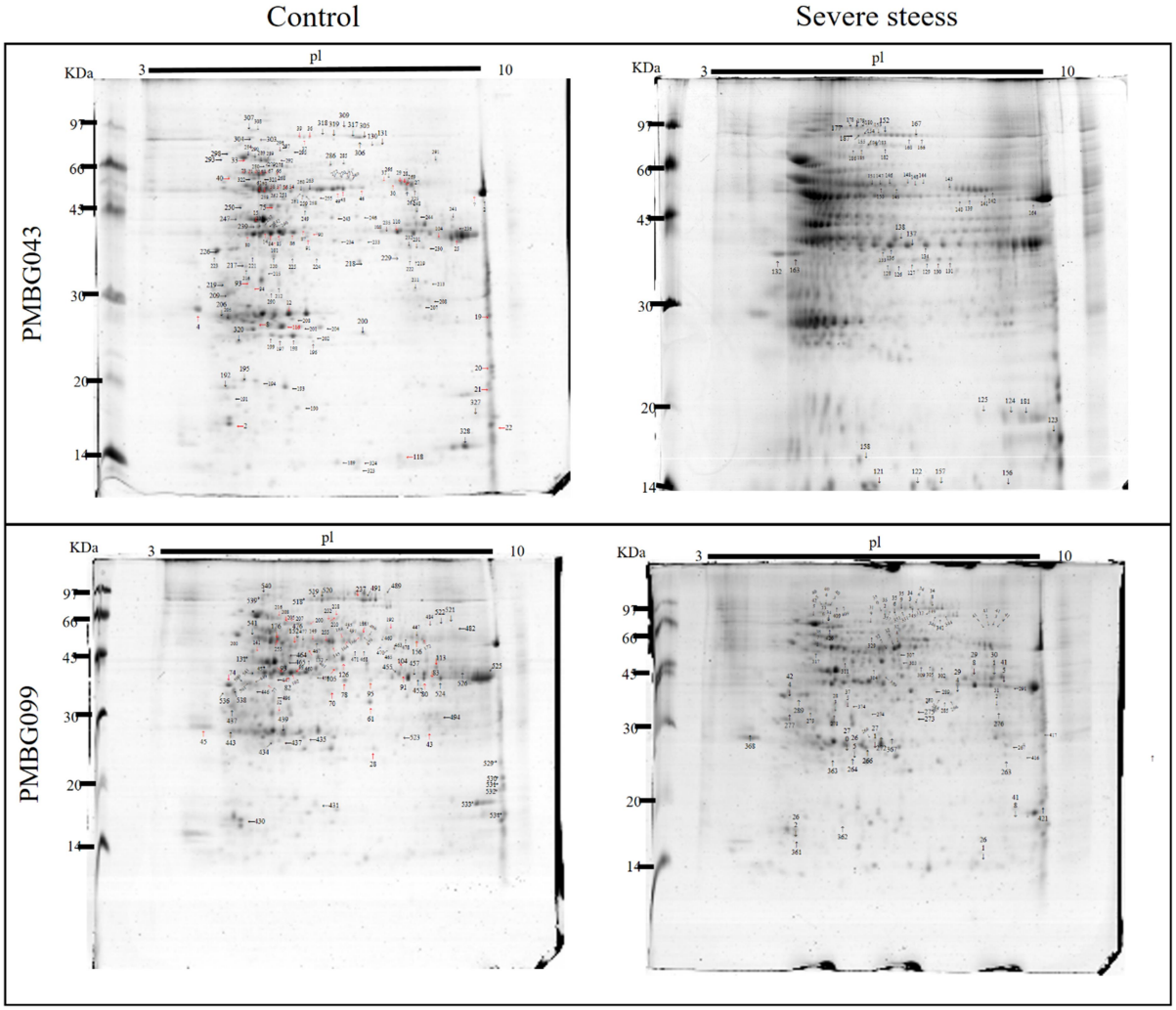


Fig. 2: Perfil proteico de raízes de *Musa acuminata* em 2D-PAGE. Amostras proteicas dos genótipos PMGB043 e PMGB099, suscetível e tolerante ao déficit hídrico, respectivamente, foram focalizadas em tiras de 13 cm com gradiente de pH 3-10 não linear (NL). Os géis foram corados com azul de *coomassie* coloidal (NEUHOFF et al. 1988). Cor das setas: vermelha, corresponde a spots diferencialmente expressos e preta, corresponde a spots exclusivamente detectados.

A comparação entre os géis do controle e tratamento do genótipo PMGB043 revelou 120 *spots* em comum, dos quais 57 *spots* foram significativamente regulados (ANOVA $p < 0.05$ e Fold ≥ 1.5). Um total de 140 *spots* foi exclusivo da condição controle e 67 *spots* foram

exclusivos do tratamento estresse severo (Fig. 3). Na comparação dos géis do genótipo PMGB099, o número de *spots* comuns entre os tratamentos foi 258. Desses, 52 *spots* proteicos apresentaram diferença significativa entre os tratamentos (ANOVA $p < 0.05$ e Fold ≥ 1.5). No gel da condição controle e estresse severo foram detectados um total de 115 e 168 *spots* exclusivos, respectivamente.

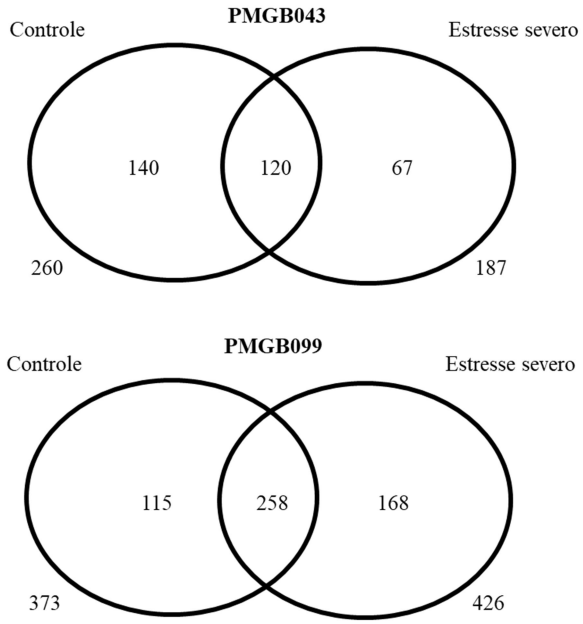


Fig. 3: Diagrama de Venn, mostrando a distribuição dos *spots* detectados nos géis das raízes de *Musa acuminata,* genótipos PMGB043 (suscetível) e PMGB099 (tolerante), em condição controle e estresse hídrico severo.

A avaliação da distribuição dos spots de *Musa acuminata* em 2D-PAGE quanto a Massa Molecular e Ponto Isoelétrico está disponível em arquivo Suplementar B1 (Fig. B.1 e B.2).

306 **3.2 Proteínas identificadas**

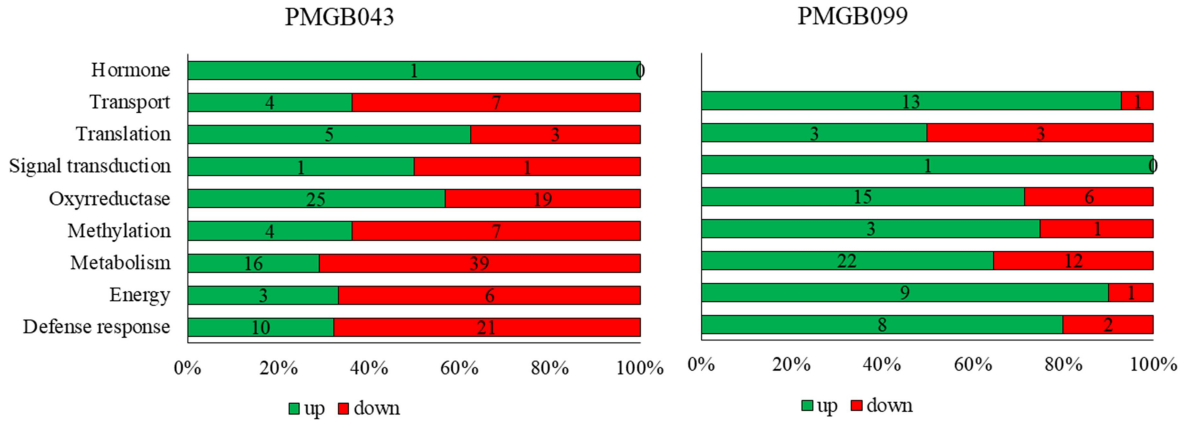
307

308 As tabelas com proteínas identificadas por genótipo (docx) e o arquivo completo
309 (xlsx) contendo os peptídeos e sequências fasta de cada proteína estão disponíveis em
310 <http://doi.org/10.5281/zenodo.2536639>. As proteínas exclusivamente detectadas e
311 diferencialmente expressas foram classificadas em categorias pelos seus processos biológicos,
312 conforme determinado pelo software Blast2GO (Tabela disponível em:
313 <http://doi.org/10.5281/zenodo.2546000>). As categorias formuladas a partir da análise do
314 software foram resposta de defesa, energia, metabolismo, metilação, transdução de sinal,
315 translação (tradução), transporte e hormônios.

316 O genótipo PMGB043 apresentou maior número de proteínas com o acúmulo reduzido
317 mediante o estresse hídrico, enquanto o genótipo PMGB099 apresentou uma maior número de
318 proteínas com aumento do acúmulo (Fig. 6). Apenas no genótipo PMGB043 houve expressão
319 de enzimas que regulam os níveis do ácido indol-3-acético (AIA) durante o estresse. Neste
320 genótipo, as proteínas relacionadas a processos de transdução e oxirredução foram up-
321 regulados em 57% e 63%, respectivamente. Proteínas relacionadas a todos os processos
322 biológicos listados para o genótipo PMGB099 foram positivamente acumuladas em
323 quantidades iguais ou superiores a 50%.

324

325



326

327 **Fig. 6: Classificação de processos biológicos para proteínas diferencialmente expressas**
328 **em respostas ao déficit hídrico identificadas nos genótipo PMGB043 e PMGB099 de**
329 ***Musa acuminata***. Comparação entre tratamentos controle e estresse severo.

330

331 A seguir uma relação de proteínas up e donw reguladas do genótipo PMGB043
332 discutidas nesse artigo (Tabela 1).

333

334 Tabela 1: Relação de proteínas identificadas através de espectrometria de massas do genótipo
335 PMGB043 de *Musa acuminata*.

ID Spot	Identified protein reference organism	Up	Down
Defense response			
27, 28, 29, 141, 142, 143, 266, 269	Catalase 1	6	2
104, 236, 244, 248	Cationic peroxidase SPC4-like	0	4
30, 199, 204	Glutathione S-transferase	1	2
33, 287, 291, 298, 303, 304	Heat shock 70 kDa protein, mitochondrial-like	1	5
93, 99, 233, 234, 312	Malate dehydrogenase	1	4
194, 195	Pathogenesis-related 10	0	2
25, 222	Peroxidase	0	2
196	Superoxide dismutase [Mn], mitochondrial	0	1
Energy			
15, 60, 242, 245	Actin 2	0	4
307, 308	Cell division cycle protein 48 homolog	0	2
71	Chaperonin CPN60-2, mitochondrial	1	0
4, 138	Fructose bisphosphate aldolase	2	0
Hormone			
78	IAA-amino acid hydrolase ILR1-like 1	1	0
Metabolism			
275, 277, 278, 279, 280, 282	2,3-bisphosphoglycerate-independent phosphoglycerate mutase	0	6
250	26S protease regulatory subunit 6A homolog	0	1

80	Adenosine kinase 2	1	0
263	Adenosylhomocysteinase-like	0	1
241	Aspartate aminotransferase, cytoplasmic	0	1
220, 224, 225	Cysteine synthase	0	3
246	Cytosolic isocitrate dehydrogenase [NADP]-like	0	1
251, 252, 253	Dihydrolipoyllysine-residue acetyltransferase component 2 of pyruvate dehydrogenase complex, mitochondrial-like	0	3
61, 132, 226, 227, 228	Fructokinase 1	1	4
213	Germin-like protein 5-1	1	1
85, 86, 102	Glutamine synthetase cytosolic isozyme 1 2	1	2
223	Horcolin-like	0	1
285	Phosphoglucomutase, cytoplasmic 2-like	1	2
87, 237	Phosphoglycerate kinase, cytosolic		↑
257	Probable gamma-aminobutyrate transaminase 3, mitochondrial	0	1
206, 208	Proteasome subunit alpha type-5-like	0	3
189, 324	Protein GOS9-like	0	2
79, 249	S-adenosylmethionine synthase 1	1	1
36, 37, 39, 152, 153, 166, 167, 168, 170, 171, 172, 305, 306, 317, 319	Sucrose synthase	10	5
261	UTP--glucose-1-phosphate uridylyltransferase	0	1
Methylation			
182, 183, 184, 185, 186, 300, 301	5-methyltetrahydropteroyltriglutamate- -homocysteine methyltransferase 2	5	2
140, 262, 325	Serine hydroxymethyltransferase 4	1	2
101	Tricetin 3',4',5'-O-trimethyltransferase-like	1	0
Oxyrreductase			
272	polyphenol oxidase, chloroplastic-like	0	1
254	6-phosphogluconate dehydrogenase, decarboxylating 1	0	2
17, 126, 243	Alcohol dehydrogenase	2	1
108, 110, 133, 134, 135, 136, 590	Glyceraldehyde 3 phosphate dehydrogenase	4	3
66, 67, 68	Ketol-acid reductoisomerase, chloroplastic-like	3	0
215, 216, 217	Lactoylglutathione lyase-like	0	3
291	L-gulonolactone oxidase 2-like isoform X1	0	1
155	Linoleate 9S-lipoxygenase 6-like	1	0
200	NAD(P)H dehydrogenase (quinone) FQR1-like	0	1
286	NADP-dependent malic enzyme	0	1
283	Polyketide cyclase dehydrase and lipid transport superfamily protein putative	0	1
144, 145, 146, 148, 149, 150, 151, 158, 270	Polyphenol oxidase, chloroplastic-like	8	1
154, 178, 187	Probable linoleate 9S-lipoxygenase 4	3	0
31	Pyruvate kinase, cytosolic isozyme	1	0
14, 46, 73	Ribulose bisphosphate carboxylase large chain Fragment	2	1
259	Succinate-semialdehyde dehydrogenase, mitochondrial	0	1
203	Triosephosphate isomerase, cytosolic	0	1
207	Tropinone reductase homolog At5g06060-like	0	1
Signal transduction			
268	Guanosine nucleotide diphosphate dissociation inhibitor 2-like	0	1
12	Histidine kinase cytokinin receptor putative	0	1
Translation			
48	40S ribosomal protein S5 Fragment	0	1
21	60S ribosomal protein L12 3	1	0
164, 177, 179, 214	Elongation factor 1 alpha	3	1
75	Eukaryotic initiation factor 4A-15	1	0

230	Eukaryotic translation initiation factor 3 subunit I	0	1
Transport			
20, 22, 49, 58, 176, 197, 255	ATP synthase subunit alpha mitochondrial Fragment	3	3
211, 288, 289, 290	V-type proton ATPase catalytic subunit A	0	4

336

337 A seguir uma relação de proteínas up e down reguladas do genótipo PMGB099
338 discutidas nesse artigo (Tabela 2).

339

340 Tabela 2: Relação de proteínas identificadas através de espectrometria de massas do genótipo
341 PMGB099 de *Musa* sp

ID Spot	Identified protein reference organism	Up	Doun
Defense response			
434	20 kDa chaperonin, chloroplastic-like	1	0
172, 413	Catalase 2	2	∞
340, 526	Cationic peroxidase SPC4-like	1	1
528	Glutathione S-transferase 3-like	0	1
70	Heat shock protein 60 isoform 5	1	0
78	Malate dehydrogenase	1	0
431	Pathogenesis-related 10	1	0
265	Pathogen-related protein-like	1	0
Energy			
264	ATP synthase subunit O, mitochondrial	1	0
315	26S protease regulatory subunit 6B homolog	1	0
104	Actin-7	1	0
403, 404, 405	Cell division cycle protein 48 homolog	3	1
414	Dynamin-related protein 5A-like	1	0
454	Fructose-bisphosphate aldolase 1, cytoplasmic-like	1	0
523	GTP-binding nuclear protein Ran3A [Musa AB Group]	0	1
289	Obg-like ATPase 1	1	0
Metabolism			
330	2,3-bisphosphoglycerate-independent phosphoglycerate mutase	0	1
307	S-adenosylmethionine synthase 3-like	1	0
205, 207, 218, 330	2,3-bisphosphoglycerate-independent phosphoglycerate mutase	2	2
301	3-ketoacyl-CoA thiolase 2, peroxisomal-like	1	0
476	Alanine aminotransferase 2	0	1
277	Aspartic proteinase oryzasin-1-like	1	0
374, 445, 496	Cysteine synthase	2	1

461	Cytosolic isocitrate dehydrogenase [NADP]	1	0
500, 501, 520, 288, 423, 443	Fructokinase-1-like	3	3
527	Germin-like protein 12-2	0	1
418	Gucleoside diphosphate kinase 1	1	0
168	Probable gamma-aminobutyrate transaminase 3, mitochondrial	1	0
446, 271, 363, 416	Proteasome subunit alpha type-1-like	4	0
259, 427	Protein GOS9-like	2	0
237, 353, 491, 519	Sucrose synthase	2	2
294	UDP-arabinopyranose mutase 1-like	1	0
152	UTP--glucose-1-phosphate uridylyltransferase	0	1
Methylation			
345	5-Methyltetrahydropteroyltriglutamate--homocysteine methyltransferase 1	1	0
472, 156	Serine hydroxymethyltransferase 4	0	2
368	Serine threonine protein kinase NAK	1	0
Oxyrreductase			
211	NADP-dependent malic enzyme	1	0
52	1-aminocyclopropane-1-carboxylate oxidase 1-like	0	1
300, 456	Alcohol dehydrogenase 1	2	0
320, 479	Aldehyde dehydrogenase family 2 member B7, mitochondrial-like isoform X4	1	1
375	Enoyl-[acyl-carrier-protein] reductase [NADH] 1, chloroplastic-like	1	0
105, 299	Glutamate dehydrogenase	2	0
524, 298, 452, 83	Glyceraldehyde 3 phosphate dehydrogenase	3	1
537, 279	Lactoylglutathione lyase-like	1	1
28	NAD(P)H dehydrogenase (quinone) FQR1-like	0	1
185	Polyphenol oxidase, chloroplastic-like	1	0
451	Pyruvate dehydrogenase E1 component subunit beta-1, mitochondrial	1	0
457	Succinate--CoA ligase [ADP-forming] Subunit beta, mitochondrial	1	0
43	Tropinone reductase homolog At5g06060-like	1	0
481	UDP-glucose 6-dehydrogenase 4-like	0	1
Signal transduction			
272	Remorin-like	1	0
Translation			
262, 538	Elongation factor 1-beta 2-like	0	2
311	Eukaryotic initiation factor 4A-15	2	0
531	Ribosomal protein 5A	0	1
534	Ribosomal protein S11 family protein	0	1
Transport			
132, 210, 346, 349, 351, 412	Adenine/guanine permease AZG2-like	6	0
323	Adenosylhomocysteinase-like	1	0
367, 415	ATP synthase subunit beta, mitochondrial	2	0

362	Phosphatidylglycerol/phosphatidylinositol transfer protein-like	1	0
331, 417	Transport protein SEC31	2	0
426	V-type proton ATPase catalytic subunit A-like	1	0
541	V-type proton ATPase subunit B 2-like isoform X1	0	1

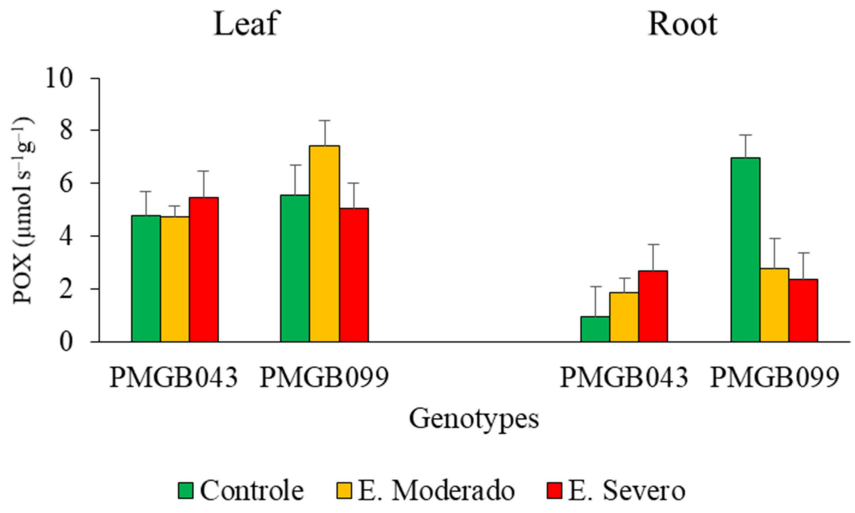
342

343 **3.4 Atividade de guaiacol peroxidase (POX, EC 1.11.1.7)**

344

345 A atividade de POX em folhas de bananeiras do genótipo PMGB043 foi superior à das
346 raízes, em todos os tratamentos (Fig. 7). Não houve diferença significativa entre os genótipos
347 e tratamento com relação a atividade de POX em folhas e raízes. Nas raízes das plantas do
348 genótipo PMGB043 ocorreu o aumento da atividade de POX, à medida que o estresse por
349 seca se intensificou. No genótipo PMGB099 nota-se um aumento da atividade de POX nas
350 folhas em estresse moderado e redução em estresse severo em relação ao controle. Nas raízes
351 das plantas em estresse severo houve redução da atividade de POX nos tratamentos de déficit
352 hídrico em relação ao controle.

353



354

355 **Fig. 7.** Atividade enzimática da peroxidase do guaiacol (POX) de genótipos de bananeira
356 submetidos a tratamentos de controle e déficit hídrico (moderado e severo). Os números
357 correspondem à média da atividade enzimática em µmol $s^{-1}g^{-1}$.

3.5 Western blotting

A imunodetecção de enzimas das raízes de bananeira está apresentada nas figures 10 e 11. Os gráficos foram normalizados com base em géis corados com azul de *comassie* (Suplementar C, Figura C.1). O acúmulo de catalase (CAT) no genótipo PMGB043 foi semelhante nos tratamentos controle e estresse severo e no genótipo PMGB099 houve redução do acúmulo nos tratamentos de déficit hídrico em relação ao controle (Fig. 8a, b).

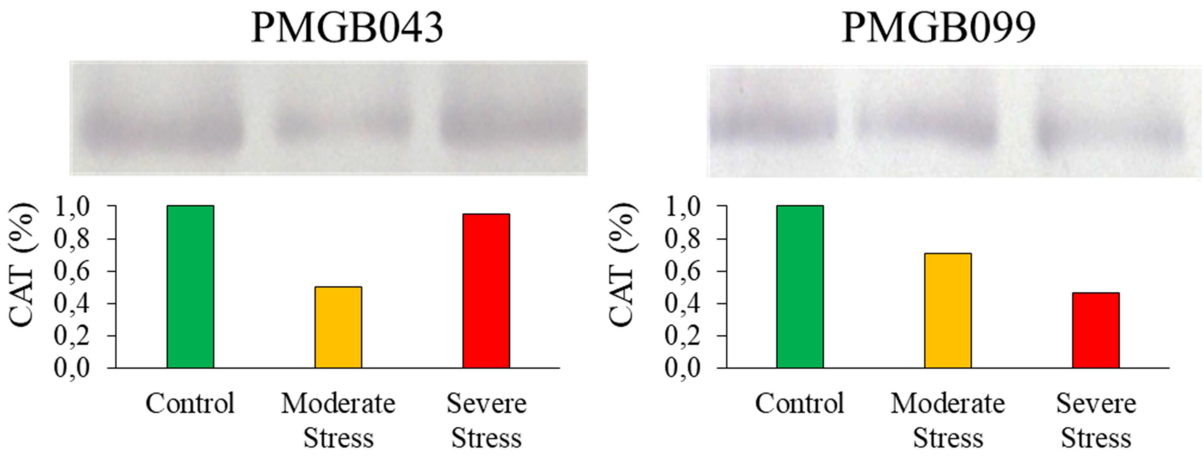


Fig. 8: Acúmulo de catalase (CAT) em raízes de genótipos de bananeira submetidos a diferentes tratamentos. a - membrana de Western blot; b - acúmulo de CAT. Legenda da cor: verde - controle; amarelo - déficit moderado; vermelho - déficit severo. 20 µg de proteína foram carregados nos géis para todos os tratamentos.

As enzimas álcool desidrogenase - ADH (42 kDa) e Catalase (57/55 kDa) foram quantificadas. O immunoblotting de Álcool Desidrogenase (ADH, EC 1.1.1.1) mostra acúmulo superior dessa enzima nos genótipos na condição controle em relação aos tratamentos de déficit hídrico nos dois genótipos (Fig. 9).

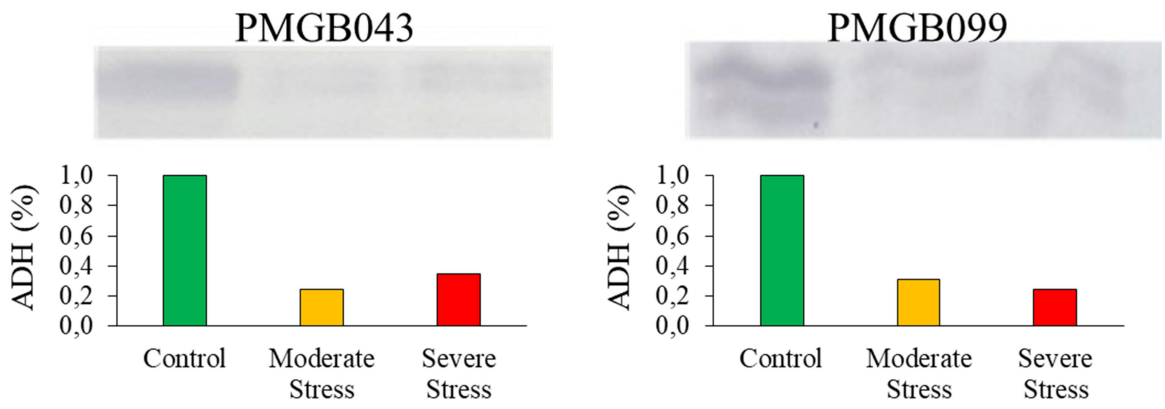


Fig. 9. Acúmulo de álcool desidrogenase (ADH) em raízes de genótipos de bananeira submetidos a diferentes tratamentos. a - membrana de Western blot; b - acumulo de ADH. Legenda da cor: verde - controle; amarelo - déficit moderado; vermelho - déficit severo. 20 µg de proteína foram carregados nos géis para todos os tratamentos.

4. DISCUSSÃO

4.1 Lavagens adicionais com acetona melhora o extrato proteico raízes de *Musa* sp.

Os testes de extração com os protocolos de Bertolde et al. 2014 e Pirovani et al. 2008 revelaram a necessidade de aumentar o número e lavagens durante o processo de extração de proteínas das raízes de bananeiras cultivadas em sistema hidropônico adaptado. Apesar de existirem protocolos de extração proteica de raízes de bananeira, a comparação entre diferentes métodos resulta em géis de baixa qualidade, repleto de estrias verticais e sem comprovação (marcação) da quantidade de *spots* detectada (VAGANAN et al. 2015). Além disso, as espécies de *Musa* sp podem variar em quantidades e tipos de compostos secundários,

polissacarídeos e outros polímeros vegetais, que interferem na qualidade dos géis utilizados para análise proteômica (SURABHI et al. 2016).

Os mapas 2D dos genótipos PMGB043 (menos tolerante à deficiência hídrica) e PMGB099 (mais tolerante à deficiência hídrica) mostraram uma mudança significativa entre genótipos e tratamentos controle e estressado. Um perfil proteômico semelhante foi observado nas variedades comerciais Prata Anã (menos tolerante à deficiência hídrica) e BRS Tropical (tolerante à deficiência hídrica), com maior detecção de *spots* proteicos nos géis do rizoma do genótipo tolerante (MATTOS-MOREIRA et al. 2018).

4.2 Proteínas reguladas positivamente em resposta ao estresse hídrico

As enzimas piruvato descarboxilase (*spots* 451) e piruvato kinase (*spot* 31, PMGB043) foram reguladas positivamente em raízes dos genótipos PMGB043 e PMGB099. O estudo do tecido meristemático da variedade Cachaco (*Musa paradisiaca* ABB), tolerante a estreses ambientais, evidencia que o estado energético do tecido afeta a indução de enzimas fermentativas sob baixo oxigênio, além disso, mecanismos para ajustar o consumo de oxigênio estão ligados à disponibilidade de piruvato (CARPENTIER et al. 2010). Assim, mesmo em condições aeróbicas, a fermentação alcoólica desempenha um papel importante na prevenção da anóxia, controlando o nível de piruvato como ocorreu em *Arabidopsis* mutantes, nocaute para álcool desidrogenase, cultivadas em sistema hidropônico (ZABALZA et al. 2009).

Os genótipos estudados apresentaram regulação positiva da enzima álcool desidrogenase (*spots* 17, 126, PMGB043; 300, 456, PMGB099) em plantas do tratamento controle. Em *Arabidopsis* submetidas a estresses ambientais, a álcool desidrogenase

acumulada positivamente foi relacionada a melhora da aclimatação e revelação de variedades tolerantes a condições adversas (DOLFERUS, et al. 1994).

Onze isoformas de sacarose sintase (SUS) foram reguladas positivamente no genótipo PMGB043 e duas no genótipo PMGB099. A SUS está relacionada a uma série de processos metabólicos, como, distribuição de sacarose nos tecidos, síntese de amido e celulose, formação de parede celular, resposta a estresses abióticos e fixação de nitrogênio (CIERESZKO 2009). Além disso, em plantas cultivadas *in vitro* foi relatado que os processos de absorção e metabolização de açúcar impulsionam a respiração, resultando em níveis limitantes de oxigênio (CARPENTIER et al. 2010).

As proteínas de choque térmico (HSPs) foi up regulada no genótipo PMGB099 (spot 70). Estudos indicam que a regulação positiva dessa chaperona molecular desencadeia mecanismos de respostas a estresse hídrico em *Musa* sp (CARPENTIER et al., 2007; MATTOS-MOREIRA et al, 2018). O sistema HSP/chaperona em condições normais de crescimento ou durante e após o estresse, determina o destino de proteínas desnaturadas ou não-nativas. Assim são responsáveis pelo enovelamento, montagem, translocação e degradação de proteínas em muitos processos celulares normais, estabilizam proteínas e membranas, e podem auxiliar no redobramento de proteínas sob condições de estresse (WANG et al. 2004).

4.3 Proteínas reprimidas devido ao estresse hídrico

Dois terços da proteínas relacionadas a resposta de defesa no genótipo PMGB043 foram reprimidas, enquanto no genótipo PMGB099 a maioria das enzimas de defesa foram up reguladas. O estresse hídrico é um dos principais fatores abióticos que podem impactar o cultivo da bananeira. Nas plantas privadas de oxigênio, devido a alagamento (BLOKHINA et

al., 2001), ou sob déficit hídrico (PLACIDE et al., 2012), o estresse oxidativo é uma resposta esperada. Assim, a produção de enzimas antioxidantes, como, por exemplo, catalase (CAT), guaiacol peroxidase (POX) e dismutase do superóxido (SOD), podem limitar os danos celulares causados pelas espécies reativas de oxigênio (ROS) durante o estresse em *Musa* spp. (SURENDAR, et al., 2013c). Todas essas enzimas fazem parte de um complexo de respostas iniciado pelas plantas, as quais desencadeiam uma cascata de eventos moleculares, e finalizam com inúmeras respostas fisiológicas, metabólicas, de crescimento e desenvolvimento (CARVALHO, 2009).

A cisteína sintase foi down regulada no genótipo mais susceptível PMGB043 (spots 224, 225, 220) e up regulada no genótipo mais tolerante PMGB99 (spots 374, 445). A cisteína desempenha um papel fundamental no metabolismo primário e secundário das plantas. Além disso, tem sido indicada como molécula sinalizadora, no citosol, desencadeando resposta imune, e na mitocôndria, atuando na desintoxicação do cianeto, essencial para o desenvolvimento de pêlos radiculares e respostas de plantas a patógenos (ROMERO et al. 2014). Um estudo com variedades de *Musa* sp indica que essa proteína está relacionada a desintoxicação de espécies reativas de oxigênio (ROS) em plantas tolerantes ao estresse hídrico (VANHOVE et al 2012).

Quatro isoformas de H^+- ATPase tipo V (V-ATPase) foram donw reguladas durante o estresse no genótipo PMGB043 e uma no genótipo PMGB099. A V-ATPase realiza o transporte de prótons dependentes de ATP do citosol para o lúmen dos compartimentos da endomembrana. Essas proteínas encontram-se relacionadas a respostas de plantas a estresses ambientais, sendo fundamentais para manutenção da homeostase (MAGNOTTA; GOGARTEN, 2002). Dentre as funções conhecidas para V-ATPase estão a separação de proteínas, transporte pela membrana, exocitose, digestão endocítica, reciclagem de

neurotransmissores e energização dos sistemas de transporte da membrana e epitélios (BEYENBACH; WIECZOREK, 2006).

4.4 Enzima relacionada a expressão de hormônios em *Musa sp* susceptíveis a estresse hídrico

A enzima amido-hidrolases ácido indolil-3-acetico (ILR1) foi identificada apenas no genótipo PMGB043. A atividade do conjugado ILR1 podem ser um dos muitos pontos reguladores na teia da homeostase das auxinas, funcionando tanto na inativação permanente quanto no armazenamento temporário desse hormônio. Em *Arabidopsis* foi relatado que alguns conjugados de ácido indolil-3-acetico (IAA) podem ser usados para determinar a localização tecidual e subcelular do IAA anexado, inibindo o alongamento da raiz (LECLERE et al. 2002).

4.5 Uso da imunodetecção e atividade de enzimas para identificação de *Musa* sp tolerante a estresses por seca e alagamento

Em plantas do genótipo PMGB099, o aumento do acúmulo de CAT no tratamento de estresse moderado, e a subsequente redução dessa enzima no déficit severo, é semelhante ao que ocorreu em gramíneas, onde um aumento transitório de CAT ocorre no período inicial de estresse, mas com a intensificação do estresse (severo) ocorre o desequilíbrio entre a formação de oxigênio ativo e sua eliminação do sistema (FU; HUANG, 2001). A piora da resposta da planta ao estresse pode estar correlacionada à perda de clorofila e à destruição das membranas celulares (FU; HUANG 2001). Outro estudo com plantas oleaginosas demostrou uma redução da atividade de CAT à medida que o estresse hídrico se intensifica,

possivelmente, devido à inibição da síntese enzimática; degradação causada por proteases peroxissomais induzidas ou devido à foto-inativação da enzima (ABEDI; PAKNIYAT 2010).

Durante o experimento, as plantas de bananeira não apresentaram sintomas visuais de hipóxia (Suplementar D, Figura D.1) como clorose nas pontas e margens das folhas (deficiência de níquel), folhas pálidas e nervuras verdes (deficiência de ferro), descoloração das folhas dos botões (deficiência de boro), folhas marrons e curtas (deficiência de zinco), entre outros sintomas (HASANUZZAMAN, et al. 2017). Além disso, apresentaram boa taxa de crescimento com aumento de massa total maior que 30% de parte aérea em relação à raiz, principalmente nas plantas controle. Isto sugere que a via fermentativa deve ter suprido a demanda de energia para suportar o crescimento por meio de maior acúmulo da Álcool desidrogenase (ADH) e da baixa resistência estomática nas plantas controle, diferente do que ocorre com genótipos de milho em condições de alagamento (BENNICELLI et al., 1998). Em variedade de cacau suscetível à hipóxia por inundação, houve aparecimento de clorose foliar, diminuição das concentrações de carboidratos nas raízes e morte de plantas após 30 dias de inundação e baixa atividade de ADH, contrastando com a variedade tolerante (BERTOLDE et al., 2012). Os diploides de bananeira mantidos por 31 dias em sistema hidropônico (tratamento controle), não apresentaram danos visuais aparentes (Suplementar, D. Fig. D.1).

Nas primeiras horas em privação de oxigênio, as plantas usam a fermentação láctica catalisada pela lactato desidrogenase (LDH) para obtenção de energia (SWEETLOVE et al., 2000). Devido ao efeito negativo do lactato sob o pH citoplasmático das plantas, essa via torna-se transitória, sendo substituída pela fermentação etanólica catalisada por pyruvate descarboxylase (PDC) (RIVOAL et al., 1991).

Em raízes do genótipo clonal de TSA-792 (*Theobroma cacau*) houve aumento da enzima PDC nas primeiras 24h, seguido do aumento da atividade de ADH após esse período (BERTOLDE et al., 2014). A PDC catalisa o primeiro passo da fermentação etanólica, e é

sugerida como a principal enzima reguladora dessa via (KÜRSTEINER et al., 2003). Isto indica que o sistema de aeração utilizado supriu parte do oxigênio para a via aeróbia e que o aumento do acúmulo de ADH ocorre lentamente para suportar as maiores demandas energéticas durante o crescimento sob hipóxia.

Esse resultado sugere que o genótipo PMGB099, que apresenta maior acúmulo de ADH, é promissor para tolerância a estresses abióticos, como déficit de O_2 estudado em variedades de *Theobroma cacao* (BERTOLDE et al., 2014), alta salinidade, relatado para plantas transgênicas de *Nicothiana tabacum* (YI et al., 2017), baixa temperatura, testada em *Zea mays* e *Oryza sativa* (CHRISTIE et al., 1991) e mais de um estresse, como observado em plantas de *Arabidopsis thaliana* submetido a salinidade e baixa temperatura (DOLFERUS et al., 1994).

5. CONCLUSÃO

As modificações realizadas no protocolo de Bertolde et al. (2014) para extração de proteínas em raízes de *Musa* sp resultaram em géis 2D de alta qualidade, livres de manchas e estrias.

Esse estudo revela que o estresse hídrico em *Musa* sp afeta o nível de expressão de várias proteínas relacionadas a processos biológicos. Além disso, os resultados indicam a necessidade de mais estudos sobre os efeitos de hipóxia por inundação em genótipos de bananeira uma vez que, os novos conhecimentos produzidos apontam a ativação de mecanismos importantes de tolerância ao estresse, como resposta antioxidante e acúmulo de ADH.

6. PERSPECTIVAS FUTURAS

- Conhecer as redes de interação proteína-proteína ativadas em situações de estresse hídrico.

7. REFERÊNCIAS

ABEDI, T., PAKNIYAT, H. 2010. Antioxidant Enzyme Changes in Response to Drought Stress in Ten Cultivars of Oilseed Rape (Brassica napus L.). Czech Journal of Genetics and Plant Breeding, 46, 27–34.

BENNICELLI, R.P., STPNIEWSKI, W., ZAKRZHEVSKY, D.A., BALAKHNINA, T.I., STPNIEWSKA, Z., LIPIEC, J. 1998. The effect of soil aeration on superoxide dismutase activity, malondialdehyde level, pigment content and stomatal diffusive resistance in maize seedlings. Environmental and Experimental Botany. 39, 203-211.

BERTOLDE, F.Z. et al. 2014. Efficient method of protein extraction from *Theobroma cacao* L . roots for two-dimensional gel electrophoresis and mass spectrometry analyses. Genetics and Molecular Research. 3, 5036-5047. doi: 10.4238/2014.July.4.19.

BERTOLDE, F.Z., ALMEIDA, A-A.F., PIROVANI, C.P. 2014. Analysis of Gene Expression and Proteomic Profiles of Clonal Genotypes from *Theobroma cacao* Subjected to Soil Flooding. PLoS One. 9, 1-15.

BERTOLDE, F.Z. et al. 2012. Physiological and biochemical responses of *Theobroma cacao* L. genotypes to flooding. Photosynthetica 50, 447-457.

565 BEYENBACH, K.W., WIECZOREK, H. (2006) The V-type H+ ATPase: molecular structure
566 and function, physiological roles and regulation. Journal of Experimental Biology. 209, 577-
567 589; doi: 10.1242/jeb.02014
568 BLOKHINA, O. B.; CHIRKOVA, T.V.; FAGERSTEDT K. V. 2001. Anoxic stress leads to
569 hydrogen peroxide formation in plant cells. Journal of Experimental Botany. 52, 1-12.

570 CARPENTIER, S.C. et al. 2007. Banana (*Musa* spp.) as a model to study the meristem
571 proteome: Acclimation to osmotic stress. Proteomics. 7, 92–105. doi
572 10.1002/pmic.200600533

573 CARPENTIER, S.C., VERTOMMEN, A., SWENNEN, R., WITTERS, E., FORTES, C.,
574 SOUZA, M.T. JR, PANIS, B.(2010) Sugar-mediated acclimation: the importance of sucrose
575 metabolism in meristems. Journal of Proteome Research. 9, 5038-46. doi:
576 10.1021/pr100321v.

577 CASTELLARIN, S.D. et al. 2007. Transcriptional regulation of anthocyanin biosynthesis in
578 ripening fruits of grapevine under seasonal water deficit. Plant, Cell and Environment. 30,
579 1381–1399 https://doi.org/10.1111/j.1365-3040.2007.01716.x

580 CHAVES, M.M. et al. 2010. Grapevine under deficit irrigation: hints from physiological and
581 molecular data. Annals of Botany. 105, 661–676. https://doi.org/10.1093/aob/mcq030

582 CHRISTIE, P. J., HAHN, M., AND WALBOT, V. 1991. Low-temperature accumulation of
583 alcohol dehydrogenase-1 mRNA and protein activity in maize and rice seedlings. Plant
584 Physiology. 95, 699-706.

585 CIERESZKO, I (2009) Sucrose metabolism in plant tissues under stress conditions: key
586 enzymes, localization and function. In: Compartmentation of Responses to Stresses in Higher
587 Plants, True or False. Cap. 8, 193-218.

588 DOLFERUS, R., DEBRUXELLES, G., DENNIS, E. S., PEACOCK, W. J. 1994. Regulation
589 of the Arabidopsis Adh-gene by anaerobic and other environmental stresses. Annals of
590 Botany. 74, 301-308.
591 FAROOQ, M. et al. 2009. Plant drought stress: effects, mechanisms and management.
592 Agronomy for Sustainable Development. 29, 185-212. DOI: 10.1051/agro:2008021

593 FU, J.; HUANG, B. 2001. Involvement of antioxidants and lipid peroxidation in the
594 adaptation of two cool-season grasses to localized drought stress. Environmental and
595 Experimental Botany 45, 105–114.
596 GRAVES, P. R., HAYSTEAD, T. A. J. 2002. Molecular Biologist's Guide to Proteomics.
597 Microbiology and Molecular Biology Reviews. 66, 39–63.
598 http://doi.org/10.1128/MMBR.66.1.39-63.2002

599 HASANUZZAMAN, M., NAHAR, K., RAHMAN, A., AL MAHMUD, J., HOSSAIN, S.,
600 ALAM, K., OKU, H., FUJITA, M. 2017. Actions of Biological Trace Elements in Plant
601 Abiotic Stress Tolerance. In: NAEEM M., ANSARI A., GILL S. S. (eds) Essential Plant
602 Nutrients. Springer. 213-274.

603 HU, W. et al. 2015. Genome-Wide Identification and Expression Analyses of Aquaporin Gene
604 Family during Development and Abiotic Stress in Banana. International Journal of Molecular
605 Sciences. 16, 19728-19751. doi:10.3390/ijms160819728

606 KÜRSTEINER, O., DUPUIS, I., KUHLEMEIER, C. 2003. The Pyruvate decarboxylase1
607 Gene of Arabidopsis Is Required during Anoxia But Not Other Environmental Stresses. Plant
608 Physiology. 132, 968-978.

609 LAEMMLI, U. K. 1970. Cleavage of structural proteins during the assembly of the head of
610 bacteriophage T4. Nature. 227, 680-685.

611 MAGNOTTA, S.M., GOGARTEN, J.P (2002) Multi site polyadenylation and transcriptional
612 response to stress of a vacuolar type H+-ATPase subunit A gene in Arabidopsis thaliana.
613 BMC Plant Biology. 2, 1-13. https://doi.org/10.1186/1471-2229-2-3

614 MATTOS-MOREIRA, L.A. et al. 2018. Differentially expressed proteins associated with
615 drought tolerance in bananas (*Musa* spp.). Acta Physiologiae Plantarum. 40, 1-15.
616 https://doi.org/10.1007/s11738-018-2638-3

617 MIAO, H. et al. 2017. Genome-wide analyses of SWEET family proteins reveal involvement
618 in fruit development and abiotic/biotic stress responses in banana. Scientific Reports.
619 Scientific Reports. 7, 1-15. doi:10.1038/s41598-017-03872-w

620 MISHRA, P. et al.. 2018. Identification of cis-regulatory elements associated with salinity and
621 drought stress tolerance in rice from co-expressed gene interaction networks. Bioinformation
622 14, 123-131. doi: 10.6026/97320630014123.

623 NEGI, S., TAK, H., GANAPATHI, T.R. 2015. Expression analysis of *MusaNAC68*
624 transcription factor and its functional analysis by overexpression in transgenic banana plants.
625 Plant Cell, Tissue and Organ Culture. 125, 59-70. doi:10.1007/s11240-015-0929-6

626 NEUHOFF, V. et al. 1988. Improved staining of proteins in polyacrylamide gels including
627 isoelectric focusing gels with clear background at nanogram sensitivity using Coomassie
628 Brilliant Blue G-250 and R-250. Electrophoresis. 9, 255-262.
629 https://doi.org/10.1002/elps.1150090603

630 OLIVEIRA, T.M. et al. 2015. Comparative study of the protein profiles of Sunki mandarin
631 and Rangpur lime plants in response to water deficit. BMC Plant Biology. 3, 15:69. doi:
632 10.1186/s12870-015-0416-6.

633 REHEM, B.C., ALMEIDA, A-AF., SANTOS, I.C., GOMES, F.P., PIROVANI, C.P.,
634 ROMERO, P. et al. (2011) Daily and seasonal patterns of leaf water relations and gas
635 Exchange of regulated deficit-irrigatedalmond trees under semiarid conditions. Envir. Exp.
636 Bot. 56: 158–173.

637 RIVOAL, J., RICARD, B., PRADET, A. 1991. Lactate Dehydrogenase in Oryza sativa L. Seedlings
638 and Roots - Identification and Partial Characterization. Plant Physiology. 95, 682-686

639 ROMERO, L.C., AROCA, A.A., LAUREANO-MARÍN, L.M., MORENO, I., GARCÍA, I.,
640 GOTOR, C. (2014) Cysteine and Cysteine-Related Signaling Pathways in Arabidopsis
641 thaliana. Molecular Plant. 7, 264-276. https://doi.org/10.1093/mp/sst168

642 PIROVANI, C.P. et al. 2008. Protein extraction for proteome analysis from cacao leaves and
643 meristems, organs infected by Moniliophthora perniciosa, the causal agent of the witches'
644 broom disease. Electrophoresis. 29, 2391-2401. doi: 10.1002/elps.200700743.

645 SALEKJALALI, M., HADDAD, R., JAFARI, B. 2012. Effects of Soil Water Shortages on
646 the Activity of Antioxidant Enzymes and the Contents of Chlorophylls and Proteins in Barley.
647 American-Eurasian Journal Of Agricultural & Environmental Sciences. 12, 57-63.

648 SANTOS, I.C. et al. 2014. Molecular, Physiological and Biochemical Responses of
649 *Theobroma cacao* L. Genotypes to Soil Water Deficit. PLoS ONE. 9, 1-31.
650 doi:10.1371/journal.pone.0115746

651 SHEKHAWAT, U.K., GANAPATHI, T.R. 2013. *MusaWRKY71* overexpression in banana
652 plants leads to altered abiotic and biotic stress responses. PLoS One. 8, 1-7. doi:
653 10.1371/journal.pone.0075506.

654 SHEKHAWAT, U.K.S., SRINIVAS, L., GANAPATHI, T.R. 2011. *MusaDHN-1*, a novel
655 multiple stress-inducible SK3-type dehydrin gene, contributes affirmatively to drought- and
656 salt-stress tolerance in banana. Planta. 234, 915–932. doi:10.1007/s00425-011-1455-3

657 SREEDHARAN. S., SHEKHAWAT, U.K.S., GANAPATHI, T.R. (2013) Transgenic banana
658 plants overexpressing a native plasma membrane aquaporin *MusaPIP1;2* display high
659 tolerance levels to different abiotic stresses. Plant Biotechnology Journal. 11, 942–952.
660 doi:10.1111/pbi.12086

661 SREEDHARAN. S., SHEKHAWAT, U.K.S., GANAPATHI, T.R. 2012. *MusaSAP1*, a
662 A20/AN1 zinc finger gene from banana functions as a positive regulator in different stress
663 responses. Plant Molecular Biology. 80, 1-15. doi:10.1007/s11103-012-9964-4

664 SURABHI, G.K., PATTANAIK, S., MOHANTY, S. 2016. A comparative method for protein
665 extraction and proteome analysis by two-dimensional gel electrophoresis from banana fruit.
666 Horticultural Biotechnology Research. 2, 8-13. doi: 10.19071/jhcbt.2016.v2.3049

667 SWEETLOVE, J., DUNFORD, R., RATCLIFFE, R. G., KRUGER, N. J. 2000. Lactate
668 metabolism in potato tubers deficient in lactatedehydrogenase activity. Plant, Cell and
669 Environment. 23, 873–881.

670 TAK, H., NEGI, S., GANAPATHI, T. R. 2017. Banana NAC transcription factor
671 *MusaNAC042* is positively associated with drought and salinity tolerance. Protoplasma. 254,
672 803–816. DOI 10.1007/s00709-016-0991-x

673 VAGANAN, M. M.et al. 2015. Evaluation of different protein extraction methods for banana
674 (*Musa* spp.) root proteome analysis by two-dimensional electrophoresis. Indian J Biochem
675 Biophys. 52:101-6.

VANHOVE, A-C. et al. 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. Frontiers in Plant Science. 3, 1-10. doi:10.3389/fpls.2012.00176

VON LOESECKE, H. W. 1949. Bananas, chemistry, physiology and technology. New York: Interscience Publishers. 1-189.

WEI. Y. et al. 2016 Heat shock transcription factors in banana: genome-wide characterization and expression profile analysis during development and stress response. Scientific Reports. 6, 1-11. doi:10.1038/srep36864

XU, Y. et al. (2014) A banana aquaporin gene, *MaPIP1;1*, is involved in tolerance to drought and salt stresses. BMC Plant Biology. 14, 1-14. doi:10.1186/1471-2229-14-59

CONCLUSÃO GERAL

Na revisão sistemática reconhecemos que a engenharia genética, a seleção *in vitro*, a aplicação exógina de hormônios vegetais e o melhoramento tradicional estão entre as principais técnicas utilizadas nos últimos 10 anos para obter genótipos de bananeira tolerantes a seca. Além disso, devido as respostas ao déficit hídrico serem genótipo dependentes, estratégias de melhoramento podem combinar cultivares AB comestíveis com *M. balbisiana* selvagem para criar novas variedades ABB produtivas, palatáveis e tolerantes. Sinalizamos a necessidade de estudos futuros sobre estresse por inundação em bananeira, mecanismos de regulação pós-traducionais ativados após períodos de estresse e herança epigenética.

O sistema hidropônico adaptado pode ser usado em testes de fenotipagem de bananeira para tolerância à seca e alagamento, com baixo custo e em grande escala. Assim, a produção dessa fruta pode ser projetada para novos ambientes, como solos inundados.

Os perfis proteômicos de variedades de bananeira submetidos ao déficit hídrico apresentam características distintas. A identificação das proteínas pode revelar potenciais marcadores de tolerância a seca, além de redes de interação proteína-proteína regulados no estresse hídrico produzido em sistema hidropônico adaptado.

REFERÊNCIAS

ALIZADEH, V. et al. 2014. Effects of Climate Change and Drought-Stress on Plant Physiology. International journal of Advanced Biological and Biomedical Research. 2, 468-472.

BIDABADI, S.S. et al. (2012). Influence of salicylic acid on morphological and physiological responses of banana (*Musa acuminata* cv. 'Berangan', AAA) shoot tips to *in vitro* water stress induced by polyethylene glycol. Plant Omics Journal. 5, 33-39.

BIDABADI, S.S. et al. (2011). Evaluation of *in vitro* Water Stress Tolerance among EMS – Induced Variants of Banana (*Musa* spp., AAA), Using "Morphological, Physiological and Molecular" Traits. Journal of Crop Science and Biotechnology. 14, 255-263. doi;10.1007/s12892-011-0073-8

HIGGINS, J.P.T. et al. 2011. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. 343. doi: https://doi.org/10.1136

719 LECLERE, S., TELLEZ, R., RAMPEY, R.A., MATSUDA, S.P., BARTEL, B.(2002)
720 Characterization of a Family of IAA-Amino Acid Conjugate Hydrolases from Arabidopsis.
721 The Journal of Biological Chemistry. 277, 20446–20452.

722 NEVES, D.M. et al. 2017. Recurrent water deficit causes epigenetic and hormonal changes in
723 citrus plants. Scientific Reports. 7, 1-11. DOI:10.1038/s41598-017-14161-x

724 ROBINSON, J.C., BOWER, J.P., 1986. Transpiration characteristics of banana leaves
725 (cultivar 'Williams') in response to progressive depletion of available soil moisture. Scientia
726 Horticulturae. 30, 289-300. https://doi.org/10.1016/0304-4238(87)90003-3

727 TURNER, D.W., FORTESCUE, J.A., THOMAS, D.S. 2007. Environmental physiology of
728 the bananas (Musa spp.). Brazilian Journal of Plant Physiology. 19, 463-484.
729 http://dx.doi.org/10.1590/S1677-04202007000400013.

730 RUKUNDO, P., CHRISTIAN, C.S., RONY, S. (2012) Development of *in vitro* technique to
731 screen for drought tolerant banana varieties by sorbitol induced osmotic stress. African
732 Journal of Plant Science. 6, 416-425. doi:10.5897/AJPS12.101

733 SOUSA, E.M.R. et al. 2018. Drought tolerance of a microcitrangemonia when treated with
734 paclobutrazol and exposed to different water conditions. Scientia Horticulturae. 238, 75-82.
735 https://doi.org/10.1016/j.scienta.2018.04.038

736 SURABHI, G.K., PATTANAIK, S., MOHANTY, S. 2016. A comparative method for protein
737 extraction and proteome analysis by two-dimensional gel electrophoresis from banana fruit.
738 Horticultural Biotechnology Research. 2, 8-13. doi: 10.19071/jhcbt.2016.v2.3049

739 VAGANAN, M.M. 2015. Evaluation of different protein extraction methods for banana
740 (*Musa* spp.) root proteome analysis by two-dimensional electrophoresis. Indian Journal of
741 Biochemistry and Biophysics. 52, 101-106. DOI: 10.1002/jsfa.5591

VANHOVE, A-C. et al. (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. Frontiers in Plant Science. 3, 1-10. doi:10.3389/fpls.2012.00176

ZABALZA, A.; VAN DONGEN, J. T.; FROEHLICH, A.; OLIVER, S. N.; FAIX, B.; GUPTA, K. J.; SCHMALZLIN, E.; IGAL, M.; ORCARAY, L.; ROYUELA, M.; GEIGENBERGER, P. (2009) Regulation of respiration and fermentation to control the plant internal oxygen concentration. Plant Physiology. 149, 1087–1098.

YI, S.Y., KU, S.S., SIM, H.J., KIM, S.K., PARK, J.H., LYU, J.I., SO E.J., CHOI, S.Y., KIM, J. AHN, M.S., KIM, S.W., PARK, H., JEONG, W.J., LIM, Y.P., MIN, S.R., LIU, J.R. 2017 An Alcohol Dehydrogenase Gene from Synechocystis sp. Confers Salt Tolerance in Transgenic Tobacco. Frontiers in Plant Science. 8, 1-13.

WANG, W., VINOCUR, B., SHOSEYOV, O., ALTMAN, A. (2004) Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. TRENDS in Plant Science. 9, 244-252. doi:10.1016/j.tplants.2004.03.006

Suplementar A: Modificações realizadas nos protocolos de extração de BERTOLDE et al. 2014.

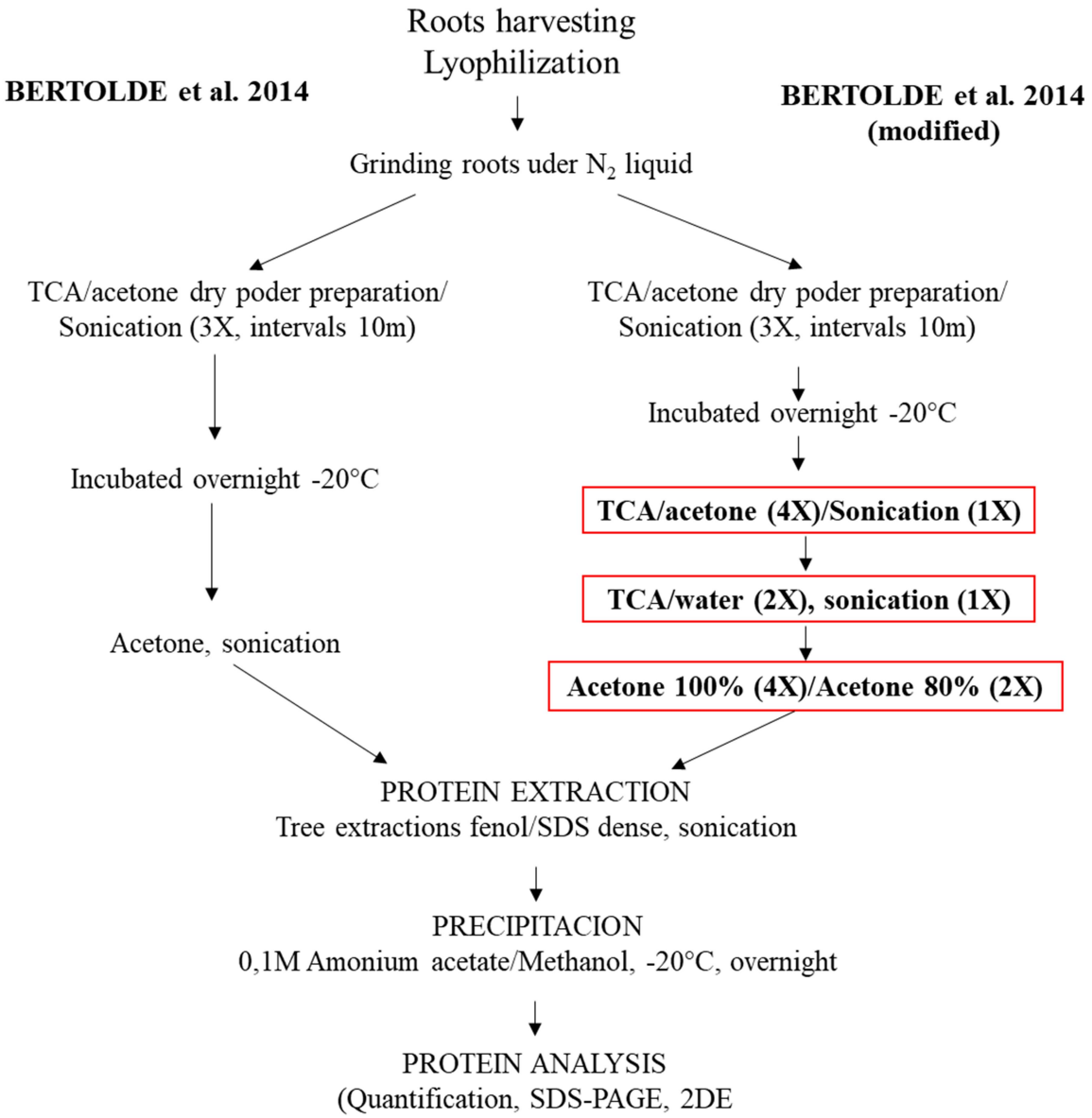


Figura A.1. Esquemas mostrando a extração de proteínas das raízes de *Musa* sp.

Suplementar B.1. Avaliação da distribuição dos *spots* de *Musa acuminata* em 2D-PAGE quanto a Massa Molecular e Ponto Isoelétrico

A avaliação da distribuição dos *spots* em relação à Massa Molecular (KDa) foi realizada entre os genótipos PMGB043 e PMGB099 e seus tratamentos controle e estresse severo (Fig. 4). A maioria dos *spots* detectados em ambos os genótipos foi visualizada na faixa de 30 a 60 kDa, sendo que os *spots* de maior massa molecular detectados no tratamento controle do genótipo PMBG043 e tratamento estressado do genótipo PMGB099.

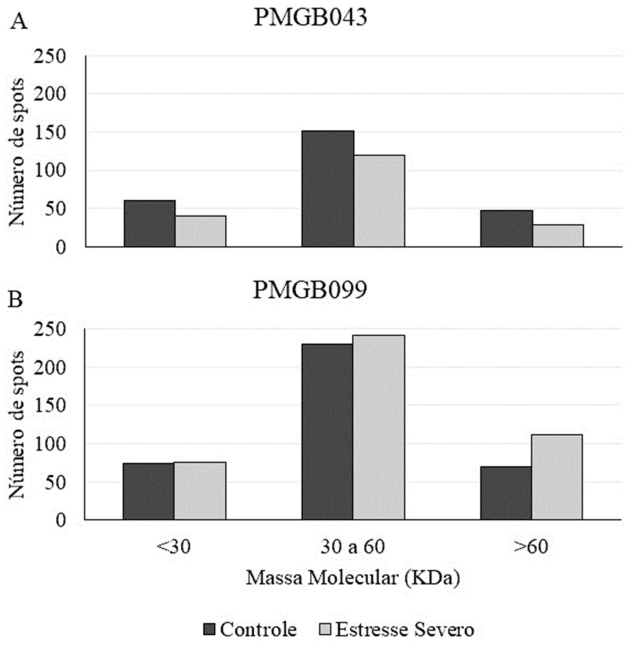


Fig. B.1: Distribuição dos *spots Musa acuminata* nos mapas 2-D, de acordo com a massa molecular (kDa). Em A, os *spots* do genótipo PMGB043 no tratamento controle e estresse hídrico severo, respectivamente. Em B, os *spots* do genótipo PMGB099 no tratamento controle e estresse hídrico severo, respectivamente.

Quanto ao ponto isoelétrico, a maioria dos *spots* foi visualizada na faixa de pH entre 4 e 8 para o genótipo PMGB043 e entre 5 a 9 para o genótipo PMGB099 (Fig. 5).

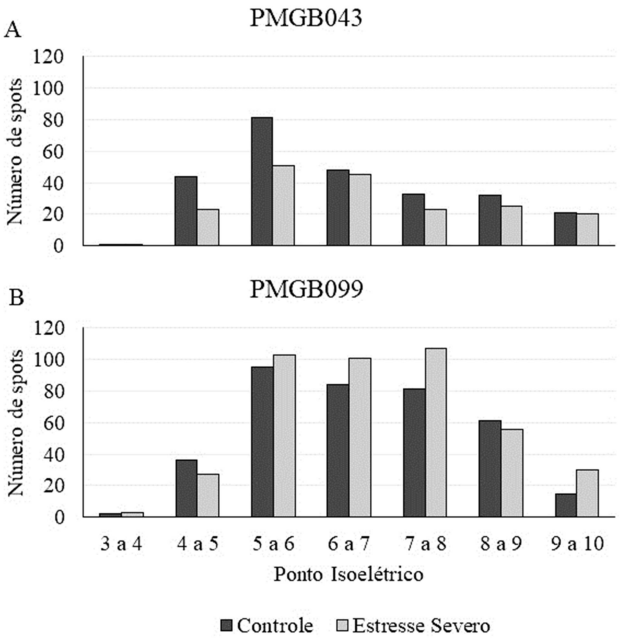


Fig. B.2: Distribuição dos *spots* de *Musa acuminata* detectados nos mapas 2-D, de acordo com o ponto isoelétrico (3-10 NL). Em A, os *spots* do genótipo PMGB043 no tratamento controle e estresse hídrico severo, respectivamente. Em B, os *spots* do genótipo PMGB099 no tratamento controle e estresse hídrico severo, respectivamente.

Suplementar C: Géis de normalização de imunodetecção de enzimas ADH e CAT.

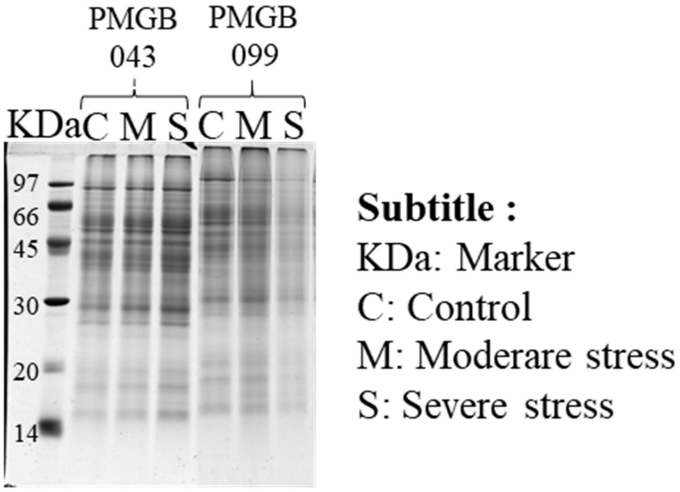


Figure C.1: Proteic profile of roots of Musa genotypes in SDS-PAGE. M – molecular mass marker with values in kDa indicated at the left isde; C, M and S refer to the treatments with water déficit.

Suplementar D.1: Appendice D.1. Musa genotypes submitted to diferente water regimes.

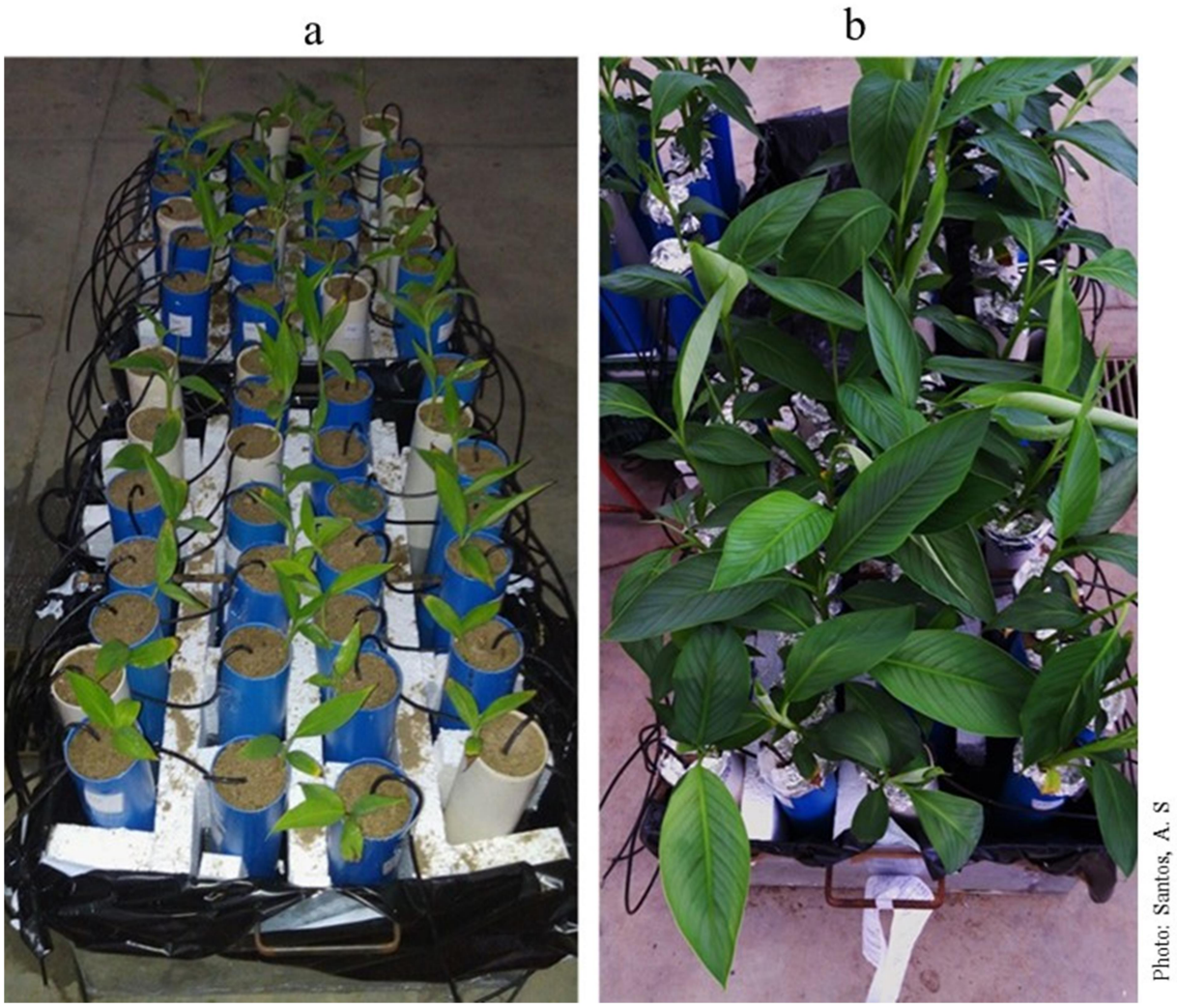


Figure D.1. Musa genotypes submitted to diferente water regimes. A) plants at the beginning of the experiment and b) plants at the final stage of the experiment.