ABSTRACT

NHANALA, STELLA ESMERALDA DA CONCEIÇÃO. Targeted Use of Crop Wild relatives for Improved Drought Tolerance in Sweetpotato [*Ipomoea batatas* (L.) Lam.]. (Under the direction of Dr. G. Craig Yencho).

Drought is a major abiotic stress causing significant yield losses in crop production. Improved drought tolerance is desirable in crop species that are drought sensitive, or in plants grown in any environment facing water stress caused by reduced rainfall. Sweetpotato [*Ipomoea batatas* (L.) Lam.] is a staple food in many developing countries, especially those in sub-Saharan Africa. Sweetpotato is often grown in arid or semi-arid areas with extended periods of drought and there is a need to improve drought tolerance in this crop in a changing climate where drought and heat stress are increasing. Crop wild relatives may harbor genes for improved drought tolerance that can be deployed in cultivated species to improve their tolerance to drought.

This research was focused on evaluating the potential of the crop wild relatives of sweetpotato as germplasm to improve drought tolerance in cultivated sweetpotato. I studied the relatedness of 52 accessions of *Ipomoea spp.* belonging to the *Batatas* complex aiming to understand the relationships between the ten species I evaluated. The *Batatas* series includes *I. batatas* (sweetpotato) and its closest relatives. A quantitative reduced representation sequencing (qRRS) method was applied for the study of the phylogenetic relationships and population structure of the *Batatas* complex. The application of a reduced representation sequencing (qRRS) method allowed the discovery of 9223 single nucleotide polymorphism (SNPs), which were used in the phylogenetic studies. The study of the phylogenetic relationships and the principal component analysis resulted in the identification of three major clades, while the population structure revealed the existence of six sub-populations within the 52 populations. I also confirmed that *I. trifida* is the closest relative of sweetpotato, and this result was in accordance

with previous studies that found that *I. trifida* was the closest relative of the cultivated species. The structure analysis revealed the presence of at least six-subpopulations. These results are important and can be applied to the breeding efforts to introgress traits of interest from the wild relatives into sweetpotato.

In addition to the study of the relatedness of the *Batatas* complex, I studied drought tolerance in this group. I evaluated drought tolerance in *Ipomoea spp.* with the goal of identifying sources of drought tolerance for sweetpotato in wild *Ipomoea spp.* that were identified with the potential to be adapted to drought-prone areas based on their ecological niches. Four cultivars of sweetpotato (*I. batatas*), and the wild species *I. cynanchifolia*, *I. leucantha*, *I. trifida*, and *I. triloba* were screened for drought tolerance in a series of greenhouse experiments. Under five levels of irrigation (control (daily irrigation), and drought periods of seven, nine, twenty-one, and fifty days) I compared: stomatal conductance; dry weight of the aboveground and belowground parts; and dry weight of the storage roots. I observed that the wild genotypes showed signs of drought, such as leaf wilting and plant desiccation before the cultivated genotypes. Compared with their wild counterparts, the *I. batatas* genotypes appeared to tolerate extended periods of drought better. I found that drought tolerance in *I. batatas* may be associated to the presence of storage roots in sweetpotato, while the wild types did not produce storage roots. Research for drought tolerance have been done, and the cultivars I selected for my study were evaluated in those previous studies as positive controls for drought tolerance. To the best of my knowledge, this research is the first of its kind to compare the drought tolerance of cultivated sweetpotato with its wild relatives. The methodologies that I applied in this study may be useful for future evaluations of wild relatives of sweetpotato for drought tolerance.

The results of the study to evaluate drought tolerance in the *Ipomoea spp.* led to a comparative transcriptomic study for drought tolerance in two cultivars of sweetpotato, 'Beauregard' and 'Resisto'. 'Beauregard' was more tolerant to drought than 'Resisto' when the *Ipomoea spp.* were screened for drought tolerance. I identified candidate genes for drought tolerance that were differentially expressed in the two cultivars. The candidate genes I identified for drought tolerance were the abscisic acid and environmental stress-inducible protein-like (TAS14), E3 ubiquitin-protein ligase RING1-like, expansin-A15-like (EXLA15), basic form of pathogenesis-related protein 1 (PRP-1), 18.8 kDa class II heat shock protein-like, and the desiccation inducible PCC13-62. The wall-associated receptor kinase-like 1 (WAK1) and Receptor-like serine/threonine-protein kinase SD1-7-like, were two genes that were identified with the recover capacity from drought in 'Beauregard' and 'Resisto. All these genes had a higher fold change in 'Beauregard' than in 'Resisto'. The results I observed at a molecular level were in general in agreement with the phenotypic response of the two cultivars when they were subjected to drought.

Targeted Use of Crop Wild relatives for Improved Drought Tolerance in Sweetpotato [*Ipomoea batatas* (L.) Lam.]

by
Stella Esmeralda da Conceição Nhanala

A dissertation submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

Horticultural Science

Raleigh, North Carolina
2021

APPROVED BY:

_____	_____
G. Craig Yencho Committee Chair	Bode A. Olukolu
_____	_____
Hamid Ashrafi	Qiuyun (Jenny) Xiang
_____	
Vasu Kuraparthy	

DEDICATION

"To my mother Ms. Natália Machele, my brothers Osvaldo and Emílio, my grandfather Vovó Cipriano, and in memory of my grandmother Vovó Teresa, and all my relatives who have been supporting me during my academic pathway."

BIOGRAPHY

Stella Esmeralda da Conceição Nhanala was born in Maputo, Mozambique. She was raised with her two brothers, by their mother Ms. Natália Machele. She attended primary school at Escola Primária 3 de Fevereiro, and then went to middle school Escola Secundária da Polana, and later she attended the high school Escola Secundária Josina Machel. Stella went to primary and secondary schools in Mozambique. She obtained her *licenciatura* (bachelor's degree) in Agronomy in 2009 from the Universidade do Algarve (Ualg) in Portugal, and Master of Science (Plant breeding, Genetics and Biotechnology) in 2014 from Michigan State University (MSU), Lansing, USA.

After finishing her bachelor training funded by a scholarship awarded by the Instituto Português de Apoio ao Desenvolvimento (IPAD), Stella returned to Mozambique and in 2010 she joined the Instituto de Investigação Agrária de Moçambique (IIAM), in Maputo. At IIAM she worked as an agricultural technician on sweetpotato, cassava, potato, and banana tissue culture (on the micropropagation of those crops). Two years after Stella joined IIAM, she began her master training at MSU funded by the MasterCard Foundation Scholarship. After finishing her masters, Stella returned to Mozambique where she continued to work at IIAM. In 2016, Stella was awarded a scholarship for her Ph.D. studies at North Carolina State University (NCSU) under the the initiative "Adapting Agriculture to Climate Change: Collecting, Protecting and Preparing Crop Wild Relatives", which is a project funded by the Crop Trust, an international organization dedicated solely to conserving and making available crop diversity.

After the completion of her Ph.D. training, Stella will return to Mozambique and use her acquired knowledge and skills to work on improving sweetpotato and other crops for adaptation

to environmental stresses, such as drought, flooding, and heat, and for nutrition by integrating phenotypic and genotypic tools to plant breeding in Mozambique.

ACKNOWLEDGMENTS

I wanted to express my deepest gratitude to my major Professor, Dr. G. Craig Yencho for his mentorship, guidance, support, training, and patience to believe in me, and keeping pushing me forward even when it did not appear that the "last effort" in my studies and/or research would be successful. Under the supervision of Dr. Yencho I had the opportunity to develop my research skills as well as to always consider other approaches to solve a problem.

I am also grateful to my committee members; Dr. Hamid Ashrafi, who provided valuable guidance during my transcriptomic profiling research project, especially with data analysis; Dr. Vasu Kuraparthy who increased my knowledge on polyploid species and their utilization in plant breeding, who has also advised on my transcriptomic profiling project; Dr. Rick Miller, who has always encouraged and challenged me to develop a deeper understanding even on matters that I was completely unfamiliar with, and these topics were relevant to my dissertation study through insightful discussions about the *Batatas* complex; Dr. Bode Olukolu, who optimized the protocol and developed the quantitative reduced representation sequencing (OmeSeq-qRRS) protocol which was applied to generate the sequenced data that was discussed in the study of the relatedness of the *Batatas* complex; Dr. Olukolu also contributed with data analysis performing the SNP filtering of the 52 accessions of *Ipomoea spp.*; and to Dr. Jenny Xiang, who introduced me to the basic evolutionary genetic concepts, analysis and practice, and which these notions were needed to understand the *Batatas* complex; Dr. Xiang also provided valuable inputs in the chapter where I studied the phylogenetic relationships of the *Batatas* complex.

I am also especially grateful to Dr. Bettina Heider and Carlos Flores from the International Potato Center (CIP), Lima, Peru who trained me on growing species of the *Batatas* complex in San Ramon, and Lima, Peru. They also generously provided plant materials for the

study of the *Batatas* complex and were willing to grow the plants and prepare and ship the samples with care to Raleigh - North Carolina State University.

My sincere appreciation goes to Dr. Maria Andrade and the CIP team in Mozambique who always provided me with help and academic content on sweetpotato research ongoing in Mozambique, which was very helpful to develop my drought studies project.

A special thanks goes to Dr. David Dickey from the Department of Statistics at NCSU, who generously and patiently helped me to understand how to thoroughly design a meaningful experiment statistically and trained me to run statistical analysis.

My extended thanks to Dr. Victor Amankwaah, Dr. Bonny Oloka, Samuel Acheampong, and Adam Canal, who were also graduate students in the sweetpotato and potato breeding program and provided help when requested. My special appreciation goes to Dr. Sharon Williamson who helped with the laboratory work for the RNA extractions; and also, a special thank you to Ana Pecota who was always willing to help me with my greenhouse work, when it was needed. I am also grateful to Dr. Xiaofei Zhang, Chris Heim, Emily Milks, Kenneth Pecota, Ragy Ibrahim, Amber Harmon, and Wendy Koch, for their encouragement and support. My appreciation also goes to all the summer interns from the Sweetpotato and Potato Breeding and Genetics Program at NCSU, who helped me with my greenhouse-based experiments.

To all who are not mentioned here and contributed to this academic achievement, my sincere thank you.

Lastly, my PhD research at NCSU was supported by the Crop Trust under the Grant Agreement GS14028. I sincerely thank them for all their support over the years. The Crop Trust is supported by the Government of Norway and their mission is to ensure the conservation and

availability of crop diversity for food security worldwide. For further information, see the Crop Trust website at https://www.croptrust.org/.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER 2

CHAPTER 3

CHAPTER 4

LIST OF FIGURES

CHAPTER 2

CHAPTER 3

CHAPTER 4

CHAPTER 1

INTRODUCTION

Sweetpotato [*Ipomoea batatas* (L.) Lam.] is a crop grown worldwide with a total production of 91.9 million tons in 2019 (FAOSTAT, 2021). The production of sweetpotato per continent is led by Asia, followed by Africa, the Americas, Oceania, and Europe. The top five producers of sweetpotato in 2019 were China, Malawi, Nigeria, Tanzania, and Uganda. These data show that the major producers of sweetpotato around the world are developing countries, and in fact, sweetpotato is a staple food in many developing countries. As a staple food, sweetpotato contributes to the local people's diet where the crop is consumed mostly as a storage root and occasionally for its leaves. While the leaves are stewed, the storage roots are processed in many ways: boiled, roasted, baked, puréed, processed as chips, and raw storage root pulp (juice). The edible parts of the plant are sources of proteins, fiber, vitamins, and minerals for humans (Padmaja, 2009; Minot, 2010; Sun et al., 2014; Laurie et al., 2015; Low et al., 2020). In some countries, sweetpotato is consumed by animals mostly as raw leaves and vines.

Sweetpotato is grown mainly in rain-fed agricultural systems, and this species tolerates short periods of drought well (Low et al., 2009; Andrade et al., 2016). However, when sweetpotato is subjected to extended drought, its yield is reduced. The percentage of yield reduction varies according to the cultivar, duration and intensity of the drought, and the plant's growth stage during drought (Van Heerden & Laurie, 2008; Solis et al., 2014; Kivuva et al., 2015; Andrade et al., 2016). Yield reduction may be more pronounced in the rain-fed systems where sweetpotato is grown if these regions are affected by long periods of drought.

Climate scientists predict prolonged and frequent periods of drought due to climate change (Carnicer et al., 2011; Knapp et al., 2015; Gizaw & Gan, 2017). Agricultural drought,

characterized as insufficient soil moisture to grow a plant under its normal metabolism, negatively impacts many crops, including sweetpotato. Food production may be at risk due to drought-related yield reductions. For regions where sweetpotato is a staple food, yield decrease may impact the availability of important nutrients such as vitamins and proteins. Therefore, it is important to improve crops like sweetpotato so that they are tolerant to long periods of drought. Breeding sweetpotato for drought tolerance will allow farmers who grow this crop in rain-fed systems or in dry areas to still cultivate sweetpotato with their usual crop-management practices. The improvement of this crop will also secure the availability of sweetpotato in the diet of those who consume this crop as a staple food; and will prevent fluctuations in sweetpotato accessibility in local markets.

The study of drought tolerance is complex, as this trait is controlled by many genes (Mitra, 2001). A phenotypic assessment, combined with the use of genomic tools, is essential to a more precise knowledge of the response of a crop to drought stress. Morphological and physiological changes in the plants can be determined visually when plants are exposed to drought. However, biochemical, and genetic changes are difficult to assess visually. To overcome this problem, genomic tools and bioinformatic analyses have been used to understand the mechanisms of drought in a wide range of species, including *Ipomoea spp*. This dissertation presents a greenhouse-based study performed to do a phenotypic assessment for drought tolerance in sweetpotato and some of its wild relatives; it is also studies the relatedness of diploid and polyploid *Ipomoea spp.* within the *Batatas* complex. The use of genomic and bioinformatic tools is applied to the study of gene expression for drought tolerance, phylogenetic relationships, and population structure analysis of the *Ipomoea spp.* belonging to the *Batatas* complex using a Quantitative Reduced Representation Sequencing method.

Sweetpotato [*Ipomoea batatas* (L.) Lam.] is a hexaploid species (2n=6x=90) that belongs to the genus *Ipomoea*. With 15 other species, sweetpotato is part of the *Batatas* complex, a heterogeneous group in terms of ploidy level, with 2n=2x, 2n=4x, and 2n=6x species (Diaz et al., 1996; Khoury et al., 2015). In addition to differences in ploidy, some of the genotypes within the *Batatas* complex are morphologically very similar leading to the difficult species delimitation in this group (Austin, 1978; Kobayashi, 1983; Austin, 1988; McDonald & Austin, 1990; Duncan & Rausher, 2013). Currently, the taxonomy of the *Batatas* complex is not completely resolved, but some taxonomists recognize a total 16 species within the *Batatas* complex group (Wood et al., 2015; Muñoz-Rodríguez et al., 2018; 2019).

The possibility of identifying wild genotypes that can be used to improve drought tolerance in cultivated sweetpotato is explored in this work. Consequently, polyploidy, hybridization, and admixture are some of the concepts that are explored in this study. The study of species with different ploidy levels was inevitable in this research project, due to the complexity of the genotypes existing in the *Batatas* complex. Plant breeders have assessed wild relatives of most staple crops as sources of diversity to improve biotic and abiotic stresses in cultivated species, and they have been successful using this strategy (Reynold et al., 2007; Pour-Aboughadareh et al., 2017; Souter et al., 2017). One goal of this work was to evaluate the potential of using these wild species approach to broaden the genetic resources available to improve drought tolerance in sweetpotato.

The possibility of identifying wild *Ipomoea spp.* genotypes that are drought tolerant demands a deep thought regarding how to breed sweetpotato, due to the variable ploidy level of these species. An implication of working with species with different ploidy levels is that there is a need to balance the ploidy level between the two species before they can intermate.

Consequently, breeders may need to incorporate alternative methods or bridge species to transfer drought-tolerant genes into sweetpotato. Therefore, the integrative use of phenotypic and genomic tools becomes particularly convenient in the case of the *Batatas* complex where interspecific crossability is unlikely.

This study was conducted with the goal of identifying possible wild relatives that could be used to improve drought tolerance in sweetpotato. Improvement in the levels of drought tolerance in sweetpotato could adapt this crop to longer periods of drought, and consequently, prevent significant yield loss. This investigation explored several components of plant breeding: pre-breeding phase, germplasm availability, selection, and genotype-by-environment (G X E) interaction. This study also addresses plant breeding cross-cutting subjects, such as plant physiology, evolution, and the application of bioinformatics tools to crop improvement. These components are discussed in four chapters of this dissertation:

1. A Study of the Relatedness of Diploid and Polyploid *Ipomoea spp.* within the *Batatas* Complex Using Quantitative Reduced Representation Sequencing (qRRS)
2. Assessment of the potential of wild *Ipomoea spp.* for the improvement of drought tolerance in cultivated sweetpotato [*Ipomoea batata* (L.) Lam.]
3. Comparative transcriptome analysis of two drought-tolerant cultivars of sweetpotato, 'Beauregard' and 'Resisto'
4. Conclusions and recommendations

LITERATURE REVIEW

Drought definition

Drought is a major environmental constraint to crop productivity, causing a variable percentage of yield loss (Venuprasad et al., 2007; Barnabás et al., 2008; Daryanto et al., 2016). The variability of yield loss depends on the crop, its growth stage, the intensity of drought event, cultivar, and the genotype-by-environment (G x E) interaction (Barnabás et al., 2008; Farooq et al., 2012; Andrade et al., 2016; Daryanto et al., 2016). Drought is defined differently depending on the context of the drought event. These contexts can be agricultural, meteorological, hydrological, and socio-economical (Dracup et al., 1980; Wilhite & Glantz, 1985; Mckee et al., 1993; Hisdal et al., 2000; Keyantash & Dracup, 2002; Lloyd-Hughes, 2014). The current review focuses on the concept of agricultural drought.

Agricultural drought is defined as an event of reduced water availability from the soil to the plant, which results in an occasional or definitive interruption of the normal cycle of development of the plant, depending on the extent of the water limitation (Passioura, 1996; Anjum et al., 2011). The phenomenon of climate change is characterized by events such as higher temperatures, frequent floods, and long periods of drought (Adams et al., 1998; Rounsevell et al., 1999; Hatfield et al., 2011; Deryng et al., 2014). With the climate changes predicted to occur during global warming in the future, there is a need for agricultural strategies to face longer periods of water scarcity.

There are drought-tolerant and drought-sensitive crops; however, even for those drought-tolerant species, extended periods of drought may result in negative effects on crop production. For instance, rice is grown primarily on well-irrigated conditions, and when it is grown in rain-fed systems, the yield loss can reach approximately 40-65% (Fukai & Cooper, 1995 Venuprasad;

et al., 2007; Farooq et al., 2009b). Sorghum has been grown in arid and semi-arid areas (Rosenow et al., 1983; Hattori et al., 2005); nevertheless, it can still suffer up to approximately 35-55% of yield reduction due to drought (Garrity et al., 1982; Assefa et al., 2010). Both drought-sensitive and drought-tolerant crops may benefit from agricultural strategies to cope with extended periods of drought. A possible agricultural adaptation is the adoption (growth) of crops that can tolerate long periods of drought. Alternatively, another strategy is the improvement of levels of drought tolerance in drought-sensitive cultivars.

Mechanisms of drought tolerance

A combination of morpho-physiological, biochemical, and molecular alterations is typically observed in plants when they are subjected to drought and plants use these mechanisms to resist water stress. Drought tolerance is associated with the following mechanisms: dehydration avoidance, drought escape, and dehydration tolerance (Hall & Schulze, 1980; Mitra et al., 2001; Price et al., 2002; Lawlor, 2013; Reddy, 2019). Though it is an underexplored strategy, one more drought tolerance mechanism has been cited: drought recovery/survival (Lou et al., 2010; Lawlor, 2013; Fang & Xion, 2015). A plant can exploit more than one of these mechanisms to resist drought.

Drought escape is a mechanism that is usually used by plants grown in drought-prone environments. Drought escape consists of reducing its life cycle, enabling the plant to complete its reproductive cycle before the environment becomes dry (Mitra el at., 2001; Lawlor, 2013; Price et al., 2002; Azhar & Rehman, 2018). For example, a short flowering time before the dry season is a strategy that plants use to avoid drought in genotypes with a short lifetime (Azhar & Rehman, 2018). Kooyers et al. (2015) and Lawlor (2013) describe the plants that circumvent drought through temporal escape mechanisms as plants having an annual cycle and with a very

efficient photosynthetic capacity. In rice, a genetic dissection study to evaluate quantitative trait loci (QTL) for associated traits when the plant was under drought showed a faster flowering time as a strategy to cope with drought (Xu et al., 2005).

Dehydration avoidance is a strategy in which plants try to keep as much water as they can under drought circumstances by avoiding water loss (stomatal closure) or by developing roots that can explore deeper soil distances (Mitra et al., 2001; Price et al., 2002; Yue et al., 2006; Lou et al., 2010; Lawlor, 2013). Annual, biennial, and perennial plants with a high water-use efficiency establish dehydration avoidance as a strategy to tolerate drought (Lawlor, 2013; Kooyers et al., 2015). In a study aiming to understand the genetic basis of drought resistance at the reproductive stage in rice, the authors identified QTLs associated with root traits, which correlated with the rapid development of the roots (Yue et al., 2006). These results suggest that the roots traits were correlated with dehydration avoidance.

Dehydration tolerance is defined as an osmotic adjustment created by the plant so that it continues to perform its function even with a low leaf relative water content (RWC) (Yue et al., 2006; Lou et al., 2010; Lawlor, 2013; Fang & Xiong, 2015; Azhar & Rehman, 2018). The osmotic adjustment ensures the cell turgor of the plant and an increase of reactive oxygen species (ROS)-scavenging enzymes. This strategy is applied by biannual and perennial plants (Kooyers et al., 2015). Xu et al. (2015) observed a delayed flowering time in rice, and they hypothesized that the delay could be the strategy of the plant to survive under stress; these authors also assumed that the flowering could occur once the plant was re-irrigated. In another study on rice, it was suggested that dehydration tolerance could be a strategy that plants used to resist drought due to osmotic adjustment and maintenance of cell-membrane stability (Yue et al., 2006).

Drought recovery/survival is a mechanism in which a plant seems irreversibly damaged by stress due to an event of severe drought but resumes its normal metabolism once the stem is re-hydrated (Lou et al., 2010; Lawlor, 2013; Fang & Xion, 2015). In fact, it is difficult to identify a plant that has experienced this strategy, as the recovery time can vary according to the genotype and the duration of the drought. Nhanala & Yencho (2020) reported that completely dry and apparently dead wild *Ipomoea spp.* could resume their full metabolism when those plants were re-irrigated after a period of severe drought; however, the ability to recover was lost when the same *Ipomoea spp.* were submitted to extreme drought. *Craterostigma plantagineum*, known as a resurrection plant, has been studied as a model species and is considered a source of desiccation tolerance genes, due to its capacity to resume its normal metabolism after it becomes desiccated (Bartels et al., 1990; Piatkowski et al., 1990; Bartels et al., 1992; Rodriguez et al., 2010; Zhang & Bartels, 2018). *Craterostigma plantagineum* has been considered for the improvement of plants to resist drought tolerance, via genetic engineering, due to the capacity of this species to recover from drought.

In conclusion, drought is a complex trait that demands the evaluation of several parameters to be understood. Plant genotype, environment, age, and the duration of the drought event are some aspects to consider when assessing the performance of a plant under drought conditions. Therefore, evaluating of the genotype in the location where the plants will be cultivated is critical, as different environments may reflect different results. Combined morphological, physiological, biochemical, and molecular analyses may be an efficient strategy to evaluate drought tolerance and assess the plant's adaptability to periods of drought. When evaluating the performance of a specific crop in a particular environment, all aspects such as age and genotype should be considered. Inferring the performance of a genotype based on studies

done in different environmental conditions is not recommended, as a cultivar's performance may reveal unexpected results.

Intensity of the drought event

The intensity of water scarcity plays a role in the damage that will result from drought stress. Depending on the restriction level, water stress may be classified into different intensities, such as moderate, severe, and extreme (Deblonde & Ledent, 2001; Liu et al., 2004; Samarah, 2005; Yadollahi et al., 2011). However, the extent of drought-induced damage that affects each crop depends on the genotype response. As mentioned before, rice and sorghum will reach their moderate, severe, and extreme stress points at a variable interval of time (days). Moderate drought may cause less damage than severe drought. Depending on the degree of the damage, the effects caused by each intensity may not be reversible.

In addition to the intensity of drought, the stage of plant growth can also determine the level of damage that the plant will suffer. In general, well-developed plants tolerate drought better than young plants, and more mature plants can recover from the damage faster than young plants. Young plants are often more susceptible to drought because they do not have enough reserves of carbohydrates. Mature plants may tolerate more prolonged periods of drought because their metabolic activities are fully functional, and they also have a source of reserves. Thus, if plants of the same genotype are compared from different ages and under the same intensity of drought, mature plants probably tolerate drought better than younger plants. Depending on the damage, young plants could die, while the old ones could survive the drought event. In sweetpotato, plant age is an important drought tolerance factor. Mature plants of sweetpotato have more storage root mass than young ones; therefore, old sweetpotato plants may be able to tolerate drought better than young plants, as the younger plants might not have a

similar reserve of carbohydrates (Nhanala & Yencho, 2020). In wheat, drought tolerance is negatively correlated to plant age. Blum & Ebercon (1981) showed that in two cultivars of wheat, the percentage of injury caused by drought rose with the increasing of number of days after emergence. These contrasting responses to drought in sweetpotato and wheat show that plants will be affected by drought in different ways. These effects will be discussed below.

Effects of drought on plants

Yield loss is one of the major impacts of drought in plants. This loss is triggered by previous morpho-physiological, biochemical, and molecular effects caused by insufficient water in plants (Valliyodan & Nguyen, 2006; Shinozaki & Yamaguchi-Shinozaki, 2007; Anjum et al., 2011; Jiménez et al., 2013; Li & Liu, 2016; Kumar et al., 2018). These morpho-physiological, biochemical, and molecular alterations affect plants in different ways, which are discussed below.

Morpho-physiological alterations

The first signs of drought in a plant are visual. These visuals signs include wilting leaves, leaf senescence from the base to the top, and eventual stem desiccation (Munné-Bosch & Alegre, 2004; Rivero et al., 2007). Decreased leaf area, relative water content, stomatal conductance, and photosynthesis are some of the alterations caused by drought stress, leading to eventual plant desiccation (Reddy et al., 2004; Anjum et al., 2011; Basu et al., 2016; Kumar et al., 2018). Alterations due to drought stress affect plants in different ways. In wheat, the grain yield was reduced due to the reduction of the leaf water potential when the plants were subjected to drought (Fischer & Maurer, 1978). Photosynthesis and dry weight were also reduced in wheat in plants that suffered dehydration (Loggini et al., 1999). In beans, parameters associated with photosynthesis (e.g., stomatal conductance, chlorophyll fluorescence, photosynthetic rate,

resistance to photoinhibition) were affected when plants were grown under drought conditions (Miyashita et al., 2005; Lizana et al., 2006; Santos et al., 2009). These studies showed that the decrease of these photosynthetic parameters under drought varies according to the genotype (Lizana et al., 2006; Santos et al., 2009). Growth and yield were reduced, even in drought-tolerant genotypes, when those plants were exposed to water stress (Bolaños & Edmeades, 1993; Kamara et al., 2003). These studies show how drought can reduce the yield of staple crops and that these losses may contribute to food security instability, depending on the severity and region.

Relative water content. The relative water content (RWC) of a plant depends on the balance of the volume of water and water lost via transpiration (Nguyen et al., 1997; Anjum et al., 2011; Zlatev & Lidon, 2012; Jiménez et al., 2013). When plants sense limited availability of water, they respond to hydric stress by preventing water loss via transpiration. Consequently, plants reduce stomatal conductance to avoid water loss, and these changes decrease the RWC of the plant and trigger the loss of cell turgor (Nxele, et al., 2017). When the RWC induces the loss of cell turgor, it is possible to "visualize" plant's effects of drought stress. Leaves wilt when the cell turgor is affected by drought stress (Ritchie et al., 1990; Kawasaki et al., 2000, Ober et al., 2005; Nxele et al., 2017). Wilted leaves can be recovered and can respond positively to re-irrigation if wilting is identified in its initial stage. The time needed to a plant to recover from wilting may vary according to species and cultivar (Briggs & Shantz, 1912). Some plants may recover from the wilting minutes after re-irrigation, while others may need several hours to recover after the re-irrigation.

The RWC directly impacts a plant's tolerance to water stress and how long it takes to express drought symptoms. The re-irrigation of wilting plants that have been under water

deprivation for an extended period may not prevent leaf senescence, and once a leaf begins senescing, that leaf will eventually drop off. Generally, a plant that experiences a slow change in RWC can still maintain its cell turgor for an extended period. Slow loss of cell turgor can be understood as a gradual change in the RWC of a plant and implying tolerance to drought (Ober, et al., 2005). However, when a slow response is associated with drought tolerance, it may be challenging to identify a critical phase when the re-irrigation can still result in a positive reaction, such as leaf recovery.

Photosynthesis. Photosynthesis results in the production of carbohydrates in a plant. The major factors affecting photosynthesis are water availability, carbon dioxide (CO_2) uptake, and sunlight (Reddy et al., 2004; Anjum et al., 2011; Zlatev & Lidon, 2012), which means that if any of these factors are limited, photosynthesis will be negatively affected. Flexas et al. (2004), Reddy et al. (2004), Cruz de Carvalho (2008), and Chaves et al. (2009) consider drought the main factor limiting photosynthesis. When stomates close or reduce transpiration to prevent water loss, and when a plant is under water stress, photosynthetic activity is decreased. Stomatal closure decreases the uptake of CO_2 that will be converted into carbohydrates. Photosynthetic pigments (chlorophyll) will also be affected due to alterations in the chloroplast.

Stomatal conductance. Stomatal closure is one of the first effects of drought in plants, and the immediate consequence of stomatal closure is a limitation in gas-exchange between the plant and the environment (Kawasaki et al., 2000; Ober et al., 2005; Anjum et al., 2011; Zlatev & Lidon, 2012). With low CO_2 and limited water availability, the photosynthetic activity in the plant becomes diminished, or, during an extended period of stress, photosynthesis can cease. Photosynthesis may recover when the plant is re-irrigated. After re-irrigation of drought-stressed plants, the stomatal conductance can reach the initial level, which means that the gas-exchange

capacity can return to plants not undergoing drought stress. Nhanala & Yencho (2020) observed a recovery of stomatal conductance after the re-irrigation of *Ipomoea spp.* The recovery speed was proportional to the duration of drought. The longer the period of drought, the greater the leaf loss, and, therefore, the greater the loss of leaf area to perform photosynthesis. The restoration of stomatal conductance will also impact the plant turgor. Wilted plants may recover the leaf turgor after re-irrigation, since the stomata reopen when they recognize that the water balance has been re-established.

Chlorophyll content. Photosynthesis occurs in chloroplasts, the structures where the chlorophyll pigments are located (Blankenship, 2014). Chlorophyll pigments respond to sunlight. Chlorophylls act as "antennas" that capture sunlight to be used during photosynthetic activity (Blankenship, 2014). Drought results in the accumulation of reactive oxygen species (ROS), and therefore, the occurrence of oxidative stress (damage) that can lead to cell death. The accumulation of ROS occurs in the chloroplasts and mitochondria (Cruz de Carvalho, 2008; Noctor et al., 2014, Das & Rovchoudhury, 2014; Nxele, et al., 2017). If chloroplasts are affected by drought, that reaction will be reflected in the chlorophyll content. Since stomatal closure results in the inhibition of uptake of CO_2 and will also prevent the release of oxygen, chlorophyll, which is in the chloroplasts, is affected during drought stress and, as a result, it degrades. The degradation of chlorophyll will impact the "capture" of sunlight.

Leaf senescence. Drought causes leaf senescence, and the dimension of the leaf loss is positively correlated with more extended periods of drought (Nhanala & Yencho, 2020). Although leaf senescence is part of a plant's natural growth cycle, it can also be a consequence of chlorophyll degradation (Buchanan-Wollaston, 1997), which results in early leaf senescence and reduced active foliar area for photosynthesis. Leaf senescence is an oxidative and

irreversible process that leads to leaf death (Buchanan-Wollaston, 1997; Munné-Bosch & Alegre, 2004; Prochazkova & Wilhelmova, 2007; Farouk, 2011). A plant that experiences an early leaf loss performs less photosynthetic activity than it is capable of. However, leaf senescence caused by drought can be a survival mechanism of plants under stress, as the abscission of the old leaves reduces the transpiration surface of the plant (Munné-Bosch & Alegre, 2004). The abscission of the old leaves allows stressed plants to have more water available for the young leaves. Young leaves have better access to sunlight than old leaves, and in drought-stressed plants, photosynthesis is more efficient in the young leaves due to their access to sunlight. Hence, leaf senescence in drought-stressed plants can be a drought-tolerance strategy and, in contrast, can lead to the reduction of available surface for photosynthesis.

Thus far, only the effects of the aerial part of the plant have been discussed. However, water, a needed element for photosynthesis, is absorbed via the roots. This means that the alterations occurring in roots should also be considered when one studies the effects of drought in plants.

Root development. It is difficult to visualize the effects of drought in roots unless they are harvested. Still, roots are also impacted by drought conditions. Tahere et al. (2000) and Gargallo-Garriga et al. (2014) observed that roots tend to develop more (in-depth) as a response to drought. Some ways to assess the effects of drought on roots is by evaluating the shoot: root ratio and the biomass of the roots (Huang & Fry, 1998; Pace et al., 1999; Tahere et al., 2000; Makbul et al., 2011). In general, drought-stressed fibrous roots tend to develop more (in length), which is caused by their need to explore deeper soil distances to obtain water. This root growth in length to absorb water is due to the scarce humidity near the ground surface during a drought event. For that reason, the root: shoot ratio will increase if a plant is stressed. For plants that have

storage roots, the behavior of the root system may be different when a plant is affected by drought. For instance, the yield tends to be reduced for storage roots under drought conditions (Solis et al., 2014; Andrade et al., 2016). Thus, the type of species and the roots that are developed in that species should be considered before determining the methodology to evaluate the effects of drought on the roots of that genotype.

Biochemical alterations

Drought also causes biochemical alterations in plants (Zhang & Kirkham, 1994; Reddy et al., 2004; Anjum et al., 2011; Sharma et al., 2012; Kumar et al., 2018). One of the major alterations during drought is the oxidative stress caused by an increase in the generation of reactive oxygen species (ROS). Due to the accumulation of ROS, an increase of ROS-scavenging enzymes, such as superoxide dismutase (SOD), catalase (CAT), and peroxidases (POD), have been observed in response to control levels of ROS (Zhang & Kirkham, 1994; Reddy et al., 2004; Anjum et al., 2011; Rosales et al., 2012; Kumar et al., 2018). This means that SOD, CAT, and POD enzymes defend the plant against oxidative stress. In wheat, an increase of POD, SOD, and CAT activities was observed in plants undergoing drought (Hameed et al., 2013; Abid et al., 2018). An anti-oxidative response was also observed in rice (Basu et al., 2010; Wang et al., 2010; Yang et al., 2014). For beans, drought increases the production of enzymes associated with oxidative stress (Zlatev et al., 2006; Rosales et al., 2012). In maize, experiments that simulated water stress also revealed that the ROS-scavenging enzymes increase when plants are under drought (Moussa & Abdel-Aziz, 2008). In summary, ROS-scavenging enzymes tend to increase in water-deprived environments; however, the extent of this increase will depend on the genotype and severity of stress.

Reactive Oxygen Species (ROS). ROS induces oxidative stress caused by stomatal closure due to drought and a decrease of photosynthesis, which causes degradation of chlorophyll (Cruz de Carvalho, 2008). ROS are produced in the chloroplast and mitochondria. When stomata close, the gas-exchange between the plant and the environment reduces, and the plant accumulates oxygen inside the chloroplast and forms free oxygen radicals that react with chlorophyll. The free radicals are used in the increased production of ROS when the free radicals react with chlorophyll. High concentrations of ROS are toxic to the plant and cause damage that leads to cell death (Sharma et al., 2012). When the plant recognizes that it is producing excessive ROS, it activates its defense mechanism to prevent cell death by increasing ROS-scavenging enzymes' production. However, this reaction depends on the genotype (cultivar), and in general, drought-sensitive cultivars produce a smaller number of ROS-scavenging enzymes than drought-tolerant cultivars. The production of ROS-scavenging enzymes may be an interesting biochemical approach to identify drought-sensitive and drought-tolerant cultivars, but this approach should not be considered solely, as morpho-physiological and molecular approaches should be included to generate a more accurate assessment of plants' responses to drought tolerance.

Molecular alterations

It is also necessary to understand the effects of drought at a molecular level. This aspect becomes particularly valuable when there is a need to evaluate a genotype under drought conditions in multiple environments. Since the genotype defines how the plant will respond to environmental conditions, molecular alterations are essential to understand the genotype-by-environment (G x E) interaction. Molecular changes caused by drought are complex. In terms of the regulatory network in response to drought, two different pathways have mostly been

discussed: the abscisic acid (ABA)-dependent pathway and the ABA-independent pathway (Shinozaki & Yamaguchi-Shinozaki,1996; Shinozaki et al., 2003; Reddy et al., 2004; Budak et al., 2013).

ABA-dependent pathway. Drought induces genes that are involved in ABA biosynthesis, resulting in the production of ABA (Shinozaki et al., 2003). Thus, during an event of water scarcity, there is an accumulation of ABA as a response to dehydration stress (Shinozaki & Yamaguchi-Shinozaki, 1996; Ramanjulu & Bartels, 2002; Reddy et al., 2004; Joshi et al., 2016). Drought-inducible genes in the ABA-dependent pathway are assumed to have ABA-responsive elements (ABREs) in the promoter region (Shinozaki & Yamaguchi-Shinozaki, 1996). Two transcription factors bind ABRE: ABRE-binding protein (AREB) and the ABRE-binding factor (ABF), which result in the activation of the ABA-dependent gene expression (Shinozaki et al., 2003). The biosynthesis of ABA triggers the ABA-responsive genes and induces stomatal closure through the reduction of guard cell turgor (Reddy et al., 2004). As discussed earlier, stomatal closure is a process that occurs to prevent water loss during dehydration stress. This indicates that the ABA-dependent pathway plays a role in the prevention of water loss due to the biosynthesis of ABA, which leads to stomatal closure. Consequently, drought tolerance via the ABA-dependent pathway is related to ROS-scavenging enzymes and dehydration tolerance mechanism via osmotic adjustment (Budak et al., 2013). The mechanisms of dehydration tolerance are discussed later in this review. Therefore, evaluating the alterations of ABA content in the plant may be a way to assess if the plant is under stress. However, it is important to consider that ABA production also occurs because of other stresses, such as cold and salinity (Shinozaki & Yamaguchi-Shinozaki, 1996; Shinozaki et al., 2003). Hence,

evaluating ABA-related changes to study drought stress should be complemented with other drought-related alterations.

ABA-independent pathway. The ABA-independent pathway, also known as the dehydration-responsive element binding (DREB) mediated pathway, was identified based on a drought-inducible gene's promoter region to dehydration. In the promoter region, a dehydration-responsive element (DRE)/C-repeat (CRT), and the transcription factors bind to the DRE/CRT: C-repeat binding factor (CBF)/ (DRE-binding protein 2) DREB2 involved in drought-inducible gene expression (Shinozaki et al., 2003). The DREB2 genes are induced by dehydration, triggering the expression of genes involved in drought tolerance (Shinozaki et al., 2003; Budak et al., 2013). The DBRE1 transcription factor also has a role in drought stress, but this is less common (Budak et al., 2013). The DREB pathway may not be associated with ROS-scavenging, and instead, could be related to Late Embryogenesis Abundant (LEA) proteins. LEA are proteins that occur during the late period of seed development, in stems, leaves, and roots when they are submitted to drought (Hong-Bo et al., 2005; Olvera-Carrilo et al., 2011). LEA protects the cytoplasm from dehydration (Hong-Bo et al., 2005). Thus, LEA proteins have a role in dehydration tolerance by protecting the membrane stability.

This review indicates that the drought-tolerance mediated pathway is determined based on the transcription factors involved in response to drought. Hence, the roles of the transcription factors AREB, ABF, and DREB are critical to understand the mechanisms of drought tolerance in plants, and this stress is regulated at a transcriptional level. Drought tolerance is controlled by several genes and more transcription factors are involved in response to dehydration. Consequently, those transcription factors may impact lesser-known pathways.

Drought understanding at the molecular level

The evaluation of the plant's alterations at the morpho-physiological level and the assessment of the changes in biochemical levels associated with drought are some of the methodologies used in drought tolerance studies. The genetic background of an organism impacts the morphology, physiology, and biochemistry of a crop plant. Nevertheless, to understand the genetic basis of drought tolerance mechanisms, studies must be performed at a molecular level, and those studies should be associated with the morpho-physiological and biochemical traits. The genetic dissection of drought tolerance involves the use of molecular markers, such as Random Amplified Polymorphic DNA (RAPDs), Restriction Fragment Length Polymorphisms (RFLPs), Simple Sequence Repeats (SSRs), and Single Nucleotide Polymorphisms (SNPs) (Tuinstra et al., 1997; Price et al., 2002; Xu et al., 2005; Yue et al., 2006, Milad et al., 2011). These markers have been applied in drought-tolerant studies using different approaches. Some of these methods commonly used for molecular studies are reviewed below.

Quantitative trait loci (QTL) mapping has been used to dissect the genetic mechanism of drought tolerance using different molecular markers. The ability to correlate drought-tolerance traits with specific genomic regions (loci) has contributed to the genetic dissection and our understanding of drought tolerance. For instance, in maize, molecular markers have been applied to identify QTLs that influence yield components, such as grain yield, ear length, ear weight, and the number of ears when plants were submitted to drought (Agrama & Moussa, 1996; Frova et al., 1999; Tuberosa et al., 2002; Tuberosa & Salvi, 2006; Marino et al., 2009). There are several examples of staple crops in which the QTL mapping approach has been applied for drought-tolerance. These crops and respective traits are:

- potato: chlorophyll content, RWC, plant height, and fresh weights (Anithakumari et al., 2012; Khan et al., 2015)
- rice: grain yield, plant height, panicle number, root length, and root mass (Lanceras et al., 2004; Kumar et al., 2007; Bhattarai & Subudhi, 2018)
- soybean: water-use efficiency, seed yield, plant height, and days to maturity (Mian et al., 1996; Specht et al., 2001; Du et al., 2009)
- sorghum: grain yield, seed weight, and stay-green, (Tuinstra et al., 1998; Kebede et al., 2001; Sanchez et al., 2002)
- wheat: accumulation of abscisic acid, grain yield, plant height, root number, root weight, days to maturity, and grain-filling duration (Quarrie et al., 1994; Kirigwi et al., 2007; Zhang et al., 2014; Gahlaut et al., 2017; Ballesta et al., 2020)

Genome-wide association studies (GWAS) is another methodology used to study drought by identifying QTLs and candidate genes for drought tolerance. Genome-wide association studies have been used to identify candidate genes associated with drought tolerance due to the GWAS's power to detect alleles associated with a phenotype. Some of the crops where the GWAS approach has been used to identify candidate genes for drought tolerance are:

- maize: grain yield, plant height, photosynthetic efficiency, and root development (Wang et al., 2016a; Wang et al., 2016b)
- rice: grain yield (Pantaliao et al., 2016)
- wheat: grain yield, days to maturity, shoot biomass, root to shoot ratio (Mathew et al., 2019)
- chickpea: grain yield, biomass, seed weight (Li et al., 2018)

In general, GWAS is performed to achieve the same goals that are accomplished by QTL mapping or a transcriptome analysis.

Transcriptome profiling is another approach that has been used to understand the genetic mechanisms of drought tolerance. Transcriptome analysis is used to understand the gene expression of plants subjected to drought. The identification of differentially expressed genes (DEGs) during a drought event and how those genes are regulated in specific plant tissue provides a broad knowledge of the impacts of drought tolerance at different time points when the plant is under stress (Hazen et al., 2005; Yamaguchi-Shinozaki & Shinozaki, 2006). Transcriptome studies are frequently performed by evaluating the responses of the genotypes to drought and by the identification of DEGs (Marino et al., 2009; Zheng et al., 2010; Lenka et al., 2011; Moumeni et al., 2011; Huang et al., 2014; Borah et al., 2017). The identification of genes that are expressed during a drought period and their level of regulation (up-regulated or down-regulated) are approaches used to determine the type of response to drought. Transcriptomic analysis allows the identification of candidate genes for drought tolerance (Turyagyenda et al., 2013; Borah et al., 2017). For example, if it is found that genes associated with drought tolerance are up-regulated during a drought event, those genes can be identified as candidate genes to drought tolerance. The cultivars in which those genes are discovered may be recognized as drought-tolerant genotypes if they tolerate drought environments and can be used for breeding purposes.

For instance, in rice, transcriptome analysis has been performed by comparing contrasting genotypes to drought tolerance, by identifying DEGs, and by discovering candidate genes for drought tolerance (Moumeni et al., 2011; Huang et al., 2014; Borah et al., 2017). In cassava, different methodologies are used to identify expressed genes under drought conditions

(Utsumi et al., 2012; Turyagyenda et al., 2013). Maize is another staple crop in which transcriptome profiling has been used to identify DEGs under drought, and the discovery of these genes has been used for crop improvement (Marino et al., 2009; Zheng et al., 2010; Min et al., 2016). Transcriptome analysis studies are essential to identify gene expression under stress conditions, as transcriptome profiling evaluates how those genes are affected at different stages of a plant's development, as well as in different tissues of the plant. The results of transcriptome analysis are especially important, as plant species are differently affected at different stages of plant development.

In terms of methodologies and technologies used to study drought at a molecular level, there has been a constant development of new tools available to perform these studies. Due to the availability of these new tools, the approach that will be applied to a specific study has to be chosen carefully. Some of the factors to consider are the size of the population to be studied, species, plant tissue, and technological ability of the user. The use of these genomic tools, integrated with phenotypic evaluations, including biochemical and physiological approaches, provides a better understanding of the mechanisms behind drought tolerance. However, none of the tools, whether phenotypic or genomic, provide a comprehensive insight into this topic by themselves. Also, independent of the tool that is used to understand drought in plants, it should be considered the genotype-by-environment (G x E) interaction. A particular genotype may utilize one mechanism of drought tolerance in a specific environment, and in another context, the same genotype will use a different mechanism of drought tolerance. For breeding purposes, these tools can be used in marker-assisted selection (MAS). MAS use of molecular markers linked to the observed alterations in a trait at morpho-physiological and biochemical levels. Another approach in which the discovery of drought-tolerant genes can be used in crop improvement is

genetic engineering, which can be used to transfer a (drought-tolerant) gene into the genotype of interest and can prevent the risk of introgression of undesirable traits.

REFERENCES

Abid, M., Ali, S., Qi, L. K., Zahoor, R., Tian, Z., Jiang, D., Snider, J.L., & Dai, T. (2018). Physiological and biochemical changes during drought and recovery periods at tillering and jointing stages in wheat (*Triticum aestivum* L.). *Scientific reports*, *8*(1), 1-15.

Adams, R. M., Hurd, B. H., Lenhart, S., & Leary, N. (1998). Effects of global climate change on agriculture: an interpretative review. *Climate research*, *11*(1), 19-30.

Agrama, H. A., & Moussa, M. E. (1996). Mapping QTLs in breeding for drought tolerance in maize (*Zea mays* L.). *euphytica*, *91*(1), 89-97.

Andrade, M. I., Naico, A., Ricardo, J., Eyzaguirre, R., Makunde, G. S., Ortiz, R., & Grüneberg, W. J. (2016). Genotype× environment interaction and selection for drought adaptation in sweetpotato (*Ipomoea batatas* [L.] Lam.) in Mozambique. *Euphytica*, *209*(1), 261-280.

Anithakumari, A. M., Nataraja, K. N., Visser, R. G., & van der Linden, C. G. (2012). Genetic dissection of drought tolerance and recovery potential by quantitative trait locus mapping of a diploid potato population. *Molecular Breeding*, *30*(3), 1413-1429.

Anjum, S. A., Xie, X. Y., Wang, L. C., Saleem, M. F., Man, C., & Lei, W. (2011). Morphological, physiological and biochemical responses of plants to drought stress. *African journal of agricultural research*, *6*(9), 2026-2032.

Assefa, Y., Staggenborg, S. A., & Prasad, V. P. (2010). Grain sorghum water requirement and responses to drought stress: A review. *Crop Management*, *9*(1), 1-11.

Austin, D. F. (1978). The *Ipomoea batatas* complex-I. taxonomy. *Bulletin of the Torrey Botanical Club*, 114-129.

Austin, D. F. (1988). Nomenclatural changes in the *Ipomoea batatas* complex (Convolvulaceae). *Taxon*, *37*(1), 184-185

Azhar, M. T., & Rehman, A. (2018). Overview on effects of water stress on cotton plants and productivity. In *Biochemical, Physiological and Molecular Avenues for Combating Abiotic Stress Tolerance in Plants* (pp. 297-316). Academic Press.

Ballesta, P., Mora, F., & Del Pozo, A. (2020). Association mapping of drought tolerance indices in wheat: QTL-rich regions on chromosome 4A. *Scientia Agricola*, *77*(2).

Barnabás, B., Jäger, K., & Fehér, A. (2008). The effect of drought and heat stress on reproductive processes in cereals. *Plant, cell & environment*, *31*(1), 11-38.

Bartels, D., Hanke, C., Schneider, K., Michel, D., & Salamini, F. (1992). A desiccation-related Elip-like gene from the resurrection plant *Craterostigma plantagineum* is regulated by light and ABA. *The EMBO journal*, *11*(8), 2771-2778.

Bartels, D., Schneider, K., Terstappen, G., Piatkowski, D., & Salamini, F. (1990). Molecular cloning of abscisic acid-modulated genes which are induced during desiccation of the resurrection plant *Craterostigma plantagineum*. *Planta*, *181*(1), 27-34.

Basu, S., Roychoudhury, A., Saha, P. P., & Sengupta, D. N. (2010). Differential antioxidative responses of indica rice cultivars to drought stress. *Plant Growth Regulation*, *60*(1), 51.

Basu, Supratim, Venkategowda Ramegowda, Anuj Kumar, and Andy Pereira. (2016). "Plant adaptation to drought stress." *F1000Research* 5.

Bhattarai, U., & Subudhi, P. K. (2018). Identification of drought responsive QTLs during vegetative growth stage of rice using a saturated GBS-based SNP linkage map. *Euphytica*, *214*(2), 38.

Blankenship, R. E. (2014). *Molecular mechanisms of photosynthesis*. (pp. 1-9) John Wiley & Sons.

Blum, A., & Ebercon, A. (1981). Cell membrane stability as a measure of drought and heat tolerance in wheat 1. *Crop Science*, *21*(1), 43-47.

Bolaños, J., & Edmeades, G. O. (1993). Eight cycles of selection for drought tolerance in lowland tropical maize. I. Responses in grain yield, biomass, and radiation utilization. *Field Crops Research*, *31*(3-4), 233-252.

Borah, P., Sharma, E., Kaur, A., Chandel, G., Mohapatra, T., Kapoor, S., & Khurana, J. P. (2017). Analysis of drought-responsive signalling network in two contrasting rice cultivars using transcriptome-based approach. *Scientific reports*, *7*, 42131.

Briggs, L. J., & Shantz, H. L. (1912). *The wilting coefficient for different plants: and its indirect determination* (No. 230). US Government Printing Office.

Buchanan-Wollaston, V. (1997). The molecular biology of leaf senescence. *Journal of experimental botany*, *48*(2), 181-199.

Budak, H., Kantar, M., & Yucebilgili Kurtoglu, K. (2013). Drought tolerance in modern and wild wheat. *The Scientific World Journal*, *2013*.

Carnicer, J., Coll, M., Ninyerola, M., Pons, X., Sánchez, G., & Peñuelas, J. (2011). Widespread crown condition decline, food web disruption, and amplified tree mortality with increased climate change-type drought. *Proceedings of the National Academy of Sciences*, *108*(4), 1474-1478.

Chaves, M. M., Flexas, J., & Pinheiro, C. (2009). Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. *Annals of botany*, *103*(4), 551-560.

Cruz de Carvalho M. H. (2008). Drought stress and reactive oxygen species: Production, scavenging and signaling. *Plant signaling & behavior*, *3*(3), 156–165. https://doi.org/10.4161/psb.3.3.5536

Daryanto, S., Wang, L., & Jacinthe, P. A. (2016). Global synthesis of drought effects on maize and wheat production. *PloS one*, *11*(5), e0156362.

Das, K., & Roychoudhury, A. (2014). Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. *Frontiers in environmental science*, *2*, 53.

Deblonde, P. M. K., & Ledent, J. F. (2001). Effects of moderate drought conditions on green leaf number, stem height, leaf length and tuber yield of potato cultivars. *European Journal of Agronomy*, *14*(1), 31-41.

Deryng, D., Conway, D., Ramankutty, N., Price, J., & Warren, R. (2014). Global crop yield response to extreme heat stress under multiple climate change futures. *Environmental Research Letters*, *9*(3), 034011.

Diaz, J., Schmiediche, P., & Austin, D. F. (1996). Polygon of crossability between eleven species of Ipomoea: section *Batatas* (Convolvulaceae). *Euphytica*, *88*(3), 189-200.

Dracup, J. A., Lee, K. S., & Paulson Jr, E. G. (1980). On the definition of droughts. *Water resources research*, *16*(2), 297-302.

Du, W., Wang, M., Fu, S., & Yu, D. (2009). Mapping QTLs for seed yield and drought susceptibility index in soybean (*Glycine max* L.) across different environments. *Journal of Genetics and Genomics*, *36*(12), 721-731.

Duncan, T. M., & Rausher, M. D. (2013). Morphological and genetic differentiation and reproductive isolation among closely related taxa in the Ipomoea series *Batatas*. *American Journal of Botany*, *100*(11), 2183-2193.

Fang, Y., & Xiong, L. (2015). General mechanisms of drought response and their application in drought resistance improvement in plants. *Cellular and molecular life sciences*, *72*(4), 673-689.

FAOSTAT. (2021) – Sweetpotato production in 2019 (http://www.fao.org/faostat/en/#data/QC , accessed on March 1, 2021).

Farooq, M., Hussain, M., Wahid, A., & Siddique, K. H. M. (2012). Drought stress in plants: an overview. In *Plant responses to drought stress* (pp. 1-33). Springer, Berlin, Heidelberg.

Farooq, M., Wahid, A., Lee, D. J., Ito, O., & Siddique, K. H. (2009). Advances in drought resistance of rice. *Critical Reviews in Plant Sciences*, *28*(4), 199-217.

Farouk, S. (2011). Ascorbic acid and α-tocopherol minimize salt-induced wheat leaf senescence. *Journal of Stress Physiology & Biochemistry*, *7*(3).

Fischer, R. A., & Maurer, R. (1978). Drought resistance in spring wheat cultivars. I. Grain yield responses. *Australian Journal of Agricultural Research*, *29*(5), 897-912.

Flexas, J., Bota, J., Loreto, F., Cornic, G., & Sharkey, T. D. (2004). Diffusive and metabolic limitations to photosynthesis under drought and salinity in C3 plants. *Plant biology*, *6*(3), 269-279.

Frova, C., Krajewski, P., Di Fonzo, N., Villa, M., & Sari-Gorla, M. (1999). Genetic analysis of drought tolerance in maize by molecular markers I. Yield components. *Theoretical and Applied Genetics*, *99*(1-2), 280-288.

Fukai, S., & Cooper, M. (1995). Development of drought-resistant cultivars using physiomorphological traits in rice. *Field Crops Research*, *40*(2), 67-86.

Gahlaut, V., Jaiswal, V., Tyagi, B. S., Singh, G., Sareen, S., Balyan, H. S., & Gupta, P. K. (2017). QTL mapping for nine drought-responsive agronomic traits in bread wheat under irrigated and rain-fed environments. *PloS one*, *12*(8), e0182857.

Gargallo-Garriga, A., Sardans, J., Pérez-Trujillo, M., Rivas-Ubach, A., Oravec, M., Vecerova, K., Urban, O., Jentsch, A., Kreyling, J., Beierkhuhnlein, C., & Parella, T. (2014). Opposite metabolic responses of shoots and roots to drought. *Scientific reports*, *4*, 6829.

Garrity, D. P., Watts, D. G., Sullivan, C. Y., & Gilley, J. R. (1982). Moisture Deficits and Grain Sorghum Performance: Evapotranspiration-Yield Relationships 1. *Agronomy Journal*, *74*(5), 815-820.

Gizaw, M. S., & Gan, T. Y. (2017). Impact of climate change and El Niño episodes on droughts in sub-Saharan Africa. *Climate Dynamics*, *49*(1-2), 665-682.

Hall, A. E., & Schulze, E. (1980). Drought effects on transpiration and leaf water status of cowpea in controlled environments. *Functional Plant Biology*, *7*(2), 141-147.

Hameed, A., Goher, M., & Iqbal, N. (2013). Drought induced programmed cell death and associated changes in antioxidants, proteases, and lipid peroxidation in wheat leaves. *Biologia Plantarum*, *57*(2), 370-374.

Hatfield, J. L., Boote, K. J., Kimball, B. A., Ziska, L. H., Izaurralde, R. C., Ort, D., Thomson, A. M. & Wolfe, D. (2011). Climate impacts on agriculture: implications for crop production. *Agronomy journal*, *103*(2), 351-370.

Hattori, T., Inanaga, S., Araki, H., An, P., Morita, S., Luxová, M., & Lux, A. (2005). Application of silicon enhanced drought tolerance in Sorghum bicolor. *Physiologia Plantarum*, *123*(4), 459-466.

Hazen, S. P., Pathan, M. S., Sanchez, A., Baxter, I., Dunn, M., Estes, B., Chang, H.S., Zhu, T., Kreps, J.A., & Nguyen, H. T. (2005). Expression profiling of rice segregating for drought tolerance QTLs using a rice genome array. *Functional & Integrative Genomics*, *5*(2), 104-116.

Hisdal, H., Tallaksen, L. M., Peters, E., Stahl, K., & Zaidman, M. (2000). Drought event definition. *ARIDE Technical Rep*, *6*, 15.

Hong-Bo, S., Zong-Suo, L., & Ming-An, S. (2005). LEA proteins in higher plants: structure, function, gene expression and regulation. *Colloids and surfaces B: Biointerfaces*, *45*(3-4), 131-135.

Huang, B., & Fry, J. D. (1998). Root anatomical, physiological, and morphological responses to drought stress for tall fescue cultivars. *Crop science*, *38*(4), 1017-1022.

Huang, L., Zhang, F., Wang, W., Zhou, Y., Fu, B., & Li, Z. (2014). Comparative transcriptome sequencing of tolerant rice introgression line and its parents in response to drought stress. *BMC genomics*, *15*(1), 1-16.

Jiménez, S., Dridi, J., Gutiérrez, D., Moret, D., Irigoyen, J. J., Moreno, M. A., & Gogorcena, Y. (2013). Physiological, biochemical and molecular responses in four Prunus rootstocks submitted to drought stress. *Tree physiology*, *33*(10), 1061-1075.

Joshi, R., Wani, S. H., Singh, B., Bohra, A., Dar, Z. A., Lone, A. A., Pareek, A. & Singla-Pareek, S. L. (2016). Transcription factors and plants response to drought stress: current understanding and future directions. *Frontiers in Plant Science*, *7*, 1029.

Kamara, A. Y., Menkir, A., Badu-Apraku, B., & Ibikunle, O. (2003). The influence of drought stress on growth, yield and yield components of selected maize genotypes. *The journal of agricultural science*, *141*(1), 43.

Kaur, G., & Asthir, B. (2017). Molecular responses to drought stress in plants. *Biologia Plantarum*, *61*(2), 201-209.

Kawasaki, S., Miyake, C., Kohchi, T., Fujii, S., Uchida, M., & Yokota, A. (2000). Responses of wild watermelon to drought stress: accumulation of an ArgE homologue and citrulline in leaves during water deficits. *Plant and Cell Physiology*, *41*(7), 864-873.

Kebede, H., Subudhi, P. K., Rosenow, D. T., & Nguyen, H. T. (2001). Quantitative trait loci influencing drought tolerance in grain sorghum (*Sorghum bicolor* L. Moench). *Theoretical and Applied Genetics*, *103*(2-3), 266-276.

Keyantash, J., & Dracup, J. A. (2002). The quantification of drought: an evaluation of drought indices. *Bulletin of the American Meteorological Society*, *83*(8), 1167-1180.

Khan, M. A., Saravia, D., Munive, S., Lozano, F., Farfan, E., Eyzaguirre, R., & Bonierbale, M. (2015). Multiple QTLs linked to agro-morphological and physiological traits related to drought tolerance in potato. *Plant molecular biology reporter*, *33*(5), 1286-1298.

Khoury, C. K., Heider, B., Castañeda-Álvarez, N. P., Achicanoy, H. A., Sosa, C. C., Miller, R. E., Scotland, R. W., Wood, J. R., Rossel, G., Eserman, L.A., Jarret, R. L., Yencho, G. C., Bernau, V., Juarez, H., Sotelo, S., Haan, S., Struil, P. C. (2015). Distributions, ex situ conservation priorities, and genetic resource potential of crop wild relatives of sweetpotato [*Ipomoea batatas* (L.) Lam., I. series Batatas]. *Frontiers in Plant Science*, *6*, 251.

Kirigwi, F. M., Van Ginkel, M., Brown-Guedira, G., Gill, B. S., Paulsen, G. M., & Fritz, A. K. (2007). Markers associated with a QTL for grain yield in wheat under drought. *Molecular Breeding*, *20*(4), 401-413.

Kivuva, B. M., Githiri, S. M., Yencho, G. C., & Sibiya, J. (2015). Screening sweetpotato genotypes for tolerance to drought stress. *Field Crops Research*, *171*, 11-22.

Knapp, A. K., Hoover, D. L., Wilcox, K. R., Avolio, M. L., Koerner, S. E., La Pierre, K. J., Loik, M.E., Luo, Y., Sala, O.E. & Smith, M. D. (2015). Characterizing differences in precipitation regimes of extreme wet and dry years: implications for climate change experiments. *Global change biology*, *21*(7), 2624-2633.

Kobayashi, M. (1983). The *Ipomoea trifida* complex closely related to sweet potato. In *Proceedings, Sixth Symposium of the International Society for Tropical Root Crops/hosted by CIP in Lima, Peru* (pp. 21-26).

Kooyers, N. J. (2015). The evolution of drought escape and avoidance in natural herbaceous populations. *Plant Science*, *234*, 155-162.

Kumar, R., Venuprasad, R., & Atlin, G. N. (2007). Genetic analysis of rainfed lowland rice drought tolerance under naturally-occurring stress in eastern India: heritability and QTL effects. *Field Crops Research*, *103*(1), 42-52.

Kumar, S., Sachdeva, S., Bhat, K. V., & Vats, S. (2018). Plant responses to drought stress: Physiological, biochemical and molecular basis. In *Biotic and abiotic Stress tolerance in plants* (pp. 1-25). Springer, Singapore.

Lanceras, J. C., Pantuwan, G., Jongdee, B., & Toojinda, T. (2004). Quantitative trait loci associated with drought tolerance at reproductive stage in rice. *Plant physiology*, *135*(1), 384-399.

Laurie, S., Faber, M., Adebola, P., & Belete, A. (2015). Biofortification of sweet potato for food and nutrition security in South Africa. *Food Research International*, *76*, 962-970.

Lawlor, D. W. (2013). Genetic engineering to improve plant performance under drought: physiological evaluation of achievements, limitations, and possibilities. *Journal of experimental botany*, *64*(1), 83-108.

Lenka, S. K., Katiyar, A., Chinnusamy, V., & Bansal, K. C. (2011). Comparative analysis of drought-responsive transcriptome in Indica rice genotypes with contrasting drought tolerance. *Plant biotechnology journal*, *9*(3), 315-327.

Li, X., & Liu, F. (2016). Drought stress memory and drought stress tolerance in plants: biochemical and molecular basis. In *Drought Stress Tolerance in Plants, Vol 1* (pp. 17-44). Springer, Cham.

Li, Y., Ruperao, P., Batley, J., Edwards, D., Khan, T., Colmer, T. D., Pang, J., Siddique, K.H. & Sutton, T. (2018). Investigating drought tolerance in chickpea using genome-wide association mapping and genomic selection based on whole-genome resequencing data. *Frontiers in Plant Science*, *9*, 190.

Liu, H. S., Li, F. M., & Xu, H. (2004). Deficiency of water can enhance root respiration rate of drought-sensitive but not drought-tolerant spring wheat. *Agricultural Water Management*, *64*(1), 41-48.

Lizana, C., Wentworth, M., Martinez, J. P., Villegas, D., Meneses, R., Murchie, E. H., Pastenes, C., Lercari, B., Vernieri, P., Horton, P. & Pinto, M. (2006). Differential adaptation of two varieties of common bean to abiotic stress: I. Effects of drought on yield and photosynthesis. *Journal of Experimental botany*, *57*(3), 685-697.

Lloyd-Hughes, B. (2014). The impracticality of a universal drought definition. *Theoretical and Applied Climatology*, *117*(3-4), 607-611.

Loggini, B., Scartazza, A., Brugnoli, E., & Navari-Izzo, F. (1999). Antioxidative defense system, pigment composition, and photosynthetic efficiency in two wheat cultivars subjected to drought. *Plant physiology*, *119*(3), 1091-1100.

Low, J. W., Ortiz, R., Vandamme, E., Andrade, M., Biazin, B., & Grüneberg, W. J. (2020). Nutrient-dense orange-fleshed sweetpotato: advances in drought-tolerance breeding and understanding of management practices for sustainable next-generation cropping systems in sub-Saharan Africa. *Frontiers in Sustainable Food Systems*. 4, 50.

Low, J., Lynam, J., Lemaga, B., Crissman, C., Barker, I., Thiele, G., G., Namanda, S., Wheatley, C. & Andrade, M. (2009). Sweetpotato in Sub-Saharan Africa. In *The sweetpotato* (pp. 359-390). Springer, Dordrecht.

Makbul, S., GÜLER, N. S., DURMUŞ, N., & GÜVEN, S. (2011). Changes in anatomical and physiological parameters of soybean under drought stress. *Turkish Journal of Botany*, *35*(4), 369-377.

Marino, R., Ponnaiah, M., Krajewski, P., Frova, C., Gianfranceschi, L., Pè, M. E., & Sari-Gorla, M. (2009). Addressing drought tolerance in maize by transcriptional profiling and mapping. *Molecular Genetics and Genomics*, *281*(2), 163-179.

Mathew, I., Shimelis, H., Shayanowako, A. I. T., Laing, M., & Chaplot, V. (2019). Genome-wide association study of drought tolerance and biomass allocation in wheat. *PloS one*, *14*(12), e0225383.

McDonald, J. A., & Austin, D. F. (1990). Changes and additions in Ipomoea section *Batatas* (Convolvulaceae). *Brittonia*, *42*(2), 116-120.

McKee, T. B., Doesken, N. J., & Kleist, J. (1993). The relationship of drought frequency and duration to time scales. In *Proceedings of the 8th Conference on Applied Climatology* (Vol. 17, No. 22, pp. 179-183).

Mian, M. A. R., Bailey, M. A., Ashley, D. A., Wells, R., Carter Jr, T. E., Parrott, W. A., & Boerma, H. R. (1996). Molecular markers associated with water use efficiency and leaf ash in soybean. *Crop Science*, *36*(5), 1252-1257.

Milad, S. I., Wahba, L. E., & Barakat, M. N. (2011). Identification of RAPD and ISSR Markers Associated with Flag Leaf Senescence under Water-stressed Conditions in Wheat ('Triticum aestivum'L.). *Australian Journal of Crop Science*, *5*(3), 337.

Min, H., Chen, C., Wei, S., Shang, X., Sun, M., Xia, R., Liu, X., Hao, D., Chen, H., & Xie, Q. (2016). Identification of drought tolerant mechanisms in maize seedlings based on transcriptome analysis of recombination inbred lines. *Frontiers in Plant Science*, *7*, 1080.

Minot, N. (2010). *Staple food prices in Malawi* (No. 1093-2016-87869). (https://ageconsearch.umn.edu/record/58558 , accessed on October 30, 2020).

Mitra, J. (2001). Genetics and genetic improvement of drought resistance in crop plants. *Current science*, 758-763.

Miyashita, K., Tanakamaru, S., Maitani, T., & Kimura, K. (2005). Recovery responses of photosynthesis, transpiration, and stomatal conductance in kidney bean following drought stress. *Environmental and experimental botany*, *53*(2), 205-214.

Moumeni, A., Satoh, K., Kondoh, H., Asano, T., Hosaka, A., Venuprasad, R., Serraj, R., Kumar, A., Leung, H. & Kikuchi, S. (2011). Comparative analysis of root transcriptome profiles of two pairs of drought-tolerant and susceptible rice near-isogenic lines under different drought stress. *BMC plant biology*, *11*(1), 1-17.

Moussa, H. R., & Abdel-Aziz, S. M. (2008). Comparative response of drought tolerant and drought sensitive maize genotypes to water stress. *Australian Journal of Crop Science*, *1*(1), 31-36.

Munné-Bosch, S., & Alegre, L. (2004). Die and let live leaf senescence contributes to plant survival under drought stress. *Functional Plant Biology*, *31*(3), 203-216.

Muñoz-Rodriguez, P., Carruthers, T., Wood, J. R., Williams, B. R., Weitemier, K., Kronmiller, B., Ellis, D., Anglin, N.L., Longway, L., Harris, S.A. and Rausher, M.D., & Scotland, R. W. (2018). Reconciling conflicting phylogenies in the origin of sweet potato and dispersal to Polynesia. *Current Biology*, *28*(8), 1246-1256.

Muñoz-Rodríguez, P., Carruthers, T., Wood, J. R., Williams, B. R., Weitemier, K., Kronmiller, B., Goodwin, Z., Sumadijaya, A., Anglin, N.L., Filer, D. and Harris, D., & Scotland, R. W. (2019). A taxonomic monograph of Ipomoea integrated across phylogenetic scales. *Nature plants*, *5*(11), 1136-1144.

Nguyen, H. T., Babu, R. C., & Blum, A. (1997). Breeding for drought resistance in rice: physiology and molecular genetics considerations. *Crop Science*, *37*(5), 1426-1434.

Nhanala, S. E. C., & Yencho, G. C. (2020). Assessment of the Potential of Wild *Ipomoea spp.* for the Improvement of Drought Tolerance in Cultivated Sweetpotato *Ipomoea batatas* (L.) Lam. Crop Science. 1-16. https://doi.org/10.1002/csc2.20363

Noctor, G., Mhamdi, A., & Foyer, C. H. (2014). The roles of reactive oxygen metabolism in drought: not so cut and dried. *Plant physiology*, *164*(4), 1636-1648.

Nxele, X., Klein, A., & Ndimba, B. K. (2017). Drought and salinity stress alters ROS accumulation, water retention, and osmolyte content in sorghum plants. *South African Journal of Botany*, *108*, 261-266.

Ober, E. S., Le Bloa, M., Clark, C. J., Royal, A., Jaggard, K. W., & Pidgeon, J. D. (2005). Evaluation of physiological traits as indirect selection criteria for drought tolerance in sugar beet. *Field Crops Research*, *91*(2-3), 231-249.

Olvera-Carrillo, Y., Luis Reyes, J., & Covarrubias, A. A. (2011). Late embryogenesis abundant proteins: versatile players in the plant adaptation to water limiting environments. *Plant signaling & behavior*, *6*(4), 586-589.

Pace, P. F., Cralle, H. T., El-Halawany, S. H., Cothren, J. T., & Senseman, S. A. (1999). Drought-induced changes in shoot and root growth of young cotton plants. *J. Cotton Sci*, *3*(4), 183-187.

Padmaja, G. (2009). Uses and nutritional data of sweetpotato. In *The sweetpotato* (pp. 189-234). Springer, Dordrecht.

Pantaliao, G. F., Narciso, M., Guimarães, C., Castro, A., Colombari, J. M., Breseghello, F., Rodrigues, L., Vianello, R.P., Borba, T.O., & Brondani, C. (2016). Genome wide association study (GWAS) for grain yield in rice cultivated under water deficit. *Genetica*, *144*(6), 651-664.

Passioura, J. B. (1996). Drought and drought tolerance. *Plant growth regulation*, *20*(2), 79-83.

Piatkowski, D., Schneider, K., Salamini, F., & Bartels, D. (1990). Characterization of five abscisic acid-responsive cDNA clones isolated from the desiccation-tolerant plant Craterostigma plantagineum and their relationship to other water-stress genes. *Plant Physiology*, *94*(4), 1682-1688.

Pour-Aboughadareh, A., Ahmadi, J., Mehrabi, A. A., Etminan, A., Moghaddam, M., & Siddique, K. H. (2017). Physiological responses to drought stress in wild relatives of wheat: implications for wheat improvement. *Acta Physiologiae Plantarum*, *39*(4), 106.

Price, A. H., Cairns, J. E., Horton, P., Jones, H. G., & Griffiths, H. (2002). Linking drought-resistance mechanisms to drought avoidance in upland rice using a QTL approach: progress and new opportunities to integrate stomatal and mesophyll responses. *Journal of experimental botany*, *53*(371), 989-1004.

Prochazkova, D., and N. Wilhelmova. (2007). "Leaf senescence and activities of the antioxidant enzymes." *Biologia plantarum* 51, no. 3: 401-406.

Quarrie, S. A., Gulli, M., Calestani, C., Steed, A., & Marmiroli, N. (1994). Location of a gene regulating drought-induced abscisic acid production on the long arm of chromosome 5A of wheat. *Theoretical and Applied Genetics*, *89*(6), 794-800.

Ramanjulu, S., & Bartels, D. (2002). Drought-and desiccation-induced modulation of gene expression in plants. *Plant, cell & environment*, *25*(2), 141-151.

Reddy, A. R., Chaitanya, K. V., & Vivekanandan, M. (2004). Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. *Journal of plant physiology*, *161*(11), 1189-1202.

Reddy, P. S. (2019). Breeding for Abiotic Stress Resistance in Sorghum. In *Breeding Sorghum for Diverse End Uses* (pp. 325-340). Woodhead Publishing.

Reynolds, M., Dreccer, F., & Trethowan, R. (2007). Drought-adaptive traits derived from wheat wild relatives and landraces. *Journal of Experimental Botany*, *58*(2), 177-186.

Ritchie, S. W., Nguyen, H. T., & Holaday, A. S. (1990). Leaf water content and gas-exchange parameters of two wheat genotypes differing in drought resistance. *Crop science*, *30*(1), 105-111.

Rivero, R. M., Kojima, M., Gepstein, A., Sakakibara, H., Mittler, R., Gepstein, S., & Blumwald, E. (2007). Delayed leaf senescence induces extreme drought tolerance in a flowering plant. *Proceedings of the National Academy of Sciences*, *104*(49), 19631-19636.

Rodriguez, M. C. S., Edsgärd, D., Hussain, S. S., Alquezar, D., Rasmussen, M., Gilbert, T., Nielsen, B.H., Bartels, D. & Mundy, J. (2010). Transcriptomes of the desiccation-tolerant resurrection plant *Craterostigma plantagineum*. *The Plant Journal*, *63*(2), 212-228.

Rodriguez, P. M. (2019). *Systematic studies of the sweet potato and its wild relatives* (Doctoral dissertation, University of Oxford).

Rosales, M. A., Ocampo, E., Rodríguez-Valentín, R., Olvera-Carrillo, Y., Acosta-Gallegos, J., & Covarrubias, A. A. (2012). Physiological analysis of common bean (*Phaseolus vulgaris* L.) cultivars uncovers characteristics related to terminal drought resistance. *Plant physiology and biochemistry*, *56*, 24-34.

Rosenow, D. T., Quisenberry, J. E., Wendt, C. W., & Clark, L. E. (1983). Drought tolerant sorghum and cotton germplasm. *Agricultural Water Management*, *7*(1-3), 207-222.

Rounsevell, M. D. A., Evans, S. P., & Bullock, P. (1999). Climate change and agricultural soils impacts and adaptation. *Climatic Change*, *43*(4), 683-709.

Samarah, N. H. (2005). Effects of drought stress on growth and yield of barley.

Sanchez, A. C., Subudhi, P. K., Rosenow, D. T., & Nguyen, H. T. (2002). Mapping QTLs associated with drought resistance in sorghum (Sorghum bicolor L. Moench). *Plant molecular biology*, *48*(5-6), 713-726.

Santos, M. G., Ribeiro, R. V., Machado, E. C., & Pimentel, C. (2009). Photosynthetic parameters and leaf water potential of five common bean genotypes under mild water deficit. *Biologia Plantarum*, *53*(2), 229-236.

Sharma, P., Jha, A. B., Dubey, R. S., & Pessarakli, M. (2012). Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *Journal of botany, 2012*.

Shinozaki, K., & Yamaguchi-Shinozaki, K. (1996). Molecular responses to drought and cold stress. *Current Opinion in Biotechnology, 7*(2), 161-167.

Shinozaki, K., & Yamaguchi-Shinozaki, K. (2007). Gene networks involved in drought stress response and tolerance. *Journal of experimental botany, 58*(2), 221-227.

Shinozaki, K., Yamaguchi-Shinozaki, K., & Seki, M. (2003). Regulatory network of gene expression in the drought and cold stress responses. *Current opinion in plant biology, 6*(5), 410-417.

Solis, J., Villordon, A., Baisakh, N., LaBonte, D., & Firon, N. (2014). Effect of drought on storage root development and gene expression profile of sweetpotato under greenhouse and field conditions. *Journal of the American Society for Horticultural Science, 139*(3), 317-324.

Souter, J. R., Gurusamy, V., Porch, T. G., & Bett, K. E. (2017). Successful introgression of abiotic stress tolerance from wild tepary bean to common bean. *Crop Science, 57*(3), 1160-1171.

Specht, J. E., Chase, K., Macrander, M., Graef, G. L., Chung, J., Markwell, J. P., Germann, M., Orf, J.H. & Lark, K. G. (2001). Soybean response to water: a QTL analysis of drought tolerance. *Crop Science, 41*(2), 493-509.

Sun, H., Mu, T., Xi, L., Zhang, M., & Chen, J. (2014). Sweet potato (*Ipomoea batatas* L.) leaves as nutritional and functional foods. *Food chemistry, 156*, 380-389.

Tahere, A. S., Yamauchi, A., Kamoshita, A., & Wade, L. J. (2000). Genotypic variation in response of rainfed lowland rice to drought and rewatering: II. Root growth. *Plant Production Science*, *3*(2), 180-188.

Tuberosa, R., & Salvi, S. (2006). Genomics-based approaches to improve drought tolerance of crops. *Trends in plant science*, *11*(8), 405-412.

Tuberosa, R., Salvi, S., SANGUINETI, M. C., Landi, P., Maccaferri, M., & Conti, S. (2002). Mapping QTLs regulating morpho-physiological traits and yield: Case studies, shortcomings and perspectives in drought-stressed maize. *Annals of Botany*, *89*(7), 941-963.

Tuinstra, M. R., Ejeta, G., & Goldsbrough, P. (1998). Evaluation of near-isogenic sorghum lines contrasting for QTL markers associated with drought tolerance. *Crop science*, *38*(3), 835-842.

Tuinstra, M. R., Grote, E. M., Goldsbrough, P. B., & Ejeta, G. (1997). Genetic analysis of post-flowering drought tolerance and components of grain development in Sorghum bicolor (L.) Moench. *Molecular Breeding*, *3*(6), 439-448.

Turyagyenda, L. F., Kizito, E. B., Ferguson, M., Baguma, Y., Agaba, M., Harvey, J. J., & Osiru, D. S. (2013). Physiological and molecular characterization of drought responses and identification of candidate tolerance genes in cassava. *AoB plants*, *5*.

Utsumi, Y., Tanaka, M. A. H. O., Morosawa, T., Kurotani, A., Yoshida, T., Mochida, K., K., Matsui, A., Umemura, Y., Ishitani, M., Shinozaki, K., & Sakurai, T. (2012). Transcriptome analysis using a high-density oligomicroarray under drought stress in various genotypes of cassava: an important tropical crop. *DNA research*, *19*(4), 335-345.

Valliyodan, B., & Nguyen, H. T. (2006). Understanding regulatory networks and engineering for enhanced drought tolerance in plants. *Current opinion in plant biology*, *9*(2), 189-195.

Van Heerden, P. D. R., & Laurie, R. (2008). Effects of prolonged restriction in water supply on photosynthesis, shoot development and storage root yield in sweet potato. *Physiologia Plantarum*, *134*(1), 99-109.

Venuprasad, R., Lafitte, H. R., & Atlin, G. N. (2007). Response to direct selection for grain yield under drought stress in rice. *Crop Science*, *47*(1), 285-293.

Wang, H. Z., Zhang, L. H., Jun, M. A., Li, X. Y., Yan, L. I., Zhang, R. P., & Wang, R. Q. (2010). Effects of water stress on reactive oxygen species generation and protection system in rice during grain-filling stage. *Agricultural sciences in China*, *9*(5), 633-641.

Wang, N., Wang, Z. P., Liang, X. L., Weng, J. F., Lv, X. L., Zhang, D. G., Yang, J., Yong, H.J., Li, M.S., Li, F.H., & Jiang, L. Y. (2016a). Identification of loci contributing to maize drought tolerance in a genome-wide association study. *Euphytica*, *210*(2), 165-179.

Wang, X., Wang, H., Liu, S., Ferjani, A., Li, J., Yan, J., Yang, X., & Qin, F. (2016b). Genetic variation in ZmVPP1 contributes to drought tolerance in maize seedlings. *Nature genetics*, *48*(10), 1233-1241.

Wilhite, D. A., & Glantz, M. H. (1985). Understanding: the drought phenomenon: the role of definitions. *Water international*, *10*(3), 111-120.

Wood, J. R., Carine, M. A., Harris, D., Wilkin, P., Williams, B., & Scotland, R. W. (2015). Ipomoea (Convolvulaceae) in Bolivia. *Kew Bulletin*, *70*(3), 31.

Xu, J. L., Lafitte, H. R., Gao, Y. M., Fu, B. Y., Torres, R., & Li, Z. K. (2005). QTLs for drought escape and tolerance identified in a set of random introgression lines of rice. *Theoretical and Applied Genetics*, *111*(8), 1642-1650.

Yadollahi, A., Arzani, K., Ebadi, A., Wirthensohn, M., & Karimi, S. (2011). The response of different almond genotypes to moderate and severe water stress in order to screen for drought tolerance. *Scientia Horticulturae*, *129*(3), 403-413.

Yamaguchi-Shinozaki, K., & Shinozaki, K. (2006). Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. *Annu. Rev. Plant Biol.*, *57*, 781-803.

Yang, P. M., Huang, Q. C., Qin, G. Y., Zhao, S. P., & Zhou, J. G. (2014). Different drought-stress responses in photosynthesis and reactive oxygen metabolism between autotetraploid and diploid rice. *Photosynthetica*, *52*(2), 193-202.

Yue, B., Xue, W., Xiong, L., Yu, X., Luo, L., Cui, K., Jin, D., Xing, Y. & Zhang, Q. (2006). Genetic basis of drought resistance at reproductive stage in rice: separation of drought tolerance from drought avoidance. *Genetics*, *172*(2), 1213-1228.

Zhang, H., Cui, F., & Wang, H. (2014). Detection of quantitative trait loci (QTLs) for seedling traits and drought tolerance in wheat using three related recombinant inbred line (RIL) populations. *Euphytica*, *196*(3), 313-330.

Zhang, J., & Kirkham, M. B. (1994). Drought-stress-induced changes in activities of superoxide dismutase, catalase, and peroxidase in wheat species. *Plant and Cell Physiology*, *35*(5), 785-791.

Zhang, Q., & Bartels, D. (2018). Molecular responses to dehydration and desiccation in desiccation-tolerant angiosperm plants. *Journal of experimental botany*, *69*(13), 3211-3222.

Zheng, J., Fu, J., Gou, M., Huai, J., Liu, Y., Jian, M., Huang, Q., Guo, X., Dong, Z., Wang, H. & Wang, G. (2010). Genome-wide transcriptome analysis of two maize inbred lines under drought stress. *Plant molecular biology*, *72*(4-5), 407-421.

Zlatev, Z. S., Lidon, F. C., Ramalho, J. C., & Yordanov, I. T. (2006). Comparison of resistance to drought of three bean cultivars. *Biologia Plantarum*, *50*(3), 389-394.

Zlatev, Z., & Lidon, F. C. (2012). An overview on drought induced changes in plant growth, water relationsand photosynthesis. *Emirates Journal of Food and Agriculture*, 57-72

CHAPTER 2

A Study of the Relatedness of Diploid and Polyploid *Ipomoea spp.* within the *Batatas* Complex using Quantitative Reduced Representation Sequencing (qRRS)

(In a format suitable for submission to Journal of Systematics and Evolution)

A Study of the Relatedness of Diploid and Polyploid *Ipomoea spp.* within the *Batatas* Complex using Quantitative Reduced Representation Sequencing (qRRS)

Stella E.C. Nhanala

[In a format suitable for submission to the Journal of Systematics and Evolution]

ABSTRACT

Sweetpotato [*Ipomoea batatas* (L.) Lam.] is a hexaploid (2n = 6x = 90) belonging to the *Batatas* species complex, a heterogenous group in terms of ploidy level consisting of 16 species. In addition to sweetpotato, *I. batatas*, fifteen other *Ipomoea spp.* are part of the *Batatas* complex, with diploid and tetraploid ploidy levels. Of these wild species, *Ipomoea trifida* (2n=2x=30, or 2n=4x=60) appears to be the closest relative of the hexaploid sweetpotato, but breeders know relatively little about the mating barriers and interfertility levels amongst the species in this complex. Improved understanding about the relatedness of the species within the *Batatas* complex will help breeders identify possible means to introgress potentially valuable traits for tolerance to abiotic and biotic stress from crop wild relative (CWR) species into cultivated *I. batatas*. In this study, we employed quantitative reduced representation sequencing (qRRS) to study the relatedness of 52 *Batatas* accessions representing 10 *Ipomoea* species present in a diallel crossing study. Our goal was to lay the foundation for an understanding of the species relationships, underlying population structure, and crossing potential of these materials for future breeding utilization. The study of the phylogenetic relationships and the genetic relatedness

within the *Batatas* series revealed three main clusters within the *Batatas* complex, with *I. trifida* and *I. batatas* coexisting within the same cluster. The 52 accessions were assigned to six sub-populations based on structure analyses. The results of the study based on genome wide single nucleotide polymorphism markers (SNPs) are congruent with previous studies regarding an autopolyploid origin from *I. trifida* and the role of hybridization in the evolution of sweetpotato. We expect that the knowledge of the genetic relatedness and the population structure in the *Batatas* complex may be useful to identify individuals that can potentially intermate in future breeding efforts and contribute to the utilization of CWR for improvement of this important food security crop.

INTRODUCTION

Cultivated sweetpotato [*Ipomoea batatas* (L.) Lam.] belongs to the *Batatas* complex or series (Austin, 1978; Diaz et al., 1996; Huang & Sun, 2000; Rajapakse et al., 2004; Khoury et al., 2015), and currently this group comprises sixteen species (Table 1) (Wood et al., 2015; Muñoz-Rodríguez et al., 2018; 2019). Sweetpotato is a hexaploid (2n=6x=90), while the other species of the *Batatas* complex are either diploid and/or tetraploid (Diaz et al., 1996; Khoury et al., 2015; Rodriguez, 2019; Guerrero-Zurita et al., 2020). Differences in ploidy levels, flowering times, mating systems, sporophytic and gametophytic incompatibilities, and geographic isolation among some species indicate barriers for hybridization in the *Batatas* complex and support presence of multiple reproductively isolated species.

The currently used classification scheme of the *Batatas* group recognizes sixteen species, with variable ploidy levels (Table 1): *Ipomoea australis*, *Ipomoea batatas (*6x, 4x*)*, *I. cordatotriloba* (2x), *I. cynanchifolia* (2x), *I. grandifolia* (2x), *I. lacunosa* (2x), *I. lactifera* (2x),

I. leucantha (2x), *I. littoralis* (2x), *I. ramosissima* (2x), *I. splendor-sylvae* (2x), *I. tabascana* (4x), *I. tenuissima* (2x), *I. tiliacea* (4x), *I. trifida* (2x, 4x), and *I. triloba* (2x), (Wood et al., 2015; Muñoz-Rodríguez et al., 2018; 2019). Floral morphology, such as outer sepal shape, corolla size, and floral color (e.g., Austin, 1978; Duncan & Rausher, 2013) have often been used to distinguish the species, but this approach has been shown to be problematic because these features have shown phenotypic plasticity in segregating populations (Duncan & Rausher, 2013), and due to the complexity of morphological variation, the species within the complex have been difficult to distinguish.

Several studies have been conducted to understand the phylogenetic relationships within the *Batatas* complex using molecular markers (Jarret et al., 1992; Duncan & Rausher, 2013; Roullier et al. 2013; Eserman, 2017; Wu et al. 2018; Muñoz-Rodriguez et al. 2018). These studies have collectively increased our understanding of this important complex. Jarret et al. (1992) employed restriction fragment length polymorphism (RFLP) markers to elucidate species relationships within the *Batatas* complex and identified *I. trifida*, and *I. tabascana* as *I. batatas'* closest relatives. It should be noted that for certain species Jarret et al. (1992) used different names than the current taxonomy. Duncan & Rausher (2013) studied the relationship of morphological and genetic differentiation in four wild *Ipomoea spp.* of the series *Batatas* using microsatellite data. Their results showed that *I. leucantha* and *I. austinii* were distinct from each other in both morphology and genetics, while *I. cordatotriloba* and *I. lacunosa* showed little genetic difference, but they were morphologically very different. These results were congruent with the results in Jarret et al. (1992), which showed that *I. cordatotriloba* and *I. lacunosa* were close to each other. *Ipomoea leucantha* and *I. austinii* were not included in the study conducted by Jarret et al. (1992). Duncan & Rausher (2013) also studied the crossability of *I.*

cordatotriloba, *I. lacunosa*, *I. leucantha*, and *I. austinii*. They found that *I. lacunosa* and *I. cordatotriloba*, *I. lacunosa* and *I. austinii*, and *I. cordatotriloba* and *I. austinii* had variable degrees of species interfertility. Roullier et al. (2013) used sequences of chloroplast noncoding regions, nuclear internal transcribed spacer (ITS) and nuclear simple sequence repeats (SSRs) in the study of 219 accessions, representing six species and found evidence supporting that sweetpotato (*I. batatas*) and *I. trifida* were closely related. They also identified two types of chloroplast DNAs with lineages derived from the hybridization of *I. batatas* and *I. trifida*.

Recently, Eserman (2017) applied a targeted enrichment method to study the evolutionary history of the series *Batatas* and found that *I. batatas* was closely related to *I. trifida*, and *I. tabascana* close to *I. triloba*. However, *I. tabascana* was found to be more distantly related to *I. batatas*, in contrast to the finding of Jarret et al. (1992). Based on the data, Eserman (2017) suggested that the origin of *I. batatas* also involved hybridization between *I. ramosissima* and *I. triloba*, or between *I. ramosissima* and *I. cordatotriloba*, suggesting that both hybridization and polyploidization were involved in the origin and evolution of sweetpotato.

More recently, Muñoz-Rodriguez et al. (2018) combined genome skimming and target DNA capture (Hyb-seq) with a larger sampling of populations representing all 16 species to determine the number of times that sweetpotato evolved and to identify the species that are related with the origin of sweetpotato. Muñoz-Rodriguez et al. also found that *I. trifida* and *I. batatas* were closely related. Based on their results they concluded that sweetpotato was an autopolyploid, with *I. trifida* being the sole progenitor and closest relative. In addition, they found evidence supporting that *I. batatas* and *I. trifida* may have hybridized resulting in two hybrid lineages of sweetpotato, with one of the lineages containing the chloroplast of *I. trifida*.

Wu et al. (2018), studied the origin and evolution of the *Batatas* complex by comparative analyses of genomes among *I. batatas, I. trifida,* and *I. triloba* as well as phylogenetic analysis of single copy region sequences from genome resequencing of other species in the complex. Their results showed high (>90%) similarity of sweetpotato to both genomes of *I. trifida* and *I. triloba* but a higher similarity to *I. trifida*. Their phylogenetic analyses recovered six distinct lineages within the *Batatas* complex and similarly showed that *I. trifida* was the closest relative of *I. batatas*.

Clearly, the use of molecular data for the study of the *Batatas* complex has advanced our understanding of species relationships within the complex and identified plausible progenitors of sweetpotato. However, disagreements exist among studies regarding relationships among other species. Jarret et al. (1992) identified *I. trifida* and *I. tabascana* as close relatives of sweetpotato, while Eserman (2017) did not find *I. tabascana* close to sweetpotato. Eserman (2017) found that the origin of *I. batatas* could be between *I. ramosissima* and *I. triloba*, or between *I. ramosissima* and *I. cordatotriloba*, which it is opposed to Muñoz-Rodriguez et al. (2018) who suggested that sweetpotato had an autopolyploid origin, probably from *I. trifida*. In this study, we employed data from genome wide regions to further elucidate species relationships within the *Batatas* complex, assess the extent of genetic admixture across species, and develop a better understanding regarding the origin of sweetpotato. Specifically, we employed 9223 polymorphic single nucleotide polymorphisms (SNPs) generated from Quantitative Reduced Representation Sequencing (qRRS). The SNP data was used to infer the phylogenetic relationships and the relatedness of 52 CWR and *I. batatas* accessions present in the gene bank at the International Potato Center, Lima, Peru, which were previously used in a diallel mating study of the *Batatas* complex in San Ramon, Peru. We expect that the knowledge of the genetic relatedness and the

population structure in the *Batatas* complex may be useful to identify individuals that can potentially intermate in future breeding efforts and contribute to the utilization of CWR for improvement of this important food security crop.

MATERIALS AND METHODS

Sampling and data generation

In this study, we focus on the germplasm materials curated in the gene bank at the International Potato Center (CIP), Lima, Peru. The plants were grown by Dr. Bettina Heider's research team at the International Potato Center (CIP), at the San Ramon Experimental Station, Chanchamayo province, Peru, located at Calle Las Margaritas; GPS coordinates: 11° 7' 17.5008" S, Longitude: 75° 21' 31.3452" W. Table 2 provides a description of the 52 accessions present in the study. *Ipomoea leucantha* was represented by only one accession. The other nine species were represented by at least two accessions. The origin of these samples is presented in Table 2, and the geographic distribution of the accessions is exhibited in Figure 1.

Leaf tissues of all 52 accessions were collected from the experimental plants, dried on silica gel, and shipped to North Carolina State University (NCSU). The origin of the accessions are provided in CIP's gene bank (http://genebank.cipotato.org/gringlobal/search.aspx#), and when the origin of the population was not available in the gene bank, the sample was labelled as "Unknown" (Table 2).

Total DNA was isolated in the NCSU Sweetpotato Molecular Genetics Laboratory (SPMGL) and next-generation sequencing (NGS) library preparation and sequencing was performed at the Genomic Sciences Laboratory (GSL), NCSU, Raleigh, NC.

DNA isolation

DNA was extracted using the Omega Mag-Bind® Plant DNA DS kit (Omega Bio-tek, Inc., Norcross, GA), according to the instructions of the manufacturer. Quality control (integrity and concentration) of the DNA was evaluated by running the DNA samples on a 1% agarose gel electrophoresis and using a NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, Delaware) for quantification.

Library construction

The preparation of the genomic library, including sample barcoding, was performed at the NCSU GSL using a quantitative reduced representation sequencing (OmeSeq-qRRS) protocol developed for highly heterozygous genomes described in Wadl et al. (2018) and Olukolu and Yencho (2020). This approach is comparable to the double digest restriction-site associated DNA sequencing (ddRADseq) method of Peterson et al. (2012) as a genotyping by sequencing (GBS) method except the method that was employed in the present study is robust for low-input DNA of about 5 ng, and it also prevents chimeric reads by avoiding a ligation-step. In contrast, the ddRADseq protocol requires library construction with about 100 ng of starting DNA, and the barcoded adapters are ligated separately to individual samples in microplate format (Peterson et al., 2012). The libraries were constructed by performing a sequential double digestion with the restriction enzymes *Nsil* and *NlaIII*, using a total of 5 - 10 ng/ µl DNA of each accession. For the sequential double digestion, 10 units of *Nsil* was applied, and followed by 10 units of *NlaIII*. Buffered and barcoded P5 and P7 Illumina adapters were integrated, respectively, after each digest by isothermal amplification (NEB Bst 2.0 WarmStart® DNA polymerase) of the dsDNA using the overhang as the priming template site. Libraries from each accession were pooled and purification of the pooled library was performed using Ampure XP MagBeads

(Beckman Coulter Inc., Brea, California) before size selection for 300 – 600 bp (including approx. 150 bp of adapter sequence) fragments using a Pippin Prep instrument (Sage Science Inc., Beverly, Massachusetts). The library containing the selected fragments were checked for quality and quantity using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, California) and enriched by 18 cycles of quantitative polymerase chain reaction (qPCR) using NEB Phusion polymerase followed by quantification using the Agilent TapeStation automated electrophoresis system (Agilent Technologies, Santa Clara, California). Sequencing was performed on a NovaSeq 6000 *Illumina* platform in two lanes of SP flow cell at 150 bp paired-end at the NCSU GSL.

Data cleaning and SNP calling

The short reads generated from Illumina sequencing were filtered using ngsComposer (https://github.com/bodeolukolu/ngsComposer), a streamlined and automated bioinformatic pipeline developed for empirical NGS data quality filtering by Kuster et al., (2021). The ngsComposer has the advantages of empirically estimated per base error rates, detection of erroneous base calls and the removal of contaminating adapter sequences, all of which contribute to reduced error rates and improved quality of GBS data. SNP calling was performed using GBSapp (https://github.com/bodeolukolu/GBSapp) based on comparison to the a high-quality genome of the *I. trifida* reference genome (Wu et al., 2018; Mollinari et al., 2020) available at: http://sweetpotato.plantbiology.msu.edu/index.shtml. The filtering of raw data used the following parameters: retaining SNPs with read depth threshold >= 6, and no missing data in any samples, minor allele frequency >= 0.02, and selection of only one SNP within an interval of approximately 50 kb for the analyses.

Phylogenetic analysis

We performed phylogenetic analyses using the 9233 SNPs obtained to understand relationships among the accessions and species using the unweighted neighbor-joining (NJ) method (Saitou and Nei, 1987), implemented in the most recent version of Darwin software v. 6.0.021 (Perrier & Jacquemoud-Collet, 2006). The analysis was performed with 1000 bootstrap replicates to estimate support of phylogenetic groupings.

Principal Component Analysis

We also conducted a principal component analysis (PCA) to determine the genetic relationships among the 52 accessions using the Darwin software, following Lee et al. (2016) and Bernard et al. (2018), which groups individuals based on genetic dissimilarities (distances).

Population structure analysis

Population structure analysis was performed using STRUCTURE version 2.3.4 (Pritchard et al., 2000), based on Bayesian statistical methods. For the analyses, a single SNP was selected from a sliding window of about 50 kb interval for the analyses, resulting in a total of 9223 SNPs across the entire genome to assess the number of gene pools (genetic groups) represented by the 52 individuals and the gene admixture between gene pools.

Because STRUCTURE was developed for study of diploid organisms, the tetraploid and hexaploid samples that were included in the study were treated as pseudo-diploids so that they could be subjected to the same analysis as the diploids in a single analysis. Based on simulation study, STRUCTURE was considered the most robust method for the analysis of genetic structure in mixed-ploidy populations (Eudy et al., 2017; Hu et al., 2019; Stiff et al., 2019). The analysis of the data with STRUCTURE were performed under the Admixture Model, K=1 to 10, a burnin period of 50,000 generations, with 500,000 *Markov Chain Monte Carlo* (MCMC) iterations after

burnin. The best K value was determined using Structure Harvester version 0.6.94 (Earl & vonHoldt, 2012), and structure bar plot was generated by selecting the highest mean probability for that K.

RESULTS

GBS data

The ngsComposer and GBSapp sequencing sequence filtering pipelines resulted in roughly 500, 000 SNPs that were linkage disequilibrium (LD), and these were trimmed to 9223 SNPs distributed across the entire genome with an average spacing of 50 kb interval.

Phylogenetic relationships

Phylogenetic analysis of the *Batatas* complex revealed that the 52 accessions were distributed in three major groups: one group strongly supported (73% bootstrap support), and two groups weakly supported (50% and 30% bootstrap support) (Figure 2). The three clades (Figure 2) consisted of the following species: Clade A contained accessions from *I. batatas*, *I. trifida*, *I. splendor-sylvae*, and *I, ramosissima* (30% bootstrap support); Clade B contained accessions from *I. triloba*, *I. grandifolia, I. cordatotriloba* , *I. leucantha, I. cynanchifolia* (50% bootstrap support); and Clade C contained accessions from *I. ramosissima* , *I. tiliacea* , and *I. triloba* (73% bootstrap support). Within cluster A, 15 accessions of *I. trifida* formed a strongly supported subclade with 98% bootstrap support, and two of the accessions of *I. trifida* (CIP 460577 and CIP 430434) were grouped with one accession of *I. ramosissima,* and all accessions of *I. batatas* were in a subclade that was weakly supported (30% bootstrap). The two accessions of *I. splendor-sylvae* were well supported as sisters (77% bootstrap). The tree also showed that

the *I. trifida* subclade was sister to the *I. batatas* clade, containing two accessions of *I. trifida* that were poorly supported (32% bootstrap support).

Within Clade B, accessions from the same species did not form monophyletic groups but four accessions of *I. triloba* formed a strongly supported subclade with one accession of *I. cordatotriloba* and one accession of *I. grandifolia* (98% bootstrap) (Figure 2).

Within Clade C, accessions from five of the six remaining accessions of *I. ramosissima* formed a well-supported subclade (76% bootstrap support) that was sister to the two accessions of *I. tiliciacea*. One accession of *I triloba* was strongly supported to be the sister of one accessions of *I. ramosissima*.

Principal Component analysis

Results of the PCA similarly exhibited three distinct clusters largely congruent to those identified in the phylogenetic analysis (Figure 3). Cluster 1 consisted of accessions of *I. trifida and I batatas*). Cluster 2 consisted of accessions of *I tiliacea* and *I. ramosissima*), while Cluster 3 consisted of accessions of *I. triloba*, *I cordatotriloba*, *I grandifolia* and *I. cynachifolia*. Exceptions were that some individuals, such as *I. splendor-sylvae* were not delimited into any of the clusters, being intermediary in genetic distance to the clusters. For example, *I. splendor-sylvae* appeared intermediate between Cluster 1 and Cluster 2. The PCA results also indicate that Clusters 1 and 2 (*I. trifida, I. batatas, and I. tiliacea* and *I. ramosissima*) were well separated by a considerable genetic distance across the Y axis, while Cluster 3 shows variation in the genetic distance that it is more pronounced along the x axis, suggesting that the factor that defines how the accessions cluster were differentially affected with each individual (accession).

Population Structure analysis

Results from the analysis with STRUCTURE showed that the optimal delta K was achieved when K=6 (Figure 4a), suggesting the presence of six genetic groups (or ancestral genomes) for the 52 accessions. The bar plot graph derived from the analysis with K = 6 indicates individuals (accessions) exhibit considerable variation in the proportion of ancestry. The genetic composition in all individuals was a mixture of three genetic groups or more (or ancestral genomes) (Figure 4b). All accessions appeared to share one of the six ancestral genomes (blue) which comprises approximately half (in most individuals) or more of their genomes. Another ancestral genome (orange) is present in most accessions, except four individuals of *I. triloba* and one individual of *I. trifida* and occupies a quarter (most) to half of their genome. The other four ancestral genomes (gray, dark brown, yellow, and green) were variously distributed among species in different proportions. The results showed that the genomic compositions of most individuals of *I. cordatotriloba*, *I. cynanchifolia*, *I. grandifolia*, *I. leucantha*, and one individual of *I. triloba* were all similar sharing the blue, orange, and dark brown ancestral genomes although vary in the relative proportions (Figure 4b), while the rest of individuals of *I. triloba* have a different admixture of ancestry containing the gray genome in place of the orange genome. The gray genome was also present as a very small proportion (<5%) in one individual of *I. cordatotriloba*, I*. grandifolia*, and *I. cynanchifolia*. The genomic composition of *I. batatas* exhibited the same pattern of ancestry admixture as those in some of *I. trifida* and one individual of *I. ramosissima* (blue, orange, gray). The rest of the individuals of *I. trifida*, however, showed an admixture of a different pattern of genomic ancestry containing four ancestral genomes (blue, orange, gray and green). The genomic compositions of the rest of the *I. ramosissima* was distinct from the others, containing blue, orange, and yellow genome ancestry,

as well as gray and/or a trace of dark brown genomes in some. The two individuals of *I. splendor-sylvae* showed an admixture of six and five genomic ancestries, respectively, while *I. tiliacea* was similar to *I. ramosissima* in having the yellow genome. The genome ancestry and grouping revealed from the Structure analysis are congruent with the clades, subclades, and clusters revealed from phylogenetic analysis and PCA, respectively.

DISCUSSION

The advent of NGS technologies has greatly benefited phylogenetic and evolutionary studies in the generation of genome-wide markers for better understanding of phylogenetic relationships and evolution of organisms including a wide range of major and minor crop species. For example, GBS has provided data for successful identification of varieties and estimate of ancestry in cassava (Rabbi et al., 2015). Genotyping by sequencing has also been used for distinguishing allele dosage in a heterozygous segregating F1 genetic mapping population of polyploidy potato (Pereira et al., 2018), and for elucidating phylogenetic relationships of other food or horticulture polyploidy or diploid crops, such as *Fothergilla spp.* (Qi et al., 2015), *Coffea spp.* (Hamon et al., 2017), *Amaranthus spp.* (Stetter & Schmid, 2017), and *Capsicum spp.* (Pereira-Dias et al., 2019). Polyploidy presents a special challenge in phylogenetic and population genetic studies due to the complexities associated with genome duplication. These studies demonstrate that SNP data generated through GBS coupled with robust bioinformatic software tailored for diploid and polyploid species will enable scientists to address a wide range of biological questions.

In sweetpotato, only a few studies employing recent next generation sequencing approaches have been conducted (Eserman, 2017; Wu et al., 2018; Muñoz-Rodríguez et al.

2018). These studies have substantially increased our understanding of the evolution of sweetpotato. Although the specific markers and sampling scale differed, our study using the qRRS method is congruent with the previous studies in finding a close relationship between *I. trifida* and *I. batatas* and six subgroups or distinct lineages within the sweetpotato species complex (i.e., the *Batatas* complex) comprising 16 taxonomic species. Although the bootstrap support in the phylogenetic tree was poorly supported in some parts, the clustering of individuals was congruent with the sorting from PCA and STRUCTURE analysis and some of the clades and subclades were well supported (Figures 2 - 4). The low support for many nodes of the phylogeny is likely a result of reticulate evolution from hybridization that resulted in admixture of genomes of different ancestry, which is discussed below.

Ipomoea splendor-sylvae was recovered as a lineage diverging out first within the complex in the nuclear phylogenies (Wu et al., 2018; Muñoz-Rodríguez et al., 2018). In our study, the accessions representing these species were grouped with the I. *batatas* - *I. trifida* clade with weak support (30%), indicating unresolved placement as a distinct lineage, though not in conflict with the previous nuclear phylogeny.

The study of Muñoz-Rodriguez et al. (2018) using plastid genome sequences and 605 single copy nuclear regions from target enrichment concluded that sweetpotato *I. batatas* is an autopolyploid with I. *trifid*a having a dual role, as the sole progenitor and the species with which sweetpotato introgressed. The hypothesized autopolyploidization of *I. trifida* resulting in the formation of *I. batatas* has also been addressed and supported by Wu et al. (2018). Our results from analyses of 9223 SNPs scattered across the entire genome corroborate this finding based on the shared admixture pattern of genome ancestry between *I. batatas* and one of the two lineages of *I. trifida* included in the study that exhibit variation in the exact proportions of each ancestral

genome among individuals. We did not observe genomic signatures of any other species investigated in this study present in *I. batatas* except those of *I. trifida* (Fig. 4). Our PCA result also shows small genetic distance among individuals of *I. trifida* and *I. batatas* (Figure 3).

Previous studies have also suggested ancient and on-going hybridization among species in the *Batatas* complex (Eserman, 2017), leading to genetic admixture in this group. In our study, we similarly identified the presence of genomic admixture in all individuals, with similar patterns within groups and different patterns among groups. Variations in the proportion of the estimated ancestry among individuals may reflect the degrees of introgressions. Although the difference in ploidy level among species in the *Batatas* species complex presents a barrier for interspecific mating, the presence of unreduced gametes would possibly have facilitated polyploidization. Further molecular genetic studies to distinguish *I. trifida* (2x, 4x) from *I. batatas* (4x, 6x) would be useful to have a better understanding of the polyploidization process involved in the evolution of *I. batatas*. Nonetheless, our results are consistent with previous studies supporting that hybridization likely has had an important role in the evolution of sweetpotato (Eserman, 2017; Muñoz-Rodriguez et al., 2018; Rodriguez, 2019).

The first *I. trifida* (CIP 460577) was listed as a tetraploid in the passport data, and it is also described as *I. batatas* "traditional cultivar/landrace" in the CIP gene bank (https://genebank.cipotato.org/gringlobal/accessiondetail.aspx?id=6453). Tetraploid *I. trifida* and tetraploid *I. batatas* have previously been morphologically confused with each other (Bohac et al., 1993). Therefore, it is possible that this accession (CIP 460577) was *I. batatas*, but misclassified as *I.* trifida. The second *I. trifida* accession (CIP 430434) has been recognized as a tetraploid in the passport data. Our results are in accordance with previous studies that have recognized *I. trifida* as the closest relative of *I. batatas* (Roullier et al., 2013; Eserman, 2017;

Rodriguez, 2019). Eserman (2017) also found that I. *ramosissima* is close to *I. batatas* and *I. trifida*. Still, *I. trifida* appears to be the closest relative to *I. batatas*, and this is supported by Jarret et al., 1992; Srisuwan et al., 2006, Eserman, 20127; Feng et al., 2018; Rodriguez, 2019.

Cluster B was composed of *I. triloba, I. cordatotriloba*, *I. grandifolia*, *I. cynachifolia,* and *I. leucantha* (the only *I. leucantha* in the study). Jarret et al. (1992) also observed that *I. triloba*, *I cordatotriloba*, and *I. grandifolia* were closely related to each other. Rodriguez (2019) found that I. *cordatotriloba* and *I. grandifolia* were even closer. These species are mostly distributed in "Zone 3" and "Zone 4" suggesting that they probably have similar ecological niches.

Cluster C was composed of: *I. ramosissima, I. tiliciacea*, and *I. triloba* (one accession). Rodriguez (2019) also found that *I. tilicacea* and *I. ramosissima* were close to one another. *I. tiliacea* and *I. triloba* are species that are distributed along the islands (Cuba and Jamaica). Probably, the adaptation of these species was related with the agroclimatic conditions of the islands.

Ipomoea cordatotriloba, *I. cynanchifolia*, *I. leucantha*, and *I. triloba* show the presence of the same sub-populations. Muñoz-Rodriguez et al. (2018) phylogeny's also show the same patterns, with *I. triloba* being a little distant of the other three species. In fact, even in the structure analysis, *I triloba* shows slight differences. Yet, this structure does not show many similarities with *I. batatas*. Roullier et al (2013) found it unlikely that *I. triloba* could have genetically contributed to the genome of sweetpotato, though Wu et al. (2018) have shown that *I triloba* is quite closely related to *I. batatas* based on their reference genome studies.

The 52 accessions included in this study represent a diverse sampling of the ecogeographical distribution of the *Batatas* complex and was based on the regions of the origin

of each accession. The whole species complex exhibits a continuous distribution in Central and south America (Figure 1) with overlapping of ecogeographical distributions between species, permitting hybridization and gene flow within the complex. Such pattern of geographic distribution suggests that ecogeographical factors may also play a role in the evolution and diversification of the *Batatas* complex .

Implications for Sweetpotato Breeding

The utilization of CWR as genetic resources for the improvement of cultivated crops is becoming increasingly important as new biotic and abiotic constraints arise due to a changing climate scenario (Harlan, 1976; Hoisington et al., 1999; Brozynska et al., 2015; Dempewolf et al., 2017; Kilian et al., 2021). Improvement of tolerance to biotic and abiotic stresses in plants has been addressed in crops such as beans (Inci & Toker, 2011; Yoshida et al., 2016), maize (Mano & Omori, 2013; de Lange et al., 2014; Moya-Raygoza, 2016), and wheat (Islam et al., 2007). This study was conducted as a component of a collaborative effort between North Carolina State University (NCSU) in Raleigh, NC, USA, and the International Potato Center (CIP) in Lima, Peru, with the CIP team leading the diallel crossing study of the 52 *Batatas* accessions in Peru, and NCSU performing the molecular analyses. The results of the diallel study performed in Peru will be reported in another forum. Differences in mating systems, geographic locations, and unsynchronized flowering time are some of the other challenges that plants (and breeders) have to overcome so that it is possible to generate interspecific hybrids. In nature, mating between the hexaploid sweetpotato and the other *Ipomoea spp.* is unlikely, though we have presented evidence that is does occur.

Some of the findings in this project may have utility for breeding programs when a controlled crossing program is undertaken, and the concept of bridge species is utilized to

overcome ploidy barriers. The current study may provide genetic background information that should allow us to better predict potentially compatible parents within the *Batatas* series. The possibility of being able to select/predict probable compatible parents based on the genetic background, and before crosses are performed is an advance for plant breeders. The utilization of molecular approaches to identify compatible parents could save time for plant breeders. In addition, these tools could allow to breeders to infer the exact genetic makeup of the intra- or interspecific hybrids.

The structure analysis of the *Batatas* complex has revealed several interesting findings that may have practical applications for the use of the crop wild relatives of sweetpotato for crop improvement. The structure analysis revealed the genetic similarity between *I. batatas* and *I. trifida*, when several times, the only difference was in the proportion of each sub-population within an accession. Thus, the utilization of *I. trifida* for the improvement of *I. batatas* may be considered in pre-breeding programs if this species are found to have important traits that are not available in the extensive cultivated and landrace germplasm.

CONCLUSIONS

In conclusion, the application of the NGS-based quantitative reduced representation sequencing (qRRS) method enabled us to develop thousands of SNP markers relatively quickly and cheaply. Phylogenetic analyses utilizing these abundant, genome-wide markers resulted in similar findings to those previously observed by others (Eserman (2017), Muñoz-Rodríguez (2018; 2019); Wu et al. (2018), Rodriguez (2019)). Here, we identified *I. trifida* as the closest relative of *I. batatas*, and population structure analyses enabled the identification of hybridization and admixture between ancestors of *I. trifida* and *I. batatas*.

The phylogenetic analysis showed three major clades and six sub-clades, with *I. trifida* and *I. batatas* being part of the same subclade. The ideal number of sub-populations for the structure analysis based on the structure analysis was six. The PCA distinguished three clusters. The results of this study suggest that the geographic location has an influence in the species distribution along the ecogeographic zones. Based on our analyses and that of others, autopolyploid and hybridization have clearly had a role in the evolution of sweetpotato.

Finally, we hope that the knowledge of the genetic relatedness and the population structure in the *Batatas* complex generated in this study will be useful to identify accessions that can potentially intermate in future breeding efforts and contribute to the utilization of CWR for improvement of this important food security crop.

ACKNOWLEDGEMENTS

We want to thank Crop Trust, the institution that funded this study. We also want to acknowledge the International Potato Center (CIP), Lima, Peru, where Dr. Bettina Heider and her team grew the plants we used in this study.

REFERENCES

Amadeu, R. (2019). AGHmatrix: An R package to compute relationship matrices for diploid and autopolyploid species.

Austin, D. F. (1978). The *Ipomoea batatas* complex - I. Taxonomy. *Bulletin of the Torrey Botanical Club*, 114-129.

Austin, D. F. (1988). Nomenclatural changes in the Ipomoea batatas complex (Convolvulaceae). *Taxon*, *37*(1), 184-185.

Bernard, A., Barreneche, T., Lheureux, F., & Dirlewanger, E. (2018). Analysis of genetic diversity and structure in a worldwide walnut (*Juglans regia* L.) germplasm using SSR markers. *PLoS One*, *13*(11), e0208021.

Bickford, D., Lohman, D. J., Sodhi, N. S., Ng, P. K., Meier, R., Winker, K., Ingram, K. K. & Das, I. (2007). Cryptic species as a window on diversity and conservation. *Trends in ecology & evolution*, *22*(3), 148-155.

Bohac, J. R., Austin, D. F., & Jones, A. (1993). Discovery of wild tetraploid sweetpotatoes. *Economic Botany*, *47*(2), 193-201.

Brozynska, M., Furtado, A., & Henry, R. J. (2016). Genomics of crop wild relatives: expanding the gene pool for crop improvement. *Plant biotechnology journal*, *14*(4), 1070-1085.

CIP's gene bank (http://genebank.cipotato.org/gringlobal/search.aspx#, accessed in February 2021)

Darwin software (https://darwin.cirad.fr/U_History.php, accessed in January 2021)

de Lange, E. S., Balmer, D., Mauch-Mani, B., & Turlings, T. C. (2014). Insect and pathogen attack and resistance in maize and its wild ancestors, the teosintes. *New Phytologist*, *204*(2), 329-341.

Dempewolf, H., Baute, G., Anderson, J., Kilian, B., Smith, C., & Guarino, L. (2017). Past and future use of wild relatives in crop breeding. *Crop Science*, *57*(3), 1070-1082.

Diaz, J., Schmiediche, P., & Austin, D. F. (1996). Polygon of crossability between eleven species of Ipomoea: section Batatas (Convolvulaceae). *Euphytica*, *88*(3), 189-200.

Duminil, J., & Di Michele, M. (2009). Plant species delimitation: a comparison of morphological and molecular markers. *Plant Biosystems*, *143*(3), 528-542.

Duncan, T. M., & Rausher, M. D. (2013). Morphological and genetic differentiation and reproductive isolation among closely related taxa in the Ipomoea series Batatas. *American Journal of Botany*, *100*(11), 2183-2193.

Earl, Dent A. and vonHoldt, Bridgett M. (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method.

Eserman, L. A. (2017). *Evolution and development of storage roots in morning glories (Convolvulaceae)* (Doctoral dissertation, uga).

Eudy, D., Bahri, B. A., Harrison, M. L., Raymer, P., & Devos, K. M. (2017). Ploidy level and genetic diversity in the genus Paspalum, group Disticha. *Crop Science*, *57*(6), 3319-3332

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular ecology*, *14*(8), 2611-2620.

Feng, J. Y., Li, M., Zhao, S., Zhang, C., Yang, S. T., Qiao, S., & Pu, Z. G. (2018). Analysis of evolution and genetic diversity of sweetpotato and its related different polyploidy wild species I. trifida using RAD-seq. *BMC plant biology*, *18*(1), 181.

GBSapp (https://github.com/bodeolukolu/GBSapp)

Genomic Tools for Sweetpotato (GT4SP) Improvement Project: http://sweetpotato.plantbiology.msu.edu/index.shtml (accessed on March 14, 2020)

Guerrero-Zurita, F., Ramírez, D. A., Rinza, J., Ninanya, J., Blas, R., & Heider, B. (2020). Potential short-term memory induction as a promising method for increasing drought tolerance in sweetpotato crop wild relatives [*Ipomoea* series *Batatas* (Choisy) DF Austin]. *Frontiers in plant science*, *11*, 1326.

Hamon, P., Grover, C. E., Davis, A. P., Rakotomalala, J. J., Raharimalala, N. E., Albert, V. A., Sreenath, H.L., Stoffelen, P., Mitchell, S.E., Couturon, E. and Hamon, S., & Guyot, R. (2017). Genotyping-by-sequencing provides the first well-resolved phylogeny for coffee (Coffea) and insights into the evolution of caffeine content in its species: GBS coffee phylogeny and the evolution of caffeine content. *Molecular phylogenetics and evolution*, *109*, 351-361.

Harlan, J. R. (1976). Genetic Resources in Wild Relatives of Crops 1. *Crop science*, *16*(3), 329-333.

Hebert, P. D., Penton, E. H., Burns, J. M., Janzen, D. H., & Hallwachs, W. (2004). Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. *Proceedings of the National Academy of Sciences*, *101*(41), 14812-14817.

Hoisington, D., Khairallah, M., Reeves, T., Ribaut, J. M., Skovmand, B., Taba, S., & Warburton, M. (1999). Plant genetic resources: what can they contribute toward increased crop productivity?. *Proceedings of the National Academy of Sciences*, *96*(11), 5937-5943.

Huang, J. C., & Sun, M. (2000). Genetic diversity and relationships of sweetpotato and its wild relatives in *Ipomoea* series *Batatas* (Convolvulaceae) as revealed by inter-simple

sequence repeat (ISSR) and restriction analysis of chloroplast DNA. *Theoretical and Applied Genetics, 100*(7), 1050-1060.

Hu, Y. N., Zhao, L., Buggs, R. J., Zhang, X. M., Li, J., & Wang, N. (2019). Population structure of *Betula albosinensis* and *Betula platyphylla*: evidence for hybridization and a cryptic lineage. *Annals of botany*, *123*(7), 1179-1189

Inci, N. E., & Toker, C. (2011). Screening and selection of faba beans (Vicia faba L.) for cold tolerance and comparison to wild relatives. *Genetic Resources and Crop Evolution, 58*(8), 1169-1175.

International Potato Center (CIP): http://genebank.cipotato.org/gringlobal/search.aspx# (accessed in June 2021)

Ipomoea trifida reference genome (available at: http://sweetpotato.plantbiology.msu.edu/index.shtml, accessed in May 2021)

Islam, S., Malik, A. I., Islam, A. K. M. R., & Colmer, T. D. (2007). Salt tolerance in a *Hordeum marinum – Triticum aestivum* amphiploid, and its parents. *Journal of Experimental Botany*, *58*(5), 1219-1229.

Jarret, R. L., Gawel, N., & Whittemore, A. (1992). Phylogenetic relationships of the sweetpotato [*Ipomoea batatas* (L.) Lam.]. *Journal of the American Society for Horticultural Science*, *117*(4), 633-637.

Khoury, C. K., Heider, B., Castañeda-Álvarez, N. P., Achicanoy, H. A., Sosa, C. C., Miller, R. E., Scotland, R.W., Wood, J.R., Rossel, G., Eserman, L.A., & Struik, P. C. (2015). Distributions, ex situ conservation priorities, and genetic resource potential of crop wild relatives of sweetpotato [*Ipomoea batatas* (L.) Lam., *I.* series *Batatas*]. *Frontiers in plant science*, *6*, 251.

Kilian, B., Dempewolf, H., Guarino, L., Werner, P., Coyne, C., & Warburton, M. L. (2021). Crop Science special issue: Adapting agriculture to climate change: A walk on the wild side. *Crop Sci*, *61*, 32-36.

Kuster, R. D., Yencho, G. C., & Olukolu, B. A. (2021). ngsComposer: an automated pipeline for empirically based NGS data quality filtering. *Briefings in Bioinformatics*.ngsComposer (https://github.com/ryandkuster/ngsComposer)

Lee, H. Y., Ro, N. Y., Jeong, H. J., Kwon, J. K., Jo, J., Ha, Y., Jung, A., Han, J.W., Venkatesh, J.; & Kang, B. C. (2016). Genetic diversity and population structure analysis to construct a core collection from a large *Capsicum* germplasm. *BMC genetics*, *17*(1), 1-13.

Mano, Y., & Omori, F. (2013). Flooding tolerance in interspecific introgression lines containing chromosome segments from teosinte (*Zea nicaraguensis*) in maize (*Zea mays* subsp. mays). *Annals of Botany*, *112*(6), 1125-1139.

Mollinari, M., Olukolu, B. A., Pereira, G. D. S., Khan, A., Gemenet, D., Yencho, G. C., & Zeng, Z. B. (2020). Unraveling the hexaploid sweetpotato inheritance using ultra-dense multilocus mapping. *G3: Genes, Genomes, Genetics*, *10*(1), 281-292.

Moya-Raygoza, G. (2016). Early development of leaf trichomes is associated with decreased damage in teosinte, compared with maize, by *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Annals of the Entomological Society of America*, *109*(5), 737-743.

Muñoz-Rodriguez, P., Carruthers, T., Wood, J. R., Williams, B. R., Weitemier, K., Kronmiller, B., Ellis, D., Anglin, N.L., Longway, L., Harris, S.A. and Rausher, M.D., & Scotland, R. W. (2018). Reconciling conflicting phylogenies in the origin of sweet potato and dispersal to Polynesia. *Current Biology*, *28*(8), 1246-1256.

Muñoz-Rodríguez, P., Carruthers, T., Wood, J. R., Williams, B. R., Weitemier, K., Kronmiller, B., Goodwin, Z., Sumadijaya, A., Anglin, N.L., Filer, D. and Harris, D., & Scotland, R. W. (2019). A taxonomic monograph of *Ipomoea* integrated across phylogenetic scales. *Nature plants*, *5*(11), 1136-1144.

Olukolu BA and Yencho GC (PCT/US20/35470): Compositions and methods related to quantitative reduced representation sequencing. May 30, 2020.

Orjeda, G., Freyre, R., & Iwanaga, M. (1991). Use of *Ipomoea trifida* germ plasm for sweet potato improvement. 3. Development of 4x interspecific hybrids between *Ipomoea batatas* (L.) Lam.(2n= 6x= 90) and *I. trifida* (HBK) G. Don.(2n= 2x= 30) as storage-root initiators for wild species. *Theoretical and Applied Genetics*, *83*(2), 159-163.

Pereira, G. S., Garcia, A. A. F., & Margarido, G. R. (2018). A fully automated pipeline for quantitative genotype calling from next generation sequencing data in autopolyploids. *BMC bioinformatics*, *19*(1), 1-10.

Pereira-Dias, L., Vilanova, S., Fita, A., Prohens, J., & Rodríguez-Burruezo, A. (2019). Genetic diversity, population structure, and relationships in a collection of pepper (*Capsicum* spp.) landraces from the Spanish centre of diversity revealed by genotyping-by-sequencing (GBS). *Horticulture research*, *6*(1), 1-13.

Perrier, X., & Jacquemoud-Collet, J. P. (2006). DARwin software: Dissimilarity analysis and representation for windows. *Website http://darwin. cirad. fr/darwin [accessed 1 March 2013]*.

Peterson, B. K., Weber, J. N., Kay, E. H., Fisher, H. S., & Hoekstra, H. E. (2012). Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. *PloS one*, *7*(5), e37135.

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, *155*(2), 945-959.

Qi, Z. C., Yu, Y., Liu, X., Pais, A., Ranney, T., Whetten, R., & Xiang, Q. Y. (2015). Phylogenomics of polyploid *Fothergilla* (Hamamelidaceae) by RAD-tag based GBS—insights into species origin and effects of software pipelines. *Journal of Systematics and Evolution*, *53*(5), 432-447.

Rajapakse, S., Nilmalgoda, S. D., Molnar, M., Ballard, R. E., Austin, D. F., & Bohac, J. R. (2004). Phylogenetic relationships of the sweetpotato in *Ipomoea* series *Batatas* (Convolvulaceae) based on nuclear β-amylase gene sequences. *Molecular phylogenetics and evolution*, *30*(3), 623-632.

Rabbi, I. Y., Kulakow, P. A., Manu-Aduening, J. A., Dankyi, A. A., Asibuo, J. Y., Parkes, E. Y., Abdoulaye, T., Girma, G., Gedil, M.A., Ramu, P. and Reyes, B., & Maredia, M. K. (2015). Tracking crop varieties using genotyping-by-sequencing markers: a case study using cassava (*Manihot esculenta* Crantz). *BMC genetics*, *16*(1), 1-11.

Rodriguez, P. M. (2019). Systematic Studies of the Sweet Potato and its Wild Relatives (Doctoral dissertation, University of Oxford).

Roullier, C., Duputié, A., Wennekes, P., Benoit, L., Bringas, V. M. F., Rossel, G., Tay, D., McKey, D., & Lebot, V. (2013). Disentangling the origins of cultivated sweet potato (*Ipomoea batatas* (L.) Lam.). *PLoS One*, *8*(5), e62707.

Saitou, N., & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular biology and evolution*, *4*(4), 406-425.

Shiotani, I., Yoshida, S., & Kawase, T. (1990). Numerical taxonomic analysis and crossability of diploid *Ipomoea* species related to the sweet potato. *Japanese Journal of Breeding*, *40*(2), 159-174.

Srisuwan, S., Sihachakr, D., & Siljak-Yakovlev, S. (2006). The origin and evolution of sweet potato (*Ipomoea batatas* Lam.) and its wild relatives through the cytogenetic approaches. *Plant Science*, *171*(3), 424-433.

Stetter, M. G., & Schmid, K. J. (2017). Analysis of phylogenetic relationships and genome size evolution of the *Amaranthus* genus using GBS indicates the ancestors of an ancient crop. *Molecular phylogenetics and evolution*, *109*, 80-92.

Steven G, N., & Subramanyam, R. (2009). Testing plant barcoding in a sister species complex of pantropical Acacia (Mimosoideae, Fabaceae). *Molecular ecology resources*, *9*, 172-180.

Stift, M., Kolář, F., & Meirmans, P. G. (2019). STRUCTURE is more robust than other clustering methods in simulated mixed-ploidy populations. *Heredity*, *123*(4), 429-441.

Structure Harvester (http://taylor0.biology.ucla.edu/structureHarvester/, accessed in May 2021)

Structure Software (https://web.stanford.edu/group/pritchardlab/structure_software/release_versions/v2.3.4/html/structure.html, accessed in April 2021)

Wadl, P. A., Olukolu, B. A., Branham, S. E., Jarret, R. L., Yencho, G. C., & Jackson, D. M. (2018). Genetic diversity and population structure of the USDA sweetpotato (*Ipomoea batatas*) germplasm collections using GBSpoly. *Frontiers in plant science*, *9*, 1166.

Wood, J. R., Carine, M. A., Harris, D., Wilkin, P., Williams, B., & Scotland, R. W. (2015). *Ipomoea* (Convolvulaceae) in Bolivia. *Kew Bulletin*, *70*(3), 31.

Wood, J. R., Muñoz-Rodríguez, P., Williams, B. R., & Scotland, R. W. (2020). A foundation monograph of *Ipomoea* (Convolvulaceae) in the New World. *PhytoKeys*, *143*, 1.

Wu, S., Lau, K. H., Cao, Q., Hamilton, J. P., Sun, H., Zhou, C., Eserman, L., Gemenet, D.C., Olukolu, B.A., Wang, H. and Crisovan, E., & Fei, Z. (2018). Genome sequences of two diploid wild relatives of cultivated sweetpotato reveal targets for genetic improvement. *Nature communications*, *9*(1), 1-12.

Yoshida, Y., Marubodee, R., Ogiso-Tanaka, E., Iseki, K., Isemura, T., Takahashi, Y., Muto, C., Naito, K., Kaga, A., Okuno, K., Ehara, H., & Tomooka, N. (2016). Salt tolerance in wild relatives of adzuki bean, *Vigna angularis* (Willd.) Ohwi et Ohashi. *Genetic Resources and Crop Evolution*, *63*(4), 627-637.

Figure legends

Figure 1. Geographic distribution of 46 *Ipomoea spp.* by defined regions "Zone 1", "Zone 2", "Zone 3", and "Zone 4". Six samples, corresponding to one *I. batatas* (origin Uganda) and five accessions from "Unknown" zones, are not represented in the map due to their unknown geographic location. The assigned zones represent the following geographic regions: Zone 1 = Central America; Zone 2 = Caribbean Islands; Zone 3 = South America (North countries); Zone 4 = South America (South countries).

Figure 2 – The phylogenetic tree resulting from analysis of 9223 SNPs using Neighbor-Joining (NJ) method, with bootstrap analysis of 1000 replicates. Numbers on branches are values of bootstrap support. Different colors represent different species. The phylogenetic tree shows three major clades: A, B, and C.

Figure 3. Result of Principal Component Analysis (PCA) of the *Batatas* complex materials studies. The PCA graph shows the clustering of the 52 *Ipomo*ea spp. accessions and revealed the existence of 3 major clusters.

Figure 4a. Delta K of a structure analysis of 52 accessions of *Ipomoea* spp. The figure shows the delta K determined for 9223 SNPs. The parameters set up to calculate the delta K were burnin in 50 000 to 500 000 MCMC, K 1 to 10, and 5 iterations.

Figure 4b. Result of STRUCTURE analysis with K= 6 (the best identified) of 52 accessions, representing 10 *Ipomoea spp.* The x-axis indicates the accessions while the y-axis each bar represents an individual, and each color represents the amount of assignment of each of the six genetic groups (or the relative proportion of genetic composition from the six different gene pools).

Tables

Table 1 – List of the sixteen species belonging to the *Batatas* series.

Order	Species	Ploidy level
1	*Ipomoea batatas*	2n=6x=90, or 2n=4x=60
2	*Ipomoea cordatotriloba*	2n=2x=30
3	*Ipomoea australis*	2n=2x=30*
4	*Ipomoea cynanchifolia*	2n=2x=30
5	*Ipomoea grandifolia*	2n=2x=30
6	*Ipomoea lacunosa*	2n=2x=30
7	*Ipomoea lactifera*	N/A**
8	*Ipomoea leucantha*	2n=2x=30
9	*Ipomoea littoralis*	2n=2x=30
10	*Ipomoea ramosissima*	2n=2x=30
11	*Ipomoea splendor-sylvae*	2n=2x=30
12	*Ipomoea tabascana*	2n=4x=60
13	*Ipomoea tenuissima*	2n=2x=30
14	*Ipomoea tiliacea*	2n=2x=30; 2n=4x=60*
15	*Ipomoea trifida*	2n=2x=30, 2n=4x=60
16	*Ipomoea triloba*	2n=2x=30

2n=2x=30*, 2n=4x=60* = Guerrero-Zurita, F., Ramírez, D. A., Rinza, J., Ninanya, J., Blas, R., & Heider, B. (2020). Potential Short-Term Memory Induction as a Promising Method for Increasing Drought Tolerance in Sweetpotato Crop Wild Relatives [*Ipomoea* series *Batatas* (Choisy) DF Austin]. *Frontiers in plant science*, *11*, 1326.

N/A** = Information not available in the passport data, or in the consulted literature.

Table 2 – List of the 52 *Ipomoea spp.* studied and the geographic regions from which they were derived based on gene bank passport data. The geographic regions were assigned according with the geographic location of the country. The assigned zones represent the following geographic regions: Zone 1 = Central America; Zone 2 = Caribbean Islands; Zone 3 = South America (North countries); Zone 4 = South America (South countries).

Sample	Species	Accession #	Country of Origin	Geographic region ("Zone #")
1	*I. batatas (Tanzania)*	*440166_1*	Uganda	Not assigned
2	*Ipomoea batatas*	*113641_086*	Unknown	Unknown
3	*Ipomoea batatas*	*105269_232*	Unknown	Unknown
4	*Ipomoea batatas (Beauregard)*	*440132_1*	Unknown	Unknown
5	*Ipomoea cordatotriloba*	*460296*	Argentina	Zone 4
6	*Ipomoea cordatotriloba**	*460345*	Paraguay	Zone 4
7	*Ipomoea cordatotriloba*	*460077*	Mexico	Zone 1
8	*Ipomoea cordatotriloba**	*460360*	Paraguay	Zone 4
9	*Ipomoea cordatotriloba**	*460585*	Argentina	Zone 4
10	*Ipomoea cordatotriloba**	*460164*	Paraguay	Zone 4
11	*Ipomoea cynachifolia*	*460149*	Brazil	Zone 3
12	*Ipomoea cynachifolia*	*460555*	Brazil	Zone 3
13	*Ipomoea grandifolia*	*460337*	Paraguay	Zone 4
14	*Ipomoea grandifolia*	*460583*	Uruguay	Zone 4
15	*Ipomoea grandifolia*	*460201*	Argentina	Zone 4
16	*Ipomoea grandifolia*	*460452*	Argentina	Zone 4
17	*Ipomoea leucantha*	*460204*	Argentina	Zone 4
18	*Ipomoea ramosissima*	*460047*	Peru	Zone 3
19	*Ipomoea ramosissima*	*460036*	Bolivia	Zone 2
20	*Ipomoea ramosissima*	*460032*	Bolivia	Zone 2
21	*Ipomoea ramosissima*	*460005*	Peru	Zone 3
22	*Ipomoea ramosissima*	*460566*	Peru	Zone 3
23	*Ipomoea ramosissima*	*460028*	Ecuador	Zone 3
24	*Ipomoea ramosissima*	*460722*	Argentina	Zone 4
25	*Ipomoea splendor-sylvae 1**	*460373*	Nicaragua	Zone 1
26	*Ipomoea splendor-sylvae 1**	*460383*	Nicaragua	Zone 1
27	*Ipomoea tiliacea*	*460528*	Cuba	Zone 2
28	*Ipomoea tiliacea*	*460531*	Cuba	Zone 2
29	*Ipomoea trifida*	*460429*	Nicaragua	Zone 1
30	*Ipomoea trifida*	*460745*	Guatemala	Zone 1
31	*Ipomoea trifida*	*460096*	Venezuela	Zone 3

Table 2 (continued).

32	*Ipomoea trifida*	*460195*	Venezuela	Zone 3
33	*Ipomoea trifida*	*460026*	Colombia	Zone 3
34	*Ipomoea trifida (4X)*	*430434*	Unknown	Unknown
35	*Ipomoea trifida*	*460021*	Venezuela	Zone 3
36	*Ipomoea trifida*	*113735_329*	Peru	Zone 3
37	*Ipomoea trifida*	*460022*	Colombia	Zone 3
38	*Ipomoea trifida*	*460663*	Mexico	Zone 1
39	*Ipomoea trifida*	*113735_258*	Peru	Zone 3
40	*Ipomoea trifida*	*113735_302*	Peru	Zone 3
41	*Ipomoea trifida*	*113735_283*	Peru	Zone 3
42	*Ipomoea trifida*	*460377*	Nicaragua	Zone 1
43	*Ipomoea trifida*	*107665_19_M19*	Peru	Zone 3
44	*Ipomoea trifida*	*107665_9_M9*	Unknown	Unknown
45	*Ipomoea trifida 2* (4X) I. batatas* in CIP database	*460577*	Ecuador	Zone 3
46	*Ipomoea triloba*	*460560*	Peru	Zone 3
47	*Ipomoea triloba*	*460309*	Paraguay	Zone 4
48	*Ipomoea triloba*	*460784*	Jamaica	Zone 2
49	*Ipomoea triloba*	*460078*	Mexico	Zone1
50	*Ipomoea triloba*	*460052*	Venezuela	Zone 3
51	*Ipomoea triloba*	*460116*	Colombia	Zone 3
52	*Ipomoea triloba*	*460517*	Ecuador	Zone 3

Accession information available at the International Potato Center (CIP) gene bank database: http://genebank.cipotato.org/gringlobal/search.aspx#.

[1*]*Ipomoea splendor-sylvae*- Identified in the CIP gene bank database as *Ipomoea umbraticola.*

[2*] *Ipomoea trifida*- Identified in the CIP gene bank database as *Ipomoea batatas.*

* *Ipomoea cordatotriloba** - is identified as *I. cordatotriloba* in the CIP gene bank (http://genebank.cipotato.org/gringlobal/search.aspx#), but it has been recognized as *I. australis*. Most recent search in the gene bank conducted on June 13, 2021. Wood et al. (2020) addressed *I. australis* as previously known as *I. cordatotriloba* var. *australis*.

Figure 1 – Geographic distribution of 46 *Ipomoea spp.* by defined regions "Zone 1", "Zone 2", "Zone 3", and "Zone 4". Six samples, corresponding to one *I. batatas* (origin Uganda) and five accessions from "Unknown" zones, are not represented in the map due to their unknown geographic location. The assigned zones represent the following geographic regions: Zone 1 = Central America; Zone 2 = Caribbean Islands; Zone 3 = South America (North countries); Zone 4 = South America (South countries).

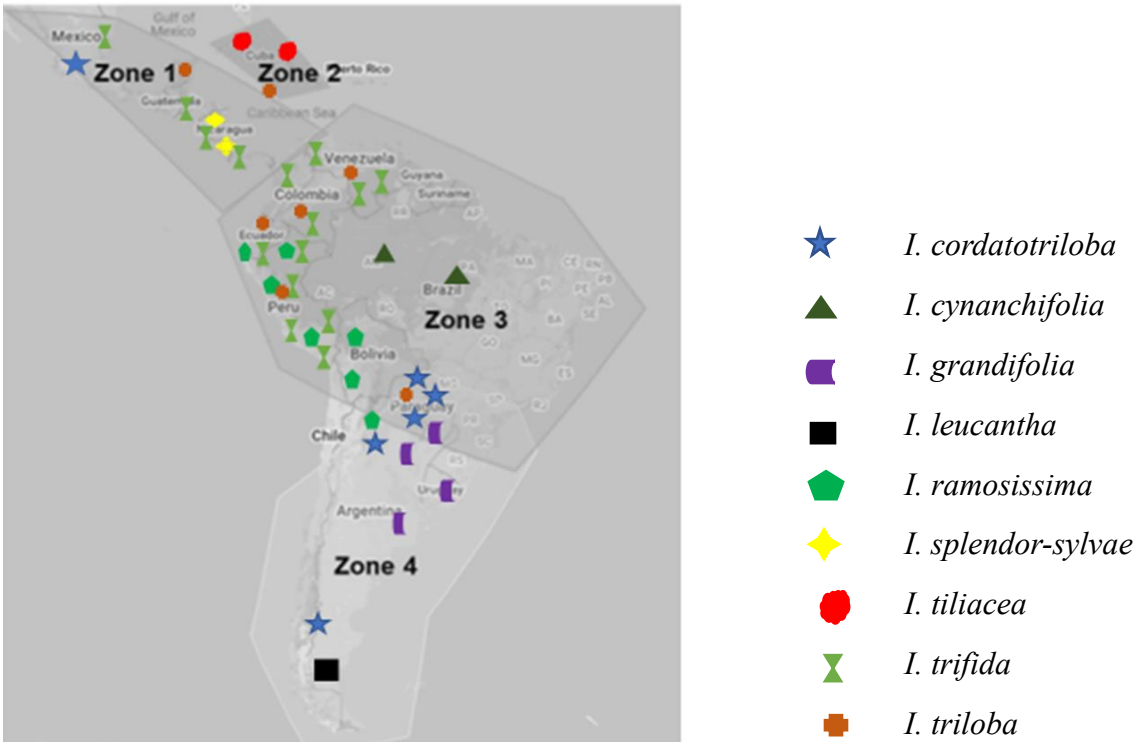


Figure 2 – The phylogenetic tree resulting from analysis of 9223 SNPs using Neighbor-Joining (NJ) method, with bootstrap analysis of 1000 replicates. Numbers on branches are values of bootstrap support. Different colors represent different species. The phylogenetic tree shows three major clades: A, B, and C.

.

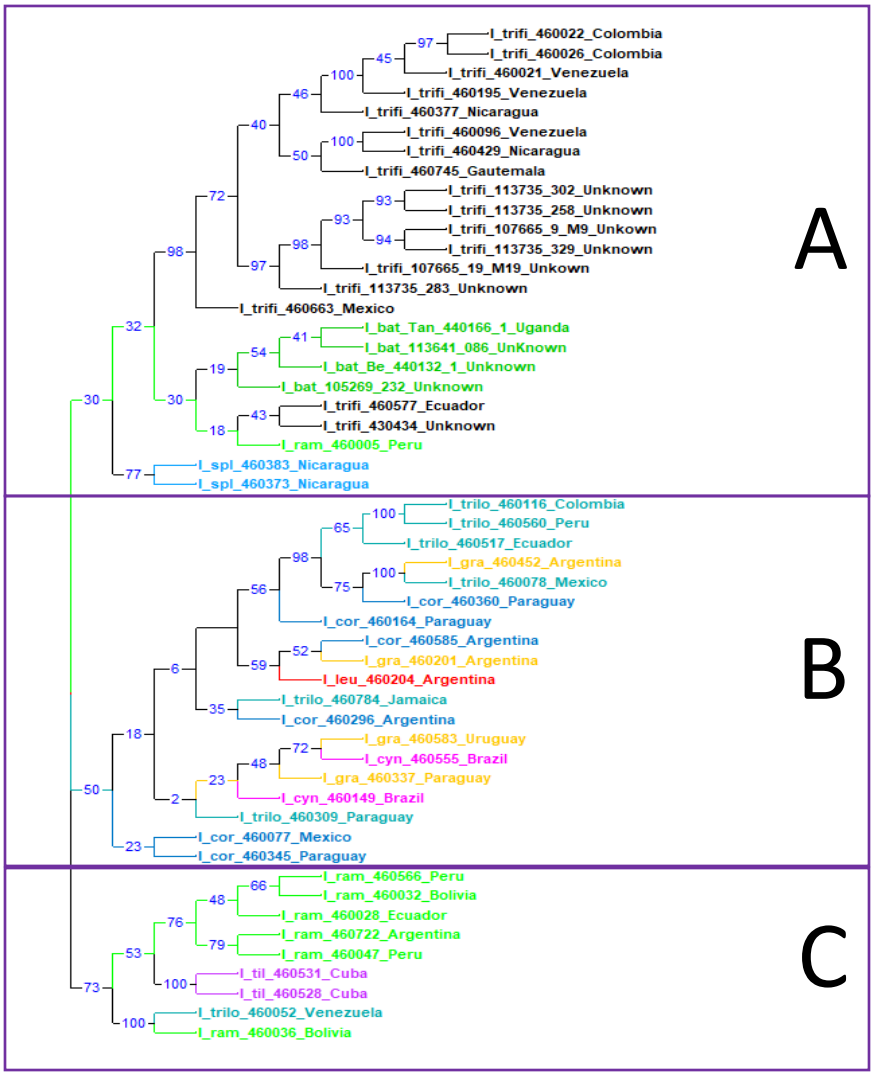


Figure 3 – Result of Principal Component Analysis (PCA) of the *Batatas* complex materials studies. The PCA graph shows the clustering of the 52 *Ipomoea* spp. accessions and revealed the existence of 3 major clusters.

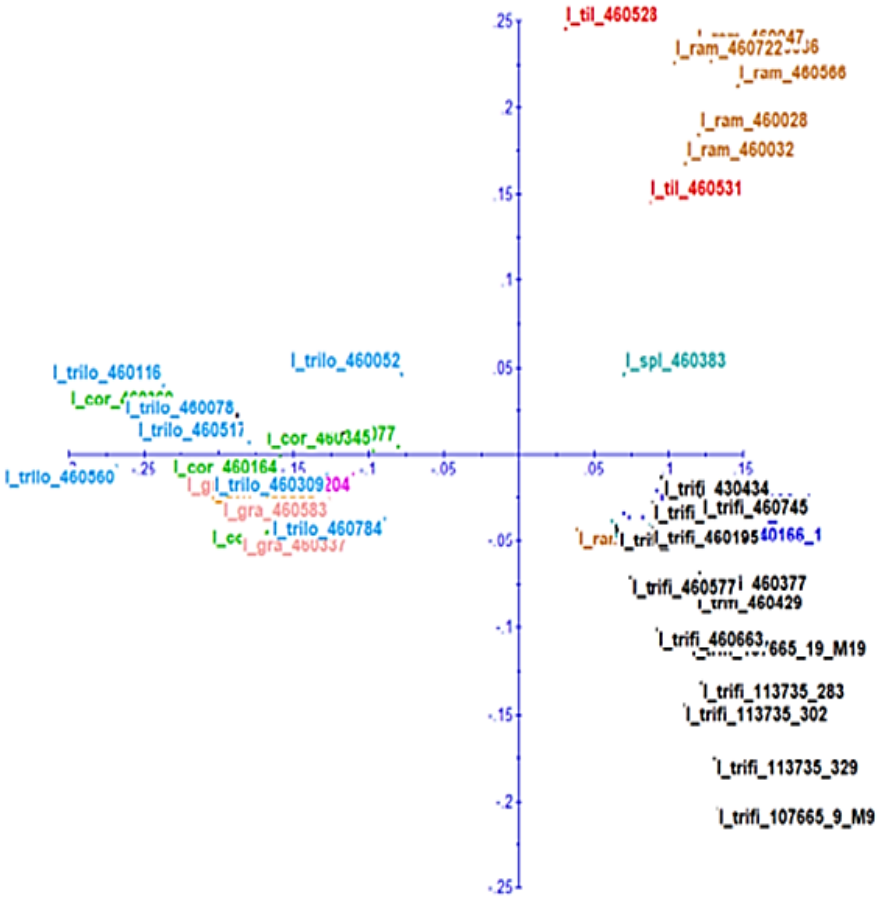


Figure 4a – Delta K of a structure analysis of 52 accessions of *Ipomoea* spp. The figure shows the delta K determined for 9223 SNPs. The parameters set up to calculate the delta K were burnin in 50 000 to 500 000 MCMC, K 1 to 10, and 5 iterations.

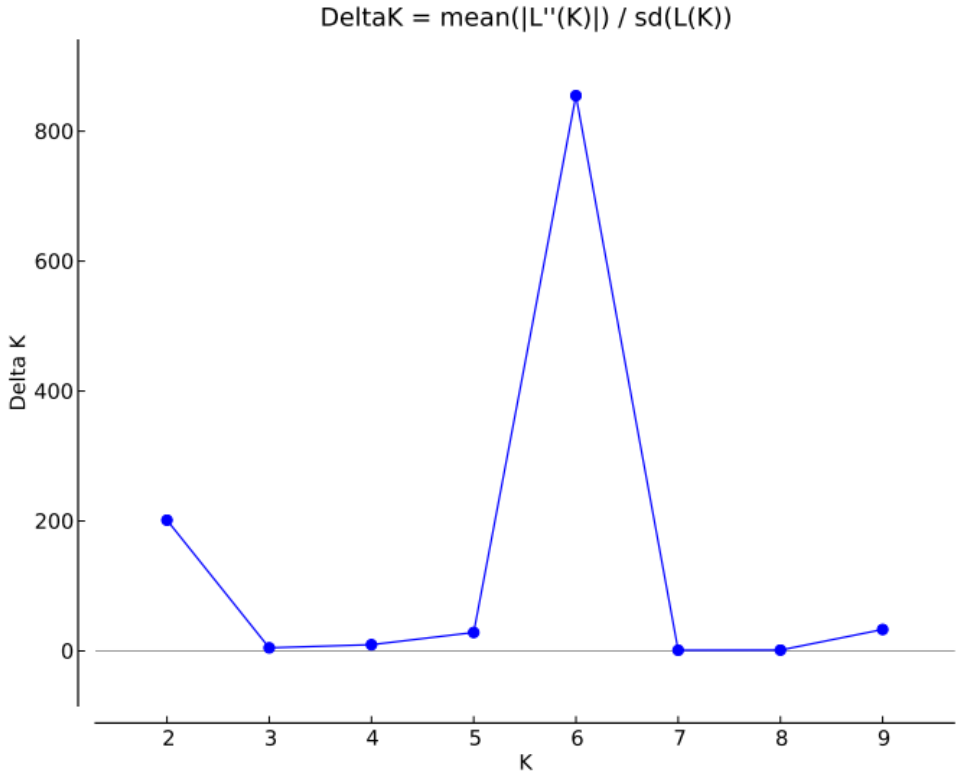
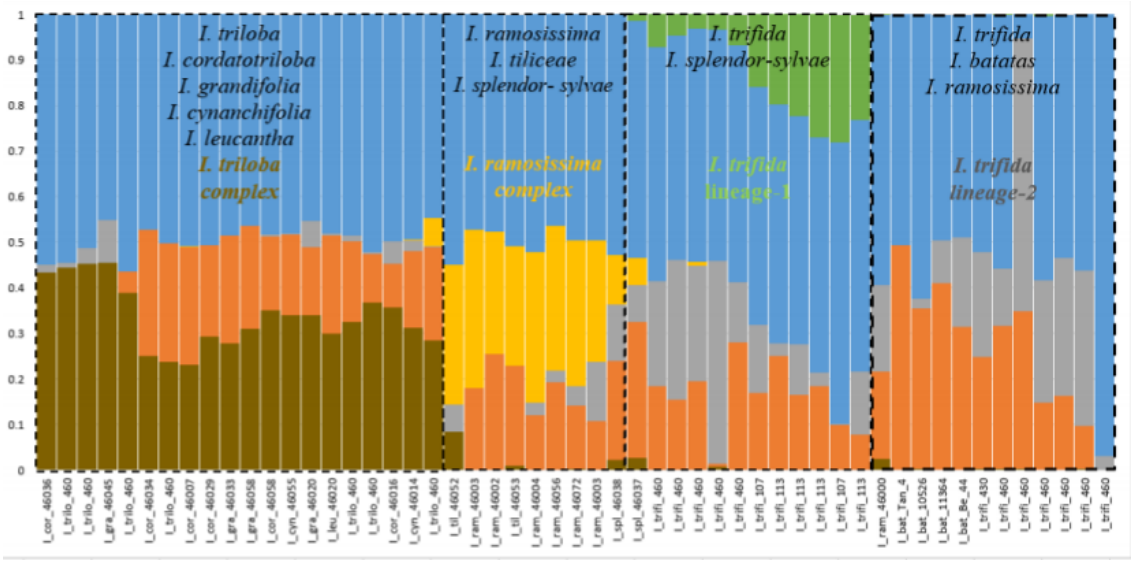


Figure 4b – Result of STRUCTURE analysis with K= 6 (the best identified) of 52 accessions, representing 10 *Ipomoea spp.* The x-axis indicates the accessions; the y-axis: each bar represents an individual, and each color represents the amount of assignment of each of the six genetic groups (or the relative proportion of genetic composition from the six different gene pools).



CHAPTER 3

Assessment of the Potential of Wild *Ipomoea spp.* for the Improvement of Drought Tolerance in Cultivated Sweetpotato *(Ipomoea batatas)* (L.) Lam

(In the format in which the paper was published in the Crop Science Journal (https://doi.org/10.1002/csc2.20363; paper submitted on May 8, 2020, accepted on September 17, 2020, and first published on October 3, 2020)

Assessment of the Potential of Wild *Ipomoea spp.* for the Improvement of Drought Tolerance in Cultivated Sweetpotato *(Ipomoea batatas)* (L.) Lam

Stella E. C. Nhanala[a,b*] and G. Craig Yencho[a*]

[a] Department of Horticultural Science, North Carolina State University, 2721 Founders Dr., Raleigh, NC 27607, USA; [b] Instituto de Investigação Agrária de Moçambique (IIAM), Av. das FPLM, nº 2698, Caixa Postal 3659, Maputo, Mozambique

*Corresponding author (craig_yencho@ncsu.edu)

Abbreviations: USDA ARS, United States Department of Agriculture Agricultural Research Service; Bea, Beauregard; Cyn, *Ipomoea cynanchifolia*; cv., Cultivar; CWR, Crop Wild Relatives; Hat, Hatteras; HFL, Horticulture Field Laboratory; Leu, *Ipomoea leucantha*; LSU, Louisiana State University; NCSU, North Carolina State University; Res, Resisto; Tba, *Ipomoea triloba*; Tfda, *Ipomoea trifida*; Tan, Tanzania;

ABSTRACT

Sweetpotato (*Ipomoea batatas* (L.) Lam) is cultivated worldwide, and it is a staple food in many developing countries. In some regions (e.g., Africa) drought is a major production constraint that results in significant yield loss. Climate change is predicted to result in even greater losses due to long periods of drought and elevated temperatures. The goal of this study was to assess the potential of wild *Ipomoea spp.* as a source of drought tolerance in cultivated

sweetpotato. We evaluated the drought tolerance of *I. batatas*, *I. cynanchifolia*, *I. leucantha*, *I. trifida* and *I. triloba* in a randomized complete block design, with five levels of simulated drought: control (daily irrigation), and no irrigation for 7, 9, 21 and 50 days. We observed that post drought re-irrigation of the wild species subjected to 21 d of stress resulted in plant recovery and an increase of the stomatal conductance of up to 99% in *I. leucantha*. However, under extreme stress (50 d) the wild plants did not respond to re-irrigation, resulting in up to 89% (*I. leucantha*) plant mortality. The wild species did not produce storage roots, while the *I. batatas* cultivars produced storage roots. Under 50 days of stress *I. batatas* had a survival rate between 44% (cv. Tanzania) and 89% (cv. Beauregard). We concluded that the wild genotypes screened may not be a valuable source of germplasm for drought tolerance and that significant levels of drought tolerance may exist in cultivated sweetpotato.

INTRODUCTION

Sweetpotato (*Ipomoea batatas* (L.) Lam) is a crop grown worldwide, and its storage roots and foliage provide a source of calories, vitamins and minerals for humans and animals (Padmaja, 2009; Pochapski et al., 2011; Mohanraj & Sivasankar, 2014). Being a good source of energy and nutrients, sweetpotato is a staple food in many Asian, sub-Saharan Africa and South Pacific countries (Grüneberg et al. 2005; Minot, 2010; Hotz et al., 2012; Mwanga et al., 2017; Low et al., 2020). Sweetpotato is also thought to be a drought tolerant crop, and due to this, it is grown in drought-prone areas (Kays & Bouwkamp,1985; Hahn et al., 1977).

Drought tolerance in plants is a complex phenomenon, and the response of a plant to drought varies according to a wide range of physiological and physical factors related to genotype (Hahn et al., 1977; Gajanayake et al., 2014a; Andrade et al., 2016). In a scenario of climate change,

with extended periods of drought being one of the impacts of environmental changes in agriculture, it is important to increase our basic knowledge of drought resistance in crop plants to prevent reduced yield in food crops.

Understanding how drought stress impacts stomatal conductance is critical to our understanding of how photosynthetic activity is affected by drought in plants. Stomatal conductance regulates plant gas exchange and plant water relations, and it is correlated with photosynthesis (Wong et al., 1979; Farquhar & Sharkey, 1982; Medrano et al., 2002; Kusumi et al., 2012). Therefore, studies of stomatal conductance may provide valuable insights into how plants survive when the uptake of carbon dioxide is reduced.

Taiz & Zeiger (2006) have noted that, in addition to stomatal conductance, leaf area plays an import role in photosynthesis, with a large leaf area implicating a high photosynthesis. Therefore, it can be speculated that leaf loss reduces the photosynthetic capacity of a plant. The opening or closure of stomata may also be as important as the ability of a plant to keep or lose its leaves when the plant is under stress. If a plant only wilts and then recovers after the period of stress, the plant may still be able to resume its normal photosynthetic capacity. However, if a plant loses a substantial portion of its leaves, probably, it will have its uptake of carbon dioxide reduced.

The yield of sweetpotato is evaluated mainly by the biomass of the storage roots (Kays & Bouwkamp,1985; Kivuva et al., 2015; Meyers et al., 2017). The formation of storage roots includes three growth stages: initiation, induction and development (Kays & Bouwkamp,1985; Du Plooy et al. 1992; Ravi et al., 2009; Villordon et al., 2009; Gajanayake et al., 2013; Gajanayake et al., 2014b; Solis et al., 2014; Meyers et al., 2017). Typically, sweetpotato storage root initiation occurs within 30 days after the crop has been planted and is characterized by the

differentiation of young and thick adventitious roots that develop into storage roots. The induction of storage roots is the beginning of the storage root formation process and the development of the storage roots is largely the result of starch accumulation. Starch is the major carbohydrate of storage roots (Kays & Bouwkamp,1985) and it is a moderately heritable trait (Amankwaah, 2019; Oloka, 2019). Sweetpotato yield can be greatly reduced when grown under deficient irrigation. Limited water uptake affects the formation of storage roots, and under these conditions, pencil roots may be formed instead of storage roots, resulting in reduced yield (Kays & Bouwkamp,1985; Meyers et al., 2017; Low et al., 2020). Regular irrigation appears to be very important for all of the storage root developmental stages of sweetpotato, but some stages may be more critical than others and each stage can be compromised if any of them are affected by irregular or no irrigation (Meyers et al., 2017).

Climate scientists predict that drought events will become prolonged and more erratic due to climate change (McDowell et al., 2008; Dai, 2013; Trenberth et al., 2014; Schlaepfer et al., 2017). These periods of water stress will undoubtedly lead to decreased yields of many of the staple crops such as sweetpotato. To prevent the reduction of yield due to extended and/or erratic periods of drought in sweetpotato it is crucial for us to develop germplasm that can serve as a source of improved drought tolerance. The utilization of crop wild relatives (CWR) of sweetpotato for improved drought tolerance could be a means to adapt its cultivated counterpart to long periods of drought.

The improvement of cultivated plants to abiotic and biotic stress via the utilization of CWR has been evaluated in many crops. Many of these studies show evidence that CWR are a valuable source of genetic diversity for breeding programs. The exploitation of CWR as a source of genetic diversity for drought tolerance purposes has been studied in food crops such as wheat

(Zaharieva et al., 2001; Placido et al., 2013), soybean (Chen et al., 2006), beans (Porch et al., 2013), barley (Suprunova et al., 2004; Honsdorf, 2014) and peanuts (Brasileiro et al. 2015). Zaharieva et al. (2001) identified accessions of *Aegilops geniculata,* a wild relative of wheat that has the potential to be used in cultivated wheat breeding programs to improve drought and heat stresses through improved leaf area and biomass production. Chen et al. (2006) identified the wild *Glycine soja* (PI 407155) as more tolerant to drought stress than its cultivated counterpart 'Essex' soybean. Honsdorf et al. (2014) evaluated introgression lines of wild barley, in order to select drought tolerant materials. They identified the line S42IL-121 as drought tolerant and suggested that S42IL – I21 could be used as a source of drought tolerant genes to improve barley. Brasileiro et al. (2015) did transcriptomic profiling of two wild relatives of peanut that resulted in the identification of drought-tolerant candidate genes such as expansin, nitrilase, NAC, and *bZIP*.

Khoury et al. (2015) used geographic and ecological habitat criteria to identify the CWR *I. Cynanchifolia, I. lacunosa, I. leucantha, I. littoralis, I. splendor-sylvae, I. trifida* and *I. triloba* with potential adaptation to drought-prone areas. The identification of genotypes of wild *Ipomoea spp.* that are tolerant to drought could be a step towards to the improvement of drought tolerance in sweetpotato.

We assessed the potential of four wild relatives identified by Khoury et al. (2015) to be exploited as a source of germplasm to improve drought tolerance in sweetpotato. We hypothesized that these wild relatives would be more tolerant to drought than the cultivated sweetpotato. The drought tolerance of eight genotypes belonging within the series *Batatas* was compared by comparing stomatal conductance, leaf loss, plant survival and biomass accumulation of wild and cultivated *Ipomoea spp.* under different levels of irrigation stress.

MATERIALS AND METHODS

Location

The study was performed during two years: May – October 2018, and May – October 2019, under greenhouse conditions at the *Horticulture Field Lab*oratory (HFL) greenhouses at North Carolina State University, Raleigh, NC***;*** GPS coordinates: 35.7847° N, 78.6821° W**.**

Plant Material

The first study was conducted during May – October 2018. We evaluated six genotypes of *Ipomoea spp.* (Table 1). One of these genotypes, PI618966, was identified as *I. trifida* in the U.S. National Plant Germplasm System (NPGS) GRIN-Global database. However, phenotypic studies conducted by members of the senior author's team at NCSU and unpublished molecular genetic studies conducted by Jan Kreuze at the International Potato Center (CIP) using 13 diagnostic SSR primers have determined that this accession, which was originally donated to the US NPGS by the CIP germplasm bank, is actually an *I. triloba* accession. In 2019, we studied eight *Ipomoea spp.* genotypes in total, and to better evaluate the yield (storage roots) of sweetpotato, we added two more cultivars of sweetpotato, Hatteras and Resisto (Table 1).

The wild species (*I. cynanchifolia*, *I. leucantha*, *I. trifida* and *I. triloba*) were identified as having the potential to be adapted to drought-prone areas based on their ecogeographic distribution by Khoury et al. (2015). To date, several *I. batatas* cultivars (cv.) have been evaluated in drought-related studies: Beauregard (Lewthwaite & Triggs, 2012; Gajanayake et al., 2014a; Taduri et al.,2017), Resisto (Van Heerden & Laurie, 2008; Laurie et al., 2015; Andrade et al., 2016) and Tanzania (Kivuva et al., 2015; Andrade et al., 2016). Heat tolerance has been positively correlated with drought tolerance in plants (Bouslama & Schapaugh, 1984; Havaux et al., 1988; Zaharieva et al., 2001). *I. batatas* cv. Hatteras was identified as a drought and heat

tolerant cultivar by Taduri et al., (2017), and it has also been regarded as a heat and drought tolerant cultivar by the Sweetpotato Breeding and Genetics Program at NCSU, who developed this cultivar (G.C. Yencho, personal observations).

Seed of the wild *Ipomoea spp*. were acquired from the U.S. National Plant Germplasm System (GRIN, 2018). Plants (ca. 15 cm in length) of the cultivated genotypes were obtained from the Sweetpotato Breeding and Genetics Program at NCSU. The seed was germinated at 22ºC in germinating mix substrate (Fafard ® Germinating Mix, Sun Gro Horticulture, Agawam, MA, USA). Germinated wild *Ipomoea spp.* plantlets and 15 cm cuttings of sweetpotato were transplanted into 1 L (one liter) pots containing 1:1 sand and soil mix. Each pot contained 1.3 Kg of soil, and the dosage of irrigation per day was 125 ml. Plants were fertigated once a week, and after one month after transplanting they were fertigated twice a week.

Experimental Design

The study was established as a randomized complete block design (RCBD). In 2018, the plants were assigned to four blocks and three treatments: Treatment 1 - control (daily irrigation), Treatment 2 - moderate (non-irrigated for 7 days (7 d)), and Treatment 3 - severe (non-irrigated during 21 days (21 d)). Within each block three plants of each genotype per treatment were established. However, six plants of *I. trifida* representing two control plants, two moderate (7 days (7d)) plants, and two (21 days (21d)) severe plants, did not survive after the transplantation making this study an unbalanced design with a population size of 210 individuals. In 2019, the plants were established with three blocks and three treatments, under the following treatments: Treatment 1 - control (daily irrigation), Treatment 2 - moderate (non-irrigated for 9 days (9 d)) and Treatment 3 - extra severe (non-irrigated for 50 days (50 d)). Within the three blocks of each

treatment, three plants of each genotype were planted, resulting in an experiment with 216 individuals.

The treatments were applied four weeks after the plants were transplanted. Due to a general lack of information regarding drought stress on wild *Ipomoea* species, the drought treatments were established based on visual observation of signs of drought (e.g., wilted leaves, and leaf loss) observed during the initial experiment. These observations resulted in the following drought treatments: moderate (7-9 d); severe (21 d); and extra severe (50 d). The moderate treatment was established as 7 to 9 d, based on the phenotypic observation of "complete" wilt status of the plant; the severe treatment was identified as the point where the wild species had completely lost their leaves and appeared totally dry to the observer; and the extra severe treatment, was defined based on the results observed in 2018, and was identified as a point where all genotypes (wild and cultivated) were completely desiccated in order to evaluate the ability of the cultivated genotypes to recover when completely dry and leafless. After the imposition of the respective drought treatment, the plants were re-irrigated until harvest.

The plants were irrigated using an automatic drip irrigation system, which was programmed to supply water to each plant daily, from 09:00 am – 09:03 am. Each plant was irrigated with 125 ml of water/day. The irrigation drippers were manually adjusted for each of the assigned treatments (moderate (7-9 d), severe (21 d) and extra severe (50 d)). Plants were fertigated once a week from transplantation up to four weeks old, and later on the plants were fertigated twice a week.

Data Collection

The following traits were measured during the experiments in 2018: 1) leaf loss - one week after re-irrigation of the severe treatment the fallen leaves were collected and weighed

using an SP401 *Ohaus Scout Pro Portable Electronic Balance (400g) (Ohaus Corporation, Pine Brook, NJ, USA*); 2) survival rate and recovery capacity - the percentage of plants surviving the treatments, and the percentage of plants that developed (recovered) new leaves from the stems of the leafless and/or dry plants under the 21 d and 50 d treatments (the plants that did not become completely dry were not recorded); 3) stomatal conductance - a fully expanded young leaf of each plant was recorded using an SC1-Leaf Porometer (Decagon Devices, Inc. Pullman, WA, USA) (stomatal conductance was recorded from: 11:00 am – 4:00 pm); 4) plant biomass – the fresh weight of the above and belowground parts of the plant; 5) Storage root count per plant – counted the number of storage roots for each plant; 6) Dry weight of the storage roots.

In 2019 we did not measure leaf loss and based on the plant survival results from 50 d, we did not evaluate plant recovery in these plants. Instead, we evaluated the plant status by associating the visual physiological status with a subjective scale where: 1 = at least 2/3 of the plant appeared to be dry; 2 = at least 2/3 of the plant had a wilted appearance; and 3 = normal plant appearance, similar to the control.

Data Analysis

Analysis of variance (ANOVA) of the data was conducted using SAS version 9.4 (SAS Institute Inc., Cary, NC) by determining the least significant means at $P \leq 0.05$ of the traits that were analyzed, using the Tukey method. A mixed model (proc mixed), with the statistical model: $Y = X\beta + Z\gamma + \mathcal{E}$, was used where: Y = response variable; X = design matrix; β = fixed-effects parameter; Z = design matrix; γ = random-effects parameter; and $\mathcal{E}$ = residual error term.

Percentage change of the traits evaluated were calculated using:

$$\%\ \text{of Stress treatment} = \frac{\text{Mean of the stress treament}}{\text{Mean of the control treatment}} * 100;\ \text{and}$$

% Change = 100% - % of the stress treatment

RESULTS

Survival rate and recovery capacity

The moderate treatments (7 d and 9 d without irrigation) resulted in some leaf loss (Figure 1); however, none of the plants grown under these levels of stress became completely dry. In the 21 d stress treatment, all of the cultivated genotypes survived, while 20% of the *I. cynanchifolia* and 8% of the *I leucantha* plants survived. These results reflected a recovery capacity from the 21 d (severe) treatment of: 80% (*I. cynanchifolia*), 92% (*I. leucantha*), and 100% (remaining wild *Ipomoea spp*.)

In terms of the 50 d stress treatment applied in 2019, all of the *I. cynanchifolia*, 89% of the *I. leucantha*, 44% of the *I. trifida* and 67% of the *I. triloba* plants died; while the wild species *I. leucantha* (11%), *I. trifida* (56%), and *I. triloba* (33%) exhibited some recovery. Even after the 50 d of stress, most of the cultivated genotypes never became completely desiccated unlike the CWR, with survival rates for Beauregard, Hatteras, Resisto and Tanzania of 89%, 78%, 33% and 44%, respectively. Due to the high rate of death of wild *Ipomoea spp.*, and due to the fact that some cultivated genotypes never achieved a death status, we did not evaluate the recovery capacity of the plants under 50 d stress.

Leaf loss

In 2018, all the genotypes were negatively affected by the 21 d treatment (Figure 1), with significant leaf shedding occurring in each of the genotypes compared to the control treatment. In general, there was a greater percentage change between the control and the 21 d treatments, but that difference was not always observed between the control and the 7 d treatments. For instance, Beauregard, lost only 1% of its leaves when comparing the control and 7 d treatments, while there was a difference of 48% between the control and the 21 d treatment. Similarly, *I.*

trifida exhibited minimal leaf loss between the control and 7 d treatment at 1%, while leaf loss between the control and the severe treatment amounted to 54%. *I. triloba* had a 17% decrease of leaf loss between the control and moderate stress treatment and had the lowest difference (23%) between the control and the 21 d treatments. For *I. cynanchifolia*, the severe treatment caused more than the double (112%) of the leaf loss when compared with the control treatment. *I. cynanchifolia* was the genotype that was the most affected by the leaf loss for both treatments: moderate (73%) and severe (112%). In general, based on leaf loss, *I. triloba* was more tolerant to long-term (21 d) drought than all other genotypes evaluated. In 2019, it was pre-determined that the third treatment would be when all the cultivated genotypes completely lost their leaves in order to evaluate their recovery ability. In 2018, the severe treatment proved to be statistically different from the control and moderate treatments. Therefore, in 2019, we inferred that the amount of leaf loss representing the extra severe treatment, i.e., complete dry and leafless plants of wild and cultivated genotypes, would also be statistically different from the other two treatments evaluated.

Stomatal conductance

Stomatal conductance was reduced both moderate and severe water stress after 7 d and 21 d of no irrigation (Figure 4a, 4b). None of the genotypes presented significant differences between the moderate and severe treatments. *I. leucantha*, and *I. triloba* did not exhibit statistically significant differences in stomatal conductance between all three treatments. Upon application of re-irrigation after the 7 d drought treatment, the stomatal conductance of all the genotypes under the 7 d drought treatment exhibited increased stomatal conductance (Figure 4b). *I. leucantha* had almost the double (99%) of the stomatal conductance of the control treatment. *I.*

triloba had an increase of approximately 6% of the percentage when the plants received the moderate treatment.

As in 2018, one week after applying the 9 d drought treatment in 2019 the stomatal conductance of all the genotypes were reduced, with a percentage decrease ranging 11% - 75% (Figure 4c). Statistical differences were observed between the control and moderate treatments within Beauregard, *I. cynanchifolia*, Hatteras, Resisto and Tanzania. Upon re-irrigation of the 9 d treatment stomatal conductance increased 20% - 56% (Figure 4d). The plants exposed to drought stress had a percentage decrease in an order of 50% -93%. Except for *I. triloba* and *I. trifida*, we observed a significant difference within the 9 d and 50 d treatments for all genotypes.

Stomatal conductance of recovered plants. After 21 d of water stress, all of the CWR had dropped their leaves and their stems appeared to be desiccated. However, roughly one month after the re-irrigation of the plants under stress for 21 d, the wild *Ipomoea spp*. produced new leaves from the seemingly desiccated stems (Figure 4e). The stomatal conductance of the recovered plants increased, with a percentage change between 30 % - 99 %. Compared to the 21 d treatment, the plants under the 50 d of stress did not recover their leaves after the re-irrigation.

Plant Status

The imposition of drought in all genotypes elicited phenotypic changes that were scorable in our greenhouse-based studies. One week after applying treatment, the 9 d treatment plants began to exhibit wilting stress (Table 2a,b).The extension of the period of drought resulted in more pronounced changes in plant phenotypic appearance in all genotypes.

Plant biomass

Biomass - aboveground. The fresh weight of the aboveground biomass was negatively affected by all the drought stress treatments (Table 3a,b). *I. trifida* was the genotype least

affected by the 7 d treatments, *while I. cynanchifolia* (11%) was the most affected by the 21 d stress (70%). *I. cynanchifolia* was negatively affected by the 50 d treatment, and none of the *I. cynanchifolia* plants survived in the extra severe treatment. *I. triloba* had the lowest survival rate under the 50 d treatment (82%).

Biomass - belowground. Belowground biomass was reduced by all the drought stress treatments (Table 3c,d). In 2018, *I. trifida* was the genotype least affected by the 7 d treatment with an increase of 41%, while *I. cynanchifolia* was the most affected by the 21 d stress (94 %). *I. cynanchifolia* was severely affected by the 50 d treatment, with none of the plants surviving this treatment.

Yield of the cultivated sweetpotatoes

Storage root count per plant. None of the wild *Ipomoea spp.* produced storage roots. For Beauregard, there was not a statistical difference between the control, 7 d and 21 d treatments (Table 4a) in terms of storage root numbers. The same results were observed for Tanzania. Tanzania storage root number was reduced in the 7 d (27%) and 21 d (91%) treatments. Beauregard was negatively affected almost to the same extent by the 7 d and 21 d treatments, with a decrease of about 16% (Table 4a). Tanzania was highly affected the by the 9 d and 50 d treatments, with the number of roots per plant being reduced to 0% when the plant was under stress for 50 d (Table 4b) . Hatteras and Resisto, two clones known for their drought tolerance had fewer storage roots at 9 d compared to the 50 d treatment. For Hatteras, the percentage decrease was of 44% (9 d) and 33% (50 d), while Resisto's productivity was reduced by half when grown under 9 d of stress, and 40% when exposed to severe stress.

Dry weight of the storage roots. Tanzania yield, as measured by dry weight, was less than all of treatments, with 21 d and 50 d treatments resulting in the most yield loss (Table 4c,d). The

50 d treatment reduced yield in all genotypes, with a percentage ranging from 92% to 100 % (Table 4d).

DISCUSSION

Our results suggest that the wild species studied were more sensitive to water stress than their cultivated counterparts and they responded to water stress differently. In response to severe drought stress *I. leucantha*, *I. trifida*, and *I. triloba* dropped their leaves, and their stems desiccated to the point that the plants were seemingly dead. However, after the drought was ended, they produced new foliage from seemingly dead tissue and exhibited some recovery.

Tanzania, *I. cynanchifolia* and I. *leucantha* wilted and lost their leaves faster than Beauregard, *I. trifida*, and *I. triloba (Figure 1)*. The effects of 7 d of stress resulted in a 24% leaf loss in Tanzania, 73% in *I. cynanchifolia* and 15% in *I. leucantha*, while the other genotypes Beauregard, *I. trifida*, and *I. triloba* increased 1%, 1% and 17 %, respectively. Taiz and Zeiger (2006), recognized leaf loss due to water stress as a mechanism to adjust a plant's leaf area to prevent water loss (via transpiration) and improve the performance of the plant in a long-term drought event. Tanzania appeared to be more sensitive to drought than the other cultivated genotypes studied. *I. cynanchifolia* and *I. leucantha*, which had 21% and 8% mortality, respectively, did not survive the 21 d stress while the other wild species recovered 100%. After 50 days of stress 100% of the *I. cynanchifolia* plants died, followed by *I. leucantha* (89%); 44% of I. *trifida* and 67% of *I.triloba* that did not recover from the 50 days of stress. .

Leaf loss in the CWR generally occurred from the bottom up (Figure 3). Krammer (1983) argued that the death of the older leaves, in terms of drought tolerance, has no effect in plants, as the rates of transpiration and photosynthesis of the old leaves contribute minimally to plant

growth. Leaf loss as a plant survival strategy is undoubtedly complex. To understand this assumption with a bit more of detail, it is important to understand the role of stomatal conductance. The wild relatives studied here could have a mechanism that allows to them to mitigate drought through the reduction of stomatal conductance to prevent water loss via transpiration. The reduction of stomatal conductance inhibits photosynthesis, as stomatal conductance is positively correlated with photosynthesis (Wong et al., 1979; Farquhar & Sharkey, 1982; Medrano et al., 2002; Kusumi et al., 2012). With the closure of stomata, plants may suffer due to the reduction of the uptake of carbon dioxide, preventing photosynthesis. The recovery of the wild species after the 21 d stress, resulted in recovered plants with higher stomatal conductance than the control and moderate plants (Figure 4e) supports the hypothesis that leaf loss might not be a strategy to protect the plant. The reason is while the plants lost their leaves to prevent water loss, they also reduced the uptake of carbon dioxide, and once the plants sensed that water stress was not an issue anymore, stomatal conductance increased in order to uptake the carbon dioxide that was suppressed during the time the plant was under stress. Except for *I. trifida*, the stomatal conductance of the recovered plants had higher values than the stomatal conductance of the plants that were under the control and moderate treatments (Figure 4e).

All the wild species that were evaluated in our study have their origin in Latin America (Table 1; GRIN, 2018), where the environmental conditions are different from where the studies were conducted (North Carolina), and they naturally grow in uncultivated conditions. Tanzania, a medium maturity African released landrace adapted to dry areas (Mwanga et al., 2001), was the first cultivated genotype showing signs of drought in our study, with a leaf loss of 24% after 7 d of stress (Figure 1), and the stomatal conductance at that same point, being reduced at about 90%

of its normal capacity (Figure 4a). All the remaining cultivated genotypes were cultivars developed in the U.S. (Table 1). Tanzania has been used as a check cultivar in drought-prone environments and it is thought to be drought tolerant (Grüneberg et al., 2015; Kivuva et al., 2015; Andrade et al., 2016). However, it did not perform as well as the other cultivated genotypes. Probably the genotype x environment interaction (G x E) is playing a role in the way how Tanzania responds to drought, as Tanzania is not adapted to the environmental and daylength conditions of North Carolina.

None of the CWR of *Ipomoea spp.* produced storage roots, and our general observations suggest that the *I. batatas* cultivars studied were more tolerant to drought than the CWR studied. Sweetpotato storage roots are mainly composed of starch (Kays & Bouwkamp,1985; Kitahara et al, 2017; Amankwaah, 2019). It is possible that the storage roots served as a source/reserve of carbohydrate that allowed the plant to continue to perform its "normal" metabolic activities even under water stress. Thus, the cultivated genotypes when under stress, used the carbon from starch as a source of reserve for the plant. This hypothesis is supported by Huber (2000) and Taiz & Zeiger (2006). Starch and sucrose are the carbohydrate end products of photosynthesis (Huber, 2000; Taiz & Zeiger, 2006). Huber (2000) recognized the role of starch in plant metabolism as a reserve of carbon. The starch that is found in sink tissues (the storage roots of sweetpotato), are a source of reserve of carbon. The wild relatives, not having this source of reserve, once they sense drought, they begin to drop their leaves as a mechanism of defense against drought (Taiz & Zeiger, 2006). Beauregard is a cultivar that matures early (Rolston et e., 1987), Hatteras has an early to mid-cycle maturity (Yencho & Pecota, 2009), Resisto has a mid to late cycle (Kapinga et al., 2010), and Tanzania is a medium to late maturity cultivar (Mwanga et al., 2001). The initiation of storage roots begins around two to three weeks after transplantation (Gajanayake et

al., 2013; Meyers et al., 2017), but this time can vary dramatically according to the cultivar. Our treatments were applied about two weeks after the early cultivars had started the initiation of storage roots. It is possible that the mid to late cultivars, began the initiation of storage roots a bit later, and the treatments (water stress) that we applied were a limiting factor in the initiation of the storage roots for those mid to late cultivars (Figure 2). Added to that, the genotype by environment (G x E) interaction also played a role in the storage root set. Thus, based on the results that we observed in terms of storage root set per plant (Figure 2), and the absence of storage roots within the wild genotypes, we interpreted that because the wild species did not have the source of carbohydrate reserves to cope with the drought tolerance they showed signs of drought earlier than the cultivated genotypes. Tanzania, a short-day adapted, drought tolerant land race from eastern Africa, that was grown under long-day conditions in these experiments, produced only a few small storage roots. Its lack of storage roots probably did not provide as much reserve as the other cultivated genotypes, and because of this it may have expressed earlier signs of drought such as leaf loss compared to the other cultivars (Figure 1). We assume therefore that storage roots play an important role in a plant's ability to tolerate water deficit stress, and that role can be even greater if the process of initiation of storage roots is not affected. Resisto, a mid to late cultivar, had the second lowest number of storage roots per plant and had the lowest survival (33%) rate under extra severe conditions, followed by Tanzania (44%), Hatteras (78%), and Beauregard (89%). These survival rates also support the hypothesis that the storage roots were a source of reserves or the plants under stress.

The wild genotypes that were evaluated in this study were diploids, while the cultivated genotypes were hexaploids. Polyploid plants are known for having certain advantages over diploid plants. Hybrid vigor and heterosis are some of the advantages of polyploids over the

diploids (Comai, 2005; Beest et al., 2012; Sattler et al., 2016). Zhang et al. (2015) observed that autotetraploid apples increased drought tolerance in apple. Yang et al. (2014) observed that autotetraploid lines of rice responded better to drought tolerance than the corresponding diploid lines. It could be that the cultivated genotypes (autoploid hexaploids) are taking advantage of their genome duplication over the diploid ones.

The role of leaf loss as a strategy to protect the plant against the stress is unclear. Based on our observation of near complete stem desiccations of some of the CWR, we speculate that the wild *Ipomoea spp.* could be resurrection plants. Resurrection plants are plants that can tolerate desiccation to 5% relative water content for extended periods and yet resume full metabolic activity on re-watering (Farrant et al., 2007). Transcriptomics studies have been done on resurrection plants and would help us to understand better the genetic mechanism behind the recovery of the wild species that were completely dry and recovered after they were irrigated. Yet, our studies suggest that in the case of sweetpotato, trying to study materials that produce storage roots may be a more feasible and productive approach to understand drought tolerance in this crop.

In conclusion, at the phenotypic level, the cultivated genotypes evaluated in our study were more tolerant to drought than the wild species studied. Also, we speculate that the storage roots of sweetpotato may play an important role into the response of cultivated sweetpotatoes to environmental stresses. Last, in terms of the yield of sweetpotato, since none of the CWR of *I. batatas* produced storage roots and they did not appear to be more tolerant of drought in general compared to the cultivated sweetpotatoes, probably these species may not be the best option to improve drought tolerance in sweetpotato.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ACKNOWLEDGMENTS

This work was undertaken as part of the initiative “Adapting Agriculture to Climate Change: Collecting, Protecting and Preparing Crop Wild Relatives”, which is supported by the Government of Norway. The project is managed by the Global Crop Diversity Trust with the Millennium Seed Bank of the Royal Botanic Gardens, Kew and implemented in partnership with national and international gene banks and plant breeding institutes around the world. For further information, see the project website: http://www.cwrdiversity.org/.

We thank Dr. Richard E. Miller for sharing his experience on the *Batatas* series, and Chris Heim for his assistance with the irrigation system used in this study.

REFERENCES

Amankwaah, V. A. (2019). Phenotyping and Genetic Studies of Storage Root Chemistry Traits in Sweetpotato. PhD Dissertation, North Carolina State University, Raleigh, NC, USA.

Andrade, M. I., Naico, A., Ricardo, J., Eyzaguirre, R., Makunde, G. S., Ortiz, R., & Grüneberg, W. J. (2016). Genotype× environment interaction and selection for drought adaptation in sweetpotato (*Ipomoea batatas* [L.] Lam.) in Mozambique. *Euphytica*, *209*(1), 261-280.

Beest, M., Le Roux, J. J., Richardson, D. M., Brysting, A. K., Suda, J., Kubešová, M., & Pyšek, P. (2012). The more the better? The role of polyploidy in facilitating plant invasions. *Annals of botany*, *109*(1), 19-45.

Bouslama, M., & Schapaugh, W. T. (1984). Stress tolerance in soybeans. I. Evaluation of three screening techniques for heat and drought tolerance 1. *Crop science*, *24*(5), 933-937.

Brasileiro, A. C., Morgante, C. V., Araujo, A. C., Leal-Bertioli, S. C., Silva, A. K., Martins, A. C., & Saraiva, M. A. (2015). Transcriptome profiling of wild Arachis from water-limited environments uncovers drought tolerance candidate genes. *Plant molecular biology reporter*, *33*(6), 1876-1892.

Chen, Y., Chen, P., & de los Reyes, B. G. (2006). Differential responses of the cultivated and wild species of soybean to dehydration stress. *Crop science*, *46*(5), 2041-2046.

Comai, L. (2005). The advantages and disadvantages of being polyploid. *Nature reviews genetics*, *6*(11), 836-846.

Dai, A. (2013). Increasing drought under global warming in observations and models. *Nature climate change*, *3*(1), 52-58.

Du Plooy, C. P., Van den Berg, A. A., Hammes, P. S., & Holtzhausen, L. C. (1992). Storage root formation at individual nodes of the sweet potato (*Ipomoea batatas* (L.) Lam). *South African Journal of Plant and Soil*, *9*(3), 136-138.

Farquhar, G. D., & Sharkey, T. D. (1982). Stomatal conductance and photosynthesis. *Annual review of plant physiology*, *33*(1), 317-345.

Farrant, J. M., Brandt, W., & Lindsey, G. G. (2007). An overview of mechanisms of desiccation tolerance in selected angiosperm resurrection plants. Plant Stress, 1(1), 72-84.

Gajanayake, B., Reddy, K. R., Shankle, M. W., & Arancibia, R. A. (2013). Early-season soil moisture deficit reduces sweetpotato storage root initiation and development. *HortScience*, *48*(12), 1457-1462.

Gajanayake, B., Reddy, K. R., Shankle, M. W., & Arancibia, R. A. (2014a). Growth, developmental, and physiological responses of two sweetpotato (Ipomoea batatas L.[Lam]) cultivars to early season soil moisture deficit. *Scientia Horticulturae*, *168*, 218-228.

Gajanayake, B., Reddy, K. R., Shankle, M. W., Arancibia, R. A., & Villordon, A. O. (2014b). Quantifying storage root initiation, growth, and developmental responses of sweetpotato to early season temperature. *Agronomy Journal*, *106*(5), 1795-1804.

GRIN The Germplasm Resources Information Network (2018) (https://npgsweb.ars-grin.gov/gringlobal/search.aspx , accessed in March 2018).

Grüneberg, W. J., Manrique, K., Zhang, D., & Hermann, M. (2005). Genotype× environment interactions for a diverse set of sweetpotato clones evaluated across varying ecogeographic conditions in Peru. *Crop Science*, *45*(6), 2160-2171.

Grüneberg, WJ; Ma, D.; Mwanga, ROM; Carey, EE; Huamani, K.; Diaz, F.; Eyzaguirre, R.; Guaf, E.; Jusuf, M.; Karuniawan, A.; Tjintokohadi, K.; Song, YS; Anil, SR; Hossain, M.;

Rahaman, E.; Attaluri, SI; Some, K.; Afuape, SO; Adofo, K.; Lukonge, E.; Karanja, L.; Ndirigwe, J.; Ssemakula, G.; Agili, S.; Randrianaivoarinovy, JM; Chiona, M.; Chipungu, F.; Laurie, SM; Ricardo, J.; Andrade, M.; Rausch Fernandes, F.; Mello, AS; Khan, MA; Labonte, DR; Yencho, GC (2015). Advances in sweetpotato breeding from 1992 to 2012. in Potato and Sweetpotato in Africa: Transforming the Value Chains for Food and Nutrition Security, eds J. Low, M. Nyongesa, S. Quinn, and M. Parker (Oxfordshire: CAB International), 3–68.

Hahn, S. K., Alvim, P. D. T., & Kozlowski, T. T. (1977). Ecophysiology of tropical crops. *Acad. Press. New York*, 237-247.

Havaux, M., Ernez, M., & Lannoye, R. (1988). Correlation between heat tolerance and drought tolerance in cereals demonstrated by rapid chlorophyll fluorescence tests. *Journal of Plant Physiology*, *133*(5), 555-560.

Honsdorf, N., March, T. J., Berger, B., Tester, M., & Pillen, K. (2014). High-throughput phenotyping to detect drought tolerance QTL in wild barley introgression lines. *PLoS One*, *9*(5).

Hotz, C., Loechl, C., de Brauw, A., Eozenou, P., Gilligan, D., Moursi, M., & Meenakshi, J. V. (2012). A large-scale intervention to introduce orange sweet potato in rural Mozambique increases vitamin A intakes among children and women. *British journal of nutrition*, *108*(1), 163-176.

Huber, S. C. (2000). Chapter 12. Starch-sucrose metabolism and assimilate partitioning, pp. 163-174. In. *Photosynthesis: A Comprehensive Treatise*, Raghavendra, A.S., Ed., Cambridge University Press, Cambridge.

Kapinga, R. S., Tumwegamire, S., Ndunguru, J., Andrade, M. I., Agili, S., & Mwanga, R. O. M. (2010). Catalogue of orange-fleshed sweetpotato varieties for Sub-Saharan Africa. International Potato Center (https://cipotato.org/wp-content/uploads/2014/08/005374.pdf, accessed on February 12, 2019).

Kays, S. J., & Bouwkamp, J. C. (1985). The physiology of yield in sweet potato. *Sweet Potato Products: A Natural Resource of the Tropics*, 79-133.

Khoury, C. K., Heider, B., Castañeda-Álvarez, N. P., Achicanoy, H. A., Sosa, C. C., Miller, R. Scotland, R.W., Wood, J.R.I., Rossel, G. Eserman, L.A., Jarret, R. L., Yencho, G. C., Bernau, V., Juarez, H., Sotelo, S., de Haan, S. & Struik, P. C. (2015). Distributions, ex situ conservation priorities, and genetic resource potential of crop wild relatives of sweetpotato [*Ipomoea batatas* (L.) Lam., I. series Batatas]. *Frontiers in Plant Science*, *6*, 251.

Kitahara, K., Nakamura, Y., Otani, M., Hamada, T., Nakayachi, O., & Takahata, Y. (2017). Carbohydrate components in sweetpotato storage roots: their diversities and genetic improvement. *Breeding science*, 16135.

Kivuva, B. M., Githiri, S. M., Yencho, G. C., & Sibiya, J. (2015). Screening sweetpotato genotypes for tolerance to drought stress. *Field Crops Research*, *171*, 11-22.

Krammer, P. J. (1983). Chapter 12 - Water Deficits in Plant Growth and Chapter 13 - Drought Tolerance and Water Use Efficiency, pp. 342 – 415. In. Water relations of plants. Academic Press, New York.

Kusumi, K., Hirotsuka, S., Kumamaru, T., & Iba, K. (2012). Increased leaf photosynthesis caused by elevated stomatal conductance in a rice mutant deficient in SLAC1, a guard cell anion channel protein. *Journal of experimental botany*, *63*(15), 5635-5644.

Laurie, R. N., Laurie, S. M., Du Plooy, C. P., Finnie, J. F., & Van Staden, J. (2015). Yield of drought-stressed sweet potato in relation to canopy cover, stem length and stomatal conductance. *Journal of Agricultural Science*, *7*(1), 201.

Lewthwaite, S. L., & Triggs, C. M. (2012). Sweet potato cultivar response to prolonged drought. *Agronomy New Zealand*, *42*, 1-10.

Low, J. W., Ortiz, R., Vandamme, E., Andrade, M., Biazin, B., & Grüneberg, W. J. (2020). Nutrient-dense orange-fleshed sweetpotato: advances in drought-tolerance breeding and understanding of management practices for sustainable next-generation cropping systems in sub-Saharan Africa. *Frontiers in Sustainable Food Systems*. 4, 50.

McDowell, N., Pockman, W. T., Allen, C. D., Breshears, D. D., Cobb, N., Kolb, T., & Yepez, E. A. (2008). Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought?. *New phytologist*, *178*(4), 719-739.

Medrano, H., Escalona, J. M., Bota, J., Gulías, J., & Flexas, J. (2002). Regulation of photosynthesis of C3 plants in response to progressive drought: stomatal conductance as a reference parameter. *Annals of botany*, *89*(7), 895-905.

Meyers, S. L., Arancibia, R. A., Shankle, M. W., Main, J., Bandara, K. G. M. C. P., & Reddy, K. R. (2017). *Sweet potato storage root initiation*. Mississippi State University Extension Service. (http://extension.msstate.edu/publications/sweetpotato-storage-root-initiation, accessed on October 29, 2018).

Minot, N. 2010. Staple food prices in Malawi. Food Security Collaborative Working Papers 58558, Michigan State University, Department of Agricultural, Food, and Resource Economics.

Mohanraj, R., & Sivasankar, S. (2014). Sweet Potato (*Ipomoea batatas* [L.] Lam)-A valuable medicinal food: A review. *Journal of medicinal food*, *17*(7), 733-741.

Mwanga, R. M., Odongo, B., Ocitti p'Obwoya, C., Gibson, R. W., & SMIT, N. M. (2001). Release of five sweetpotato cultivars in Uganda. *HortScience*, *36*(2), 385-386.

Mwanga, R. O., Andrade, M. I., Carey, E. E., Low, J. W., Yencho, G. C., & Grüneberg, W. J. (2017). Sweetpotato (*Ipomoea batatas* L.). In *Genetic improvement of tropical crops* (pp. 181-218). Springer, Cham.

Oloka, B. M. (2019). Genetic Linkage Map Construction and QTL Analysis of Important Pest and Agronomic Traits in Two Bi-parental Sweetpotato SNP Mapping Populations. PhD. Dissertation, North Carolina State University, Raleigh, NC, USA.

Padmaja, G. (2009). Uses and nutritional data of sweetpotato. In *The sweetpotato* (pp. 189-234). Springer, Dordrecht.

Placido, D. F., Campbell, M. T., Folsom, J. J., Cui, X., Kruger, G. R., Baenziger, P. S., & Walia, H. (2013). Introgression of novel traits from a wild wheat relative improves drought adaptation in wheat. *Plant Physiology*, *161*(4), 1806-1819.

Pochapski, M. T., Fosquiera, E. C., Esmerino, L. A., dos Santos, E. B., Farago, P. V., Santos, F. A., & Groppo, F. C. (2011). Phytochemical screening, antioxidant, and antimicrobial activities of the crude leaves' extract from Ipomoea batatas (L.) Lam. *Pharmacognosy magazine*, *7*(26), 165.(https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3113358/?tool=pmcentrez&report=abstract , accessed on February 16, 2020).

Porch, T. G., Beaver, J. S., Debouck, D. G., Jackson, S. A., Kelly, J. D., & Dempewolf, H. (2013). Use of wild relatives and closely related species to adapt common bean to climate change. *Agronomy*, *3*(2), 433-461.

Ravi, V., Naskar, S., Makeshkumar, T., Babu, B., & Krishnan, B. P. (2009). Molecular physiology of storage root formation and development in sweet potato (*Ipomoea batatas* (L.) Lam.). *J Root Crops*, *35*(1), 1-27.

Rolston, L.H., Clark, C.A., Cannon, J.M., Randle, W.M., Rilery, E.G., Wilson, P.W., & Robbins, M.L. 1987. 'Beauregard' sweetpotato. *HortScience* 22:1338-1339.

Sattler, M. C., Carvalho, C. R., & Clarindo, W. R. (2016). The polyploidy and its key role in plant breeding. *Planta*, *243*(2), 281-296.

Schlaepfer, D. R., Bradford, J. B., Lauenroth, W. K., Munson, S. M., Tietjen, B., Hall, S. A., & Lkhagva, A. (2017). Climate change reduces extent of temperate drylands and intensifies drought in deep soils. *Nature communications*, *8*(1), 1-9.

Solis, J., Villordon, A., Baisakh, N., LaBonte, D., & Firon, N. (2014). Effect of drought on storage root development and gene expression profile of sweetpotato under greenhouse and field conditions. *Journal of the American Society for Horticultural Science*, *139*(3), 317-324.

Suprunova, T., Krugman, T., Fahima, T., Chen, G., Shams, I., Korol, A., & Nevo, E. (2004). Differential expression of dehydrin genes in wild barley, *Hordeum spontaneum*, associated with resistance to water deficit. *Plant, cell & environment*, *27*(10), 1297-1308.

Taduri, S., Lone, A., Meyers, S.L., Shankle, M.W. and Reddy, K.R. (2017, October 24). *Sweetpotato Cultivar Responses to Interactive Effects of Temperature, Drought, and Carbon Dioxide*. Poster session presented at the Managing Global Resources For a Secure Future,

Tampa, FL. (https://scisoc.confex.com/crops/2017am/webprogram/Paper107834.html, accessed on February 14, 2019).

Taiz, L., & Zeiger, E. (2006). Plant physiology, 4th edition. Sunderland: Sinauer Associates. 700pp.

Trenberth, K. E., Dai, A., Van Der Schrier, G., Jones, P. D., Barichivich, J., Briffa, K. R., & Sheffield, J. (2014). Global warming and changes in drought. *Nature Climate Change*, *4*(1), 17-22.

Van Heerden, P. D. R., & Laurie, R. (2008). Effects of prolonged restriction in water supply on photosynthesis, shoot development and storage root yield in sweet potato. *Physiologia Plantarum*, *134*(1), 99-109.

Villordon, A., LaBonte, D., & Firon, N. (2009). Development of a simple thermal time method for describing the onset of morpho-anatomical features related to sweetpotato storage root formation. *Scientia horticulturae*, *121*(3), 374-377.

Wong, S. C., Cowan, I. R., & Farquhar, G. D. (1979). Stomatal conductance correlates with photosynthetic capacity. *Nature*, *282*(5737), 424-426.

Yang, P. M., Huang, Q. C., Qin, G. Y., Zhao, S. P., & Zhou, J. G. (2014). Different drought-stress responses in photosynthesis and reactive oxygen metabolism between autotetraploid and diploid rice. *Photosynthetica*, *52*(2), 193-202.

Yencho, G. C. & Pecota, K.V. (2009). Progress Report to North Carolina SweetPotato Commission. (https://potatoes.ncsu.edu/pdf/NCSPCBRDRPT08.pdf, accessed on February 12, 2019).

Zaharieva, M., Gaulin, E., Havaux, M., Acevedo, E., & Monneveux, P. (2001). Drought and heat responses in the wild wheat relative *Aegilops geniculata* Roth. *Crop Science*, *41*(4), 1321-1329.

Zhang, F., Xue, H., Lu, X., Zhang, B., Wang, F., Ma, Y., & Zhang, Z. (2015). Autotetraploidization enhances drought stress tolerance in two apple cultivars. *Trees*, *29*(6), 1773-1780

Tables

Table 1- Genotypes evaluated in the study, breeding program or country origin, and years evaluated.

Species	**Genotype ID**	**Origin**	**Year (s)**
I. batatas	cv. Beauregard	USA - LSU	2018/2019
I. batatas	cv. Tanzania	East African Landrace - Uganda	2018/2019
I. batatas	cv. Resisto	USDA-ARS	2019
I. batatas	cv. Hatteras	USA - NCSU	2019
I. cynanchifolia	PI 549093	Peru	2018/2019
I. leucantha	PI 518481	Mexico	2018/2019
I. trifida	PI 540724	Mexico	2018/2019
I. triloba	PI 618966	Mexico	2018/2019

Table 2 – Frequency of the status of the *Ipomoea spp.* plants studied. Each numerical value was assigned to a specific plant status: normal (3), wilted (2) and, dry (1). The data include the frequency of the status of the plants for control, 9 d and 50 d treatments during the first week (2a); and the frequency during the second week (2b). Values were derived from a sample of 216 individuals.

2a. Frequency of the status of the plants one week (8 – 10 days) after treatment - Year 2019

	Status 3 = Normal			Status 2 = Wilted			Status 1 = Dry		
	Control	9 d	50 d	Control	9 d	50 d	Control	9 d	50 d
Bea	9	2	0	0	7	9	0	0	0
Cyn	9	2	0	0	6	6	0	1	3
Hat	9	3	0	0	6	8	0	0	1
Leu	9	0	0	0	7	8	0	2	1
Res	9	3	0	0	6	9	0	0	0
Tan	9	3	0	0	6	9	0	0	0
Tba	9	2	0	0	6	9	0	1	0
Tfda	9	3	0	0	6	8	0	0	1
Total	72	18	0	0	50	66	0	4	6
Percentage	33	8	0	0	23	31	0	2	3

2b. Frequency of the status of the plants two weeks (16 – 18 days) after treatment - Year 2019

	Status 3 =Normal			Status 2= Wilted			Status 1= Dry		
	Control	9 d	50 d	Control	9 d	50 d	Control	9 d	50 d
Bea	9	8	0	0	1	7	0	0	2
Cyn	9	8	0	0	1	0	0	0	9
Hat	9	9	0	0	0	8	0	0	1
Leu	9	8	0	0	1	4	0	0	5
Res	9	8	0	0	1	6	0	0	3
Tan	9	7	0	0	2	7	0	0	2
Tba	9	9	0	0	0	4	0	0	5
Tfda	9	9	0	0	0	5	0	0	4
Total	72	66	0	0	6	41	0	0	31
Percentage	33	31	0	0	3	19	0	0	14

Bea = Beauregard; Cyn = *I. cynanchifolia*; Hat = Hatteras; Leu = *I. leucantha*; Res =Resisto; Tan = Tanzania; Tba = *I. triloba*; Tfda = *I. trifida*.

Table 3 - Mean ± standard error of aboveground and belowground fresh biomass of the *Ipomoea spp*. studied. Tables present averages calculated for the control, 7 d, 9 d, 21 d and 50 d treatments. The results include observation of the aboveground weight of the 7 d and 21 d stress (3a); aboveground weight of the 9 d and 50 d stress (3b); belowground weight of the 7 d and 21 d stress (3c); and the belowground weight of the 9 d and 50 d of stress (3d). Percent change was calculated based on the means of the control treatment for the respective genotype.

3a. Biomass - aboveground (g) - Year 2018					
	Control	7 d		21 d	
	Mean	Mean	% Change	Mean	% Change
Bea	46.16 ± 2.47 cd	38.94 ± 2.47 cde	15.63	36.20 ± 2.47 cdef	21.57
Tan	77.89 ± 2.47 a	59.68 ± 2.58 b	23.37	46.21 ± 2.47 cd	40.66
Cyn	48.34 ± 2.47 bc	33.25 ± 2.47 defg	31.21	14.33 ± 2.47 hi	70.35
Leu	48.29 ± 2.47 bc	32.76 ± 2.47 efg	32.16	14.82 ± 2.47 hi	69.30
Tba	36.55 ± 2.47 cdef	23.84 ± 2.47 fgh	34.78	10.21 ± 2.47 i	72.05
Tfda	38.32 ± 2.65 cde	33.93 ± 2.67 defg	11.44	20.29 ± 2.67 ghi	47.03

3b. Biomass - aboveground (g) - Year 2019					
	Control	9 d		50 d	
	Mean	Mean	% Change	Mean	% Change
Bea	80.06 ± 5.02 bcd	72.70 ± 5.02 cde	9.20	41.29 ± 5.29 efg	48.42
Cyn	67.90 ± 5.02 cdef	48.86 ± 5.02 ef	28.03	NE*	
Hat	84.02 ± 5.29 abc	70.27 ± 5.29 cde	16.36	35.19 ± 5.64 fg	58.11
Leu	67.84 ± 5.02 cdef	45.94 ± 5.02 efg	32.28	10.40 ± 14.17 fg	84.67
Res	75.25 ± 5.02 bcde	80.77 ± 5.02 bcd	7.34	39.16 ± 8.70 efg	47.95
Tan	108.61 ± 5.02 a	101.87 ± 5.02 ab	6.20	40.79 ± 7.42 efg	62.44
Tba	53.80 ± 5.02 def	45.36 ± 5.02 efg	15.67	9.76 ± 8.70 g	81.84
Tfda	64.37 ± 5.64 cdef	59.83 ± 5.64 cdef	7.06	15.57 ± 6.57 fg	75.8

3c. Biomass - belowground (g) - Year 2018					
	Control	7 d		21 d	
	Mean	Mean	% Change	Mean	% Change
Bea	35.22 ± 5.54 a	16.42 ± 5.54 abc	53.38	11.02 ± 5.54 abc	68.72
Tan	13.13 ± 5.54 abc	5.25 ± 5.54 bc	60.03	2.35 ± 5.54 bc	82.13
Cyn	31.37 ± 5.54 ab	12.12 ± 5.54 abc	61.38	1.79 ± 5.54 c	94.30
Leu	12.22 ± 5.54 abc	4.75 ± 5.54 bc	61.11	1.47 ± 5.54 c	87.94
Tba	10.07 ± 5.54 abc	2.60 ± 5.54 bc	74.16	1.42 ± 5.54 c	85.88
Tfda	19.26 ± 5.64 abc	9.46 ± 5.58 abc	50.89	5.88 ± 5.71 abc	69.45

Table 3 (continued).

3d. Biomass - belowground (g) - Year 2019					
	Control	9 d		50 d	
	Mean	Mean	% Change	Mean	% Change
Bea	12.21 ± 5.51 d	13.27 ± 5.51 d	8.73	13.41 ± 5.78 d	9.81
Cyn	95.58 ± 5.51 ab	45.94 ± 5.51 cd	51.93	NE*	
Hat	8.47 ± 5.78 d	8.98 ± 5.78 d	6.03	10.25 ± 6.16 d	20.90
Leu	42.72 ± 5.51 cd	24.46 ± 5.51 d	42.73	7.40 ± 15.11 d	82.68
Res	13.14 ± 5.51 d	11.24 ± 5.51 d	14.45	8.86 ± 9.55 d	32.54
Tan	24.38 ± 5.51 d	29.83 ± 5.51 d	22.32	15.84 ± 8.07 d	35.01
Tba	44.35 ± 5.51 cd	22.64 ± 5.51 d	48.95	4.60 ±9.55 d	89.63
Tfda	104.68 ± 5.51 a	70.36 ± 5.51 bc	32.78	16.65 ± 7.12 d	84.09

NE* = Mean not estimated due to no survival of none of the plants grown under the treatment.

Common letters within columns represent no significant difference at $\alpha = 0.05$ (Tukey method).

Bea = Beauregard; Cyn = *I. cynanchifolia*; Hat = Hatteras; Leu = *I. leucantha*; Res = Resisto; Tan = Tanzania; Tba = *I. triloba*; Tfda = *I. trifida*.

Table 4 – Mean ± standard error of storage root yield of the cultivated *I. batatas*. The data include the storage root count per plant and dry weights for control, 7 d, 9 d, 21 d, and 50 d the treatments. Storage root count per plant for 7 d and 21 d (3a); storage root count per plant for 9 d and 50 d (3b); dry weight of the storage roots for 7 d and 21 d (3c); dry weight of the storage roots for 9 d and 50 d (3d). Percent change was calculated based on the means of the control treatment for the respective genotype.

4a. Storage root count per plant - Year 2018

	Control	7 d		21 d	
	Mean	Mean	% Change	Mean	% Change
Bea	3.07 ± 0.31 a	3.08 ± 0.31 a	0.14	2.57 ± 0.31 a	16.31
Tan	0.91 ± 0.31 b	0.66 ± 0.31 b	27.42	0.08 ± 0.31 b	91.01

4b. Storage root count per plant - Year 2019

	Control	9 d		50 d	
	Mean	Mean	% Change	Mean	% Change
Bea	4.66 ± 0.45 ab	3.88 ± 0.45 abc	16.67	3.25 ± 0.48 abcd	30.36
Hat	5.55 ± 0.45 a	3.11 ± 0.45 bcd	44.00	3.71 ± 0.52 abc	33.14
Res	3.33 ± 0.48 abcd	1.66 ± 0.48 cde	49.99	2.00 ± 0.79 bcde	39.99
Tan	1.11 ± 0.45 de	0.33 ± 0.45 e	70.00	0.00 ± 0.68 e	100.00

4c. Dry weight of the storage roots (g) - Year 2018

	Control	7 days		21 days	
	Mean	Mean	% Change	Mean	% Change
Bea	22.6579 ± 1.1841 a	11.8751 ± 1.1841 b	47.59	6.5313 ± 1.1841 bc	71.17
Tan	1.6835 ± 1.1841 cd	1.3739 ± 1.1841 cd	18.39	0.03915 ± 1.1841 d	97.67

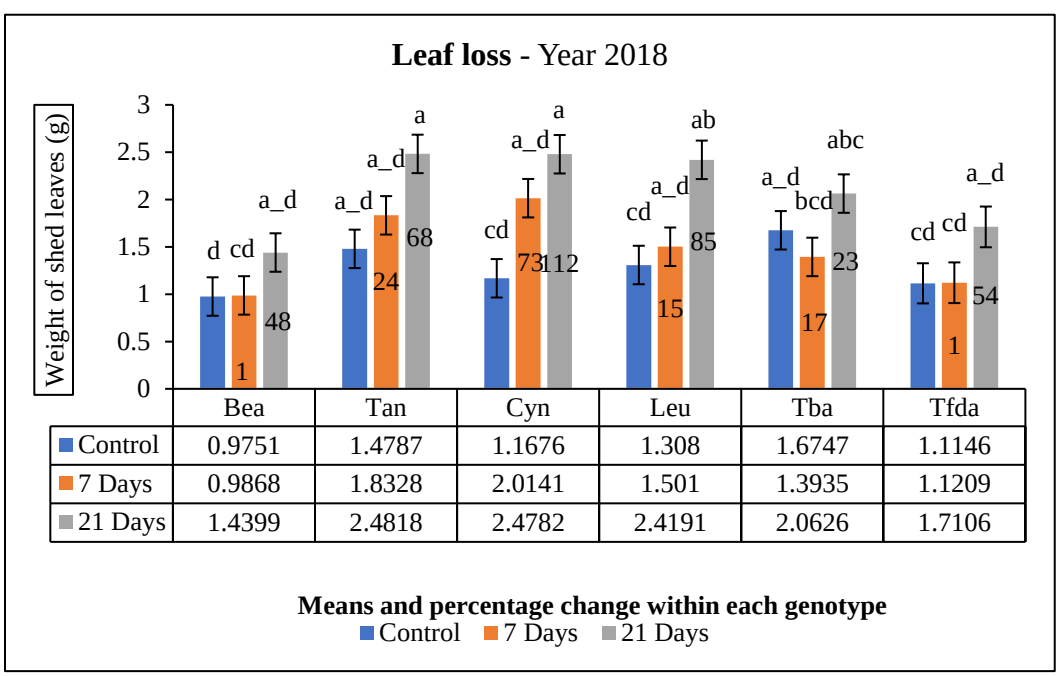
4d. Dry weight of the storage roots (g) - Year 2019

	Control	9 days		50 days	
	Mean	Mean	% Change	Mean	% Change
Bea	70.14 ± 8.21 ab	47.05 ± 8.21 abc	32.92	5.40 ± 8.41 c	92.29
Hat	83.61 ± 8.21 a	49.57 ± 8.21 abc	40.70	3.94 ± 8.81 c	95.28
Res	32.27 ± 8.41 bc	11.28 ± 8.41 c	65.02	1.43 ± 14.22 c	95.96
Tan	9.50 ± 8.21 c	0.45 ± 8.21 c	95.20	0.00 ± 11.24 c	100.00

Common letters within columns represent no significant difference at $\alpha = 0.05$ (Tukey method). Bea = Beauregard; Hat = Hatteras; Res = Resisto; Tan = Tanzania.

Figures

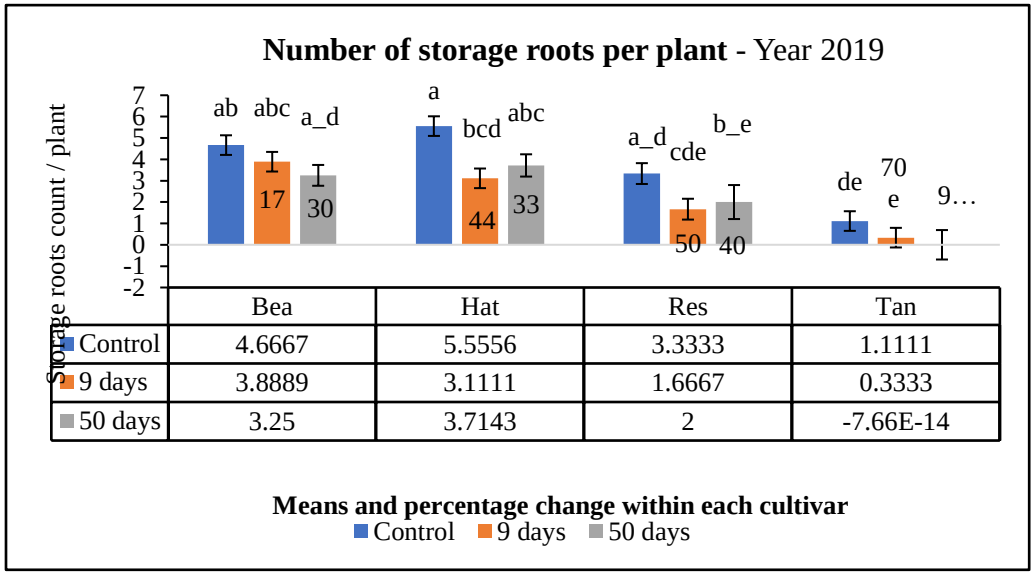
Figure 1 – Leaf loss of *Ipomoea spp*. studied. The figure shows the averages calculated for the control, 7 d, and 21 d treatments. The results include observation of the shed leaves for the control, 7d, and 21 d treatments.



	Bea	Tan	Cyn	Leu	Tba	Tfda
Control	0.9751	1.4787	1.1676	1.308	1.6747	1.1146
7 Days	0.9868	1.8328	2.0141	1.501	1.3935	1.1209
21 Days	1.4399	2.4818	2.4782	2.4191	2.0626	1.7106

Common letters within genotypes represent no significant difference at α = 0.05 (Tukey method). Bea = Beauregard; Cyn = *I. cynanchifolia*; Leu = *I. leucantha*; Tan = Tanzania; Tba = *I. triloba*; Tfda = *I. trifida*.

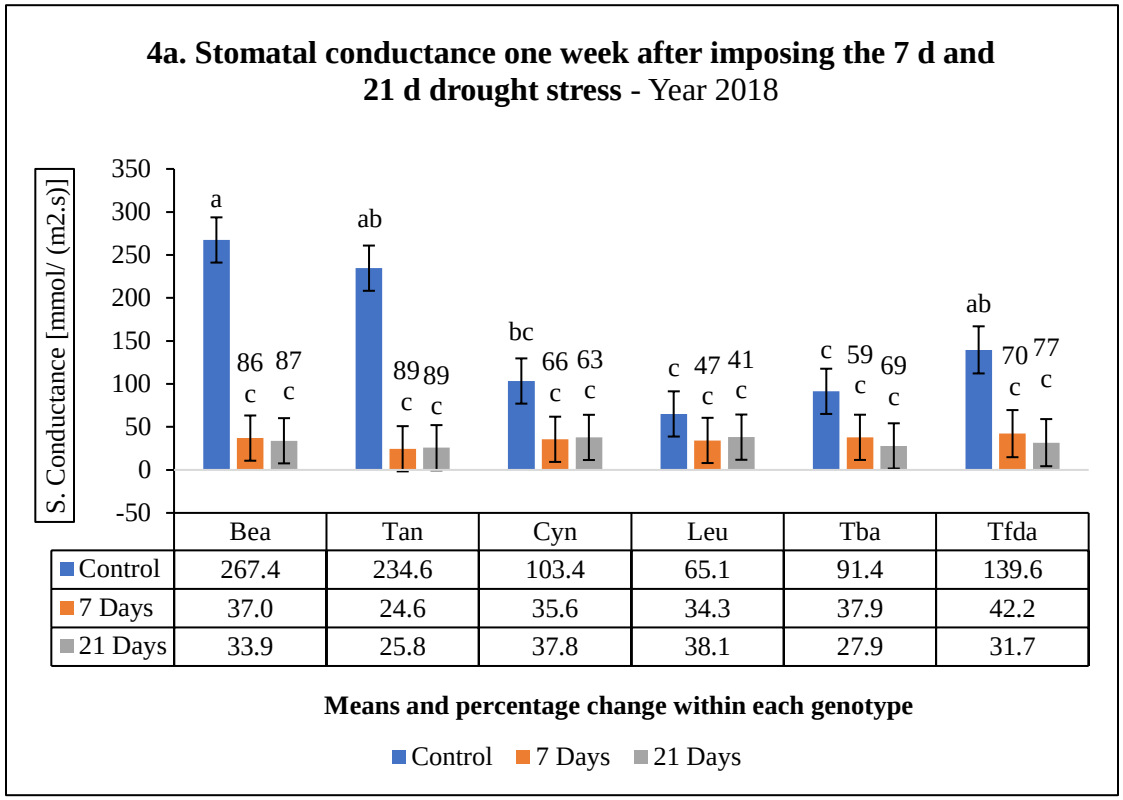
Figure 2 – Storage root yield of the cultivated *I. batatas* studied. The figure shows the storage root count per plant for control, 9 d, and 50 d treatments.



Common letters within genotypes represent no significant difference at α = 0.05 (Tukey method). Bea = Beauregard; Cyn = *I. cynanchifolia*; Hat = Hatteras; Leu = *I. leucantha*; Res = Resisto; Tan = Tanzania; Tba = *I. triloba*; Tfda = *I. trifida*.

Figure 3 – Signs of drought stress of *Ipomoea spp.* studied**.** The figure shows *I. batatas*, cultivars Tanzania and Hatteras and wild *I. leucantha* with signs of 15 days of drought stress.

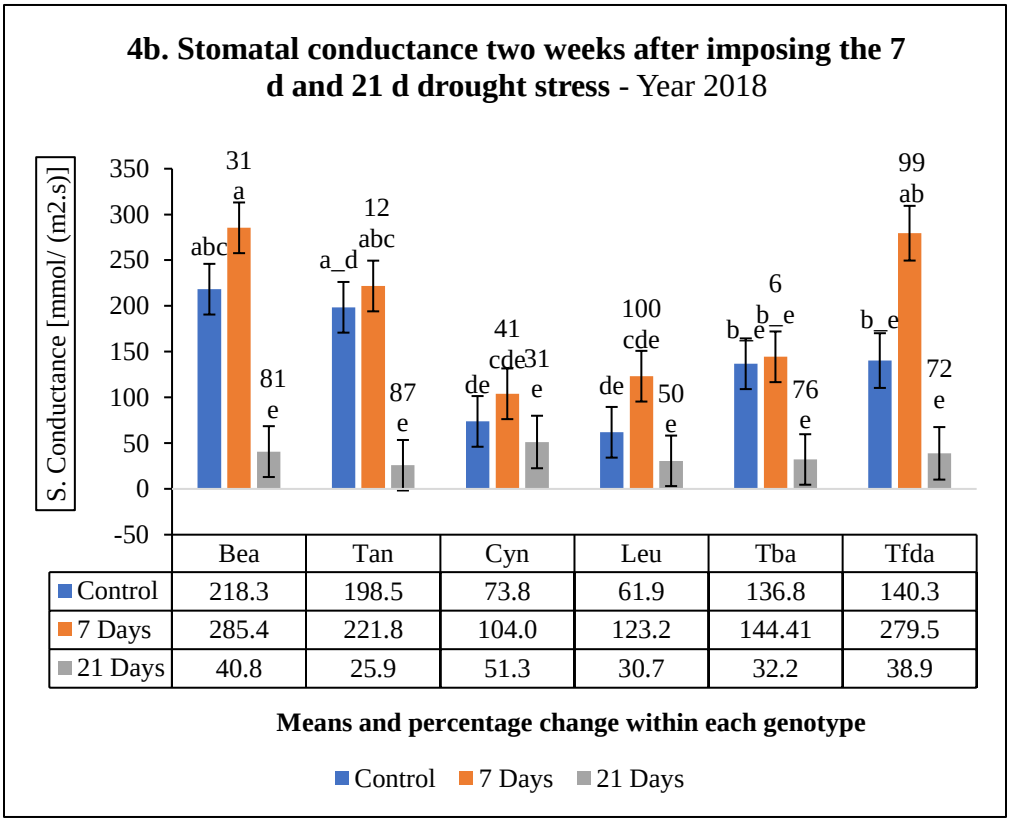
Figure 4 – Stomatal conductance of *Ipomoea spp.* studied. Averages of stomatal conductance at different time points of the study are provided for the control, 7 d, 9 d, 21 d and 50 d treatments. The results include observation of the stomatal conductance one week after imposing the 7 d and 21 d stress (4a); two weeks after imposing the 7 d and 21 d stress (4b); one week after imposing the 9 d and 50 d stress (4c); two weeks after imposing the 9 d and 50 d stress (4d); and stomatal conductance of the plants recovered from 21 d stress (4e).



	Bea	Tan	Cyn	Leu	Tba	Tfda
Control	267.4	234.6	103.4	65.1	91.4	139.6
7 Days	37.0	24.6	35.6	34.3	37.9	42.2
21 Days	33.9	25.8	37.8	38.1	27.9	31.7

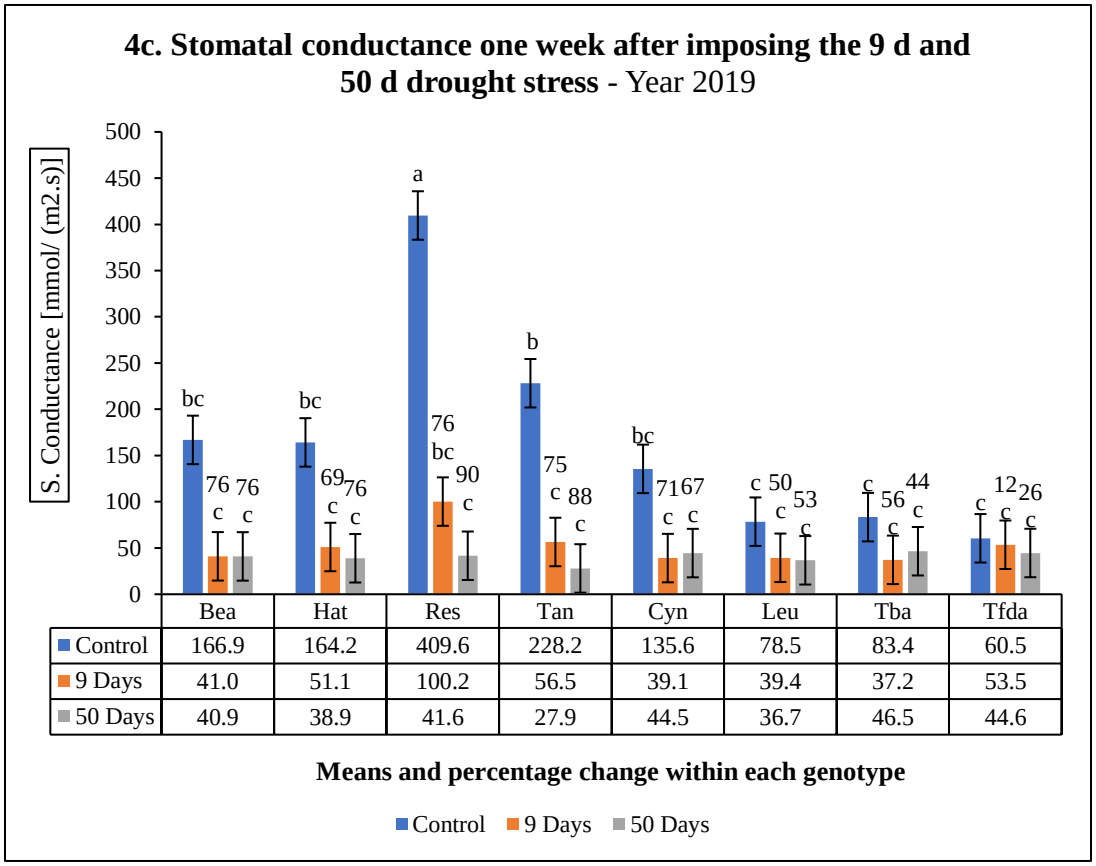
Common letters represent no significant difference at α = 0.05 (Tukey method). Bea = Beauregard; Cyn = *I. cynanchifolia*; Leu = *I. leucantha*; Tan = Tanzania; Tba = *I. triloba*; Tfda = *I. trifida*.

Figure 4 (continued).



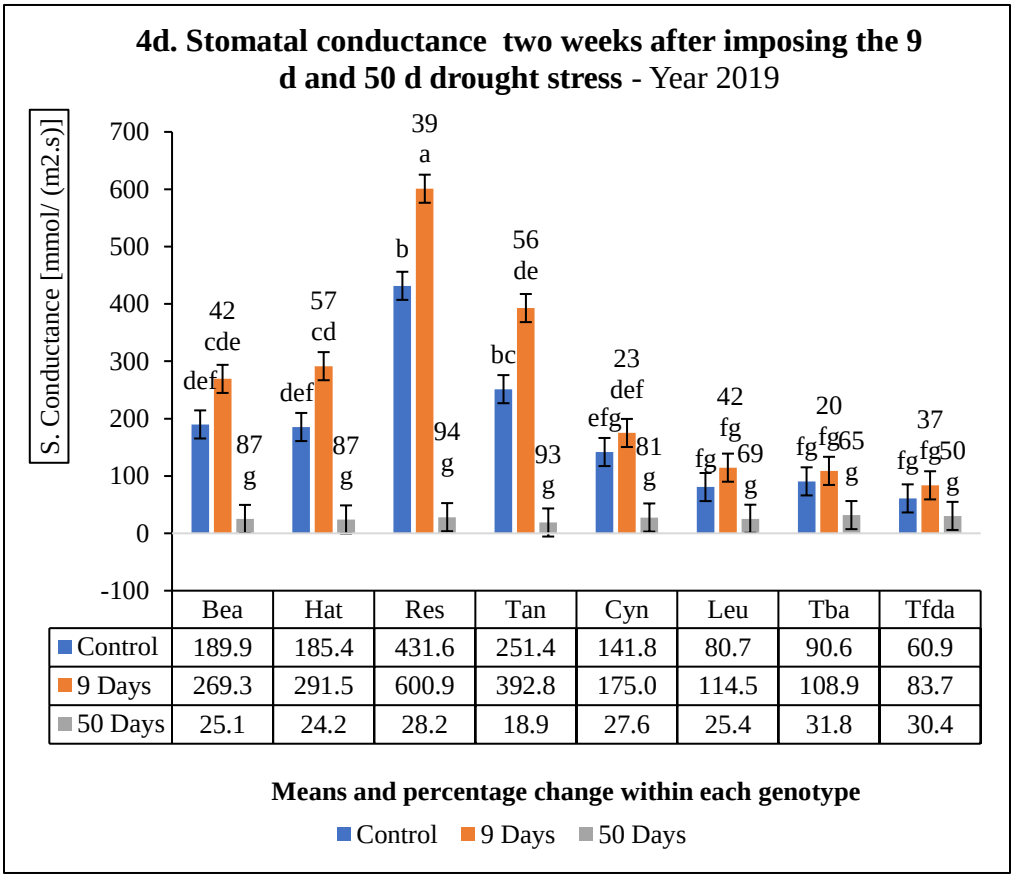
Common letters represent no significant difference at α = 0.05 (Tukey method). Bea = Beauregard; Cyn = *I. cynanchifolia*; Leu = *I. leucantha*; Tan = Tanzania; Tba = *I. triloba*; Tfda = *I. trifida*.

Figure 4 (continued).



Common letters represent no significant difference at α = 0.05 (Tukey method). Bea = Beauregard; Cyn = *I. cynanchifolia*; Hat = Hatteras; Leu = *I. leucantha*; Res = Resisto; Tan = Tanzania; Tba = *I. triloba*; Tfda = *I. trifida*.

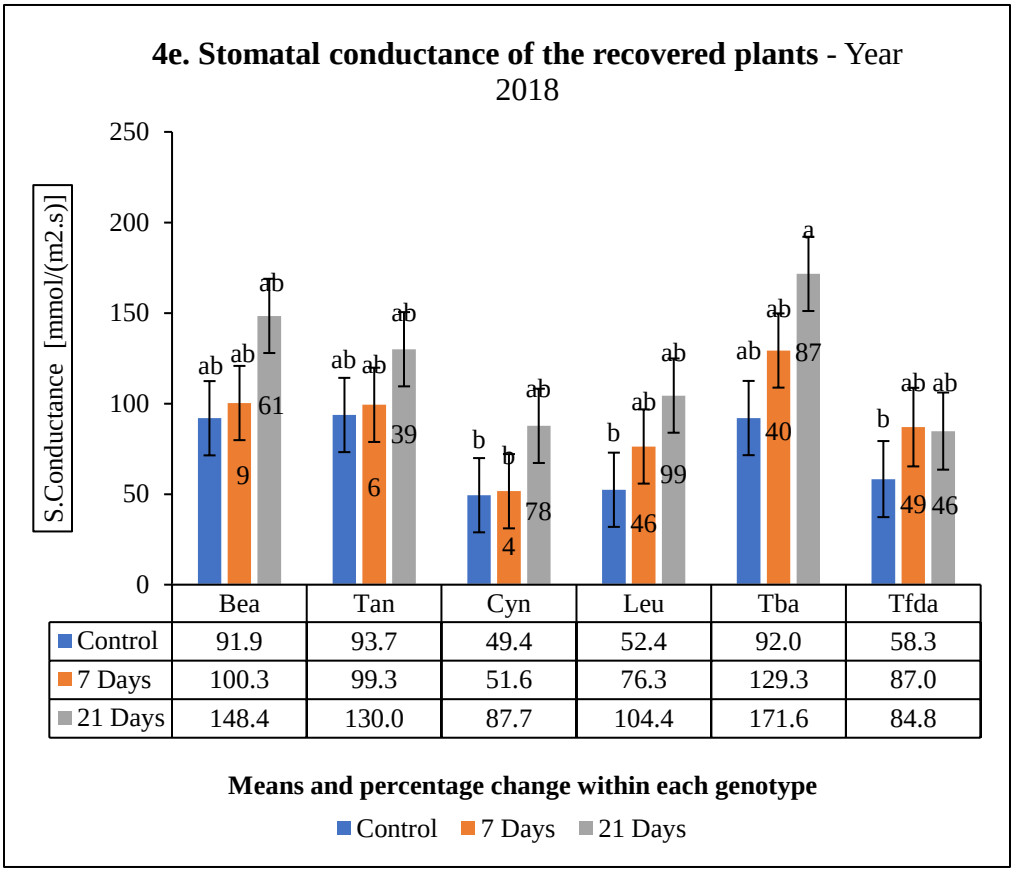
Figure 4 (continued).



	Bea	Hat	Res	Tan	Cyn	Leu	Tba	Tfda
Control	189.9	185.4	431.6	251.4	141.8	80.7	90.6	60.9
9 Days	269.3	291.5	600.9	392.8	175.0	114.5	108.9	83.7
50 Days	25.1	24.2	28.2	18.9	27.6	25.4	31.8	30.4

Common letters represent no significant difference at α = 0.05 (Tukey method).
Bea = Beauregard; Cyn = *I. cynanchifolia*; Hat = Hatteras; Leu = *I. leucantha*; Res = Resisto; Tan = Tanzania; Tba = *I. triloba*; Tfda = *I. trifida*.

Figure 4 (continued).



	Bea	Tan	Cyn	Leu	Tba	Tfda
Control	91.9	93.7	49.4	52.4	92.0	58.3
7 Days	100.3	99.3	51.6	76.3	129.3	87.0
21 Days	148.4	130.0	87.7	104.4	171.6	84.8

Common letters represent no significant difference at α = 0.05 (Tukey method). Bea = Beauregard; Cyn = *I. cynanchifolia*; Leu = *I. leucantha*; Tan = Tanzania; Tba = *I. triloba*; Tfda = *I. trifida*.

CHAPTER 4

Comparative transcriptome analysis of drought tolerance in the sweetpotato cultivars 'Beauregard' and 'Resisto'

(In a format suitable for submission to Journal Elsevier)

Comparative transcriptome analysis of drought tolerance in the sweetpotato cultivars 'Beauregard' and 'Resisto'

Stella E. C. Nhanala[a,b], Hamid Ashrafi [a], Qiuyun (Jenny) Xiang[c], Sharon Williamson [a], Bode Olukolu [d], and G. Craig Yencho[a]

[a] Department of Horticultural Science, North Carolina State University, 2721 Founders Dr., Raleigh, NC 27607, USA; [b] Instituto de Investigação Agrária de Moçambique (IIAM), Av. das FPLM, nº 2698, Caixa Postal 3659, Maputo, Mozambique; [c] Department of Plant and Microbial Biology, North Carolina State University, Campus Box 7612, Raleigh, NC 27695-7612; [d] Entomology and Plant Pathology Department at UTIA University of Tennessee – Institute of Agriculture, 370 Plant Biotechnology Building, 2505 E J Chapman Drive, Knoxville, TN 37996, USA.

*Corresponding author (craig_yencho@ncsu.edu)

ABSTRACT

Sweetpotato (*Ipomoea batatas*), a crop grown predominantly in hot, tropical environments, is often grown in drought-prone agricultural systems, and is generally considered to be well adapted to hot and dry conditions. However, episodes of drought have been more frequent, and their duration has been extended, particularly in Sub-Saharan Africa, where the crop is an important staple food.

Due to this, there is a need to improve drought tolerance in sweetpotato. In a previous greenhouse study that compared drought tolerance of wild relatives to cultivated sweetpotato, two cultivated varieties, 'Beauregard' and 'Resisto', were observed to be more drought tolerant than the wild relatives *I. trifida*, *I. triloba*, *I. cynanchifolia*, and *I. leucantha*. In the current study, a comparative transcriptomic analysis for drought tolerance of 'Beauregard' and 'Resisto' was conducted. The comparative transcriptome analyses revealed differential gene expression of several known gene families. We investigated the expression patterns of six genes that were potentially associated with drought tolerance, namely: 1) abscisic acid and environmental stress-inducible protein-like gene (TAS14); 2) E3 ubiquitin-protein ligase RING1-like gene; 3) expansin-A15-like gene (EXLA15); 4) basic form of a pathogenesis-related protein 1 gene (PRP-1); 5) 18.8 kDa class II heat shock protein-like gene; and 6) the desiccation inducible PCC13-62 gene. Different levels of gene expression were observed between the two cultivars during different periods of drought when comparisons were performed using an FDR *p-value* < 0.05 and absolute fold change ≥ 2. In general, 'Beauregard' showed a greater expression level for these genes than 'Resisto'. 'Beauregard' also had a consistent pattern of expression, with long periods of drought resulting in higher gene expression levels. The cv. 'Resisto', however, was not consistent in terms of the linearity between time of exposure to drought and the level of overexpression of the genes. The results of these studies provide additional insights into the molecular mechanisms regarding drought tolerance in sweetpotato and provide a starting point for further studies related to drought tolerance in this important crop.

INTRODUCTION

Sweetpotato (*Ipomoea batatas*) is grown worldwide. In 2019, the top five producers of sweetpotato were China, Malawi, Nigeria, Tanzania, and Uganda (FAOSTAT, 2021). Sweetpotato is grown primarily in developing countries, where subsistence agriculture is a common practice, and the irrigation systems are frequently rain-fed. Some landraces and cultivars of sweetpotato are adapted to poorly irrigated soils. However, many economically important genotypes cannot tolerate drought, and different intensities of drought cause variable degrees of yield loss in sweetpotato (Van Heerden & Laurie, 2008; Solis et al., 2014; Nhanala & Yencho, 2020).

The effects of drought on plant development can be assessed in different ways. The most common methodologies used to evaluate the impact of drought in plants typically consist of morphological and physiological studies (Fischer & Maurer, 1978; Roth et al., 2013; Kumar et al., 2014). However, understanding the molecular mechanisms of drought responses/resistance revealed by these methodologies requires comparative molecular studies. The identification of candidate genes for drought tolerance as a complement of morpho-physiological studies can provide valuable information and viable target genes for crop improvement. However, for drought tolerance, identifying candidate genes is complex, as dehydration stress is often controlled by multiple genes (Blum, 2011; Krannich et al., 2015). In sweetpotato, transcriptomic studies have been conducted for different purposes. In most cases, these studies were primarily focused on identifying differential genes expressed during storage root development (Wang et al., 2010; Tao et al., 2012; Xie et al., 2012; Firon et al., 2013; Ponniah et al., 2017; Zhang et al., 2017). For instance, Wang et al. (2010) studied the formation and development of roots in sweetpotato by characterizing storage root transcriptome and by developing expressed sequence

tag-derived simple sequence repeat (EST-derived SSR) markers. The authors assigned about 2500 genes to metabolic pathways associated with the metabolism of carbohydrates and the biosynthesis of secondary metabolites. Candidate genes with the potential of mediating resistance to abiotic stresses (drought, salt, cold, heat, or osmotic stress) in different tissues (young leaves, mature leaves, stems, fibrous roots, and storage roots) were identified by Tao et al. (2012) when the authors performed a *de novo* combined transcriptome assembly strategy. Some of the candidate genes that were identified for stress tolerance were: late embryogenesis abundant proteins (LEA), early-responsive to dehydration stress protein (ERD), aquaporin (AQP), abscisic acid responsive elements-binding factor (AREB), Mn-superoxide dismutase (MnSOD), vacuolar H^+-pyrophosphatase (PPase), ascorbate peroxidase (APX), and polyphenol oxidase (PPO).

Firon et al. (2013) studied the molecular mechanisms involved in the initiation of storage roots by comparing the transcriptome of storage roots and fibrous roots. They found that about 8000 contigs were differentially expressed between the two types of roots; and the differential expression was associated with, lignin biosynthesis, carbohydrate metabolism, and the biosynthesis of starch. Tolerance to salt in roots of sweetpotato was studied by performing a transcriptome study of a salt-sensitive and a salt-tolerant cultivar of sweetpotato (Zhang et al., 2017). They observed that between the two cultivars, there was differential expression of genes involved in the biosynthesis of jasmonic acid, and that the signaling pathway of jasmonic acid was associated with salt tolerance in sweetpotato.

Transcriptome analysis of drought stress has led to the identification of candidate genes associated with drought tolerance in crops such as beans, cassava, maize, potato, and wheat (Turyagyenda et al., 2013; Wu et al., 2014; Xu et al., 2014; Gong et al., 2015; Li et al., 2017).

Transcriptome studies to understand molecular mechanisms of drought tolerance in sweetpotato have been performed primarily in laboratory experiments, where dry soil is simulated with artificial growth media solutions (Lau et al., 2018; Hong et al., 2019; Arisha et al., 2020). For example, Solis et al. (2014) performed transcriptome analysis under greenhouse conditions and applied the quantitative real-time polymerase chain reaction (qRT-PCR) analysis of 19 genes in sweetpotato plants grown using a water-soluble osmotic gradient solution to simulate different drought levels. Ten out of the 19 genes chosen for study were found to be up-regulated, with a significant fold increase in three genes: a homeobox protein (IbHB2), a protein similar to the *Arabidopsis thaliana* cytokinin response factor 1 (IbCRF1), and an abscisic acid-responsive elements-binding factor (IbAREB). Lau et al. (2018) applied the RNA-seq approach, based on the reference genome of *Ipomoea trifida*, the closest wild relative of sweetpotato (Muñoz-Rodriguez et al., 2018; Wu et al., 2018), to identify drought tolerance candidate genes, including up-regulated genes [abscisic acid and environmental stress-inducible protein (TAS14) and dehydration-responsive-element-binding protein 2A (DREB2A)], and down-regulated genes [slow anion channel-associated 1 (SLAC1) and light-harvesting chlorophyll A/B-binding6 (LHCB6)]. Hong et al. (2019) performed a *de novo* transcriptome analysis and found that the abscisic acid (ABA), ethylene, and jasmonic acid pathways were involved in drought tolerance.

Nhanala & Yencho (2020) evaluated four wild *Ipomoea* species and four cultivars for drought tolerance and found that the wild genotypes were more sensitive to drought than the cultivated sweetpotato. Based on those results, we decided to focus on further studies of the drought tolerance of cultivated sweetpotato. A comparative transcriptomic study for drought tolerance will provide an understanding of the gene expression of the selected cultivars when

they are submitted to water deprivation and show how the differences in the gene expression can influence the phenotypic responses of the two cultivars to drought stress. In the present study, comparative transcriptome profiling was performed with two cultivars of sweetpotato, namely 'Beauregard' and 'Resisto'. The two cultivars exhibited different phenotypic responses (e.g., stomatal conductance) when the plants were exposed to dehydration and showed different survival rates to 50 days of drought (Nhanala & Yencho, 2020). Ribonucleic Acid-sequencing (RNA-seq) analysis was utilized to study the gene expression in sweetpotato when exposed to drought. Because previous studies have shown differential responses to drought in 'Beauregard' and 'Resisto' (Ricardo, 2011; Ricardo & Andrade, 2013; Nhanala & Yencho, 2020), we hypothesized that these two genotypes would show differences in the expression of genes associated with drought tolerant traits. An evaluation of the phenotypic traits was conducted to show that the drought treatments affected the plants. Our study was performed under greenhouse conditions, where the plants were grown in sand and soil mix, and with a daily water irrigation. Drought was imposed on plants, in the greenhouse, by interrupting the irrigation for seven days, and the results of this study have provided additional insights into the molecular mechanisms regarding drought tolerance in sweetpotato.

MATERIALS AND METHODS

Plant Materials

We evaluated the drought tolerance of two sweetpotato cultivars, 'Beauregard' and 'Resisto', identified as drought tolerant in a previous study (Nhanala & Yencho, 2020). The plants were grown from August-September 2020 in the Horticulture Field Laboratory (HFL) greenhouses at North Carolina State University (NCSU), Raleigh, NC (35.7847° N, 78.6821°

W). Twelve plants, six each of ‘Beauregard’ and ‘Resisto’ were grown in the greenhouse. Plant cuttings roughly 15 cm in length were transplanted into 1 L pots containing 1:1 sand and soil mix. Each pot contained 1.3 kg of soil. Plants were fertigated once a week during the first 21 days after transplanting (DAT), and the fertilization dosage was increased to twice a week after 21 DAT. Two drought treatments were set up with water restrictions for 24 hours, and 48 hours. After seven days of drought, in total, the plants were re-irrigated. Plants were grown under ambient light and temperature, in a ventilated greenhouse.

Experimental design for drought treatment

Control plants were treated daily in an uninterrupted manner with a water volume of ~ 150 ml/plant until the harvest time. Plants that were exposed to drought were irrigated daily until four weeks DAT. At four weeks DAT, the daily irrigation was interrupted for seven days. After seven days of drought stress, the plants were re-irrigated until the harvest time.

Each treatment (and genotype) was represented by three biological replicates, with a total number of 12 plants (Table 1) to account for control and drought-treated plants. The treatments were applied at the time of the initiation of the storage roots, which is about four weeks, depending on the cultivar. The collection of leaf tissue and recording of the phenotypic data was performed in the greenhouse during four time points: T0 = 24 hours before drought; T1 = 24 hours after imposing drought; T2 = 48 hours after imposing drought, and T3 = 24 hours after re-irrigation (Figure 1).

The status (wilted plant vs. normal appearance) of the plants was observed during the time that they were exposed to drought. The volume of water per plant was recorded during each time point, as well as the reduction of water/plant when they were subjected to drought. To estimate the volume of reduction of water/plant after each daily irrigation, water was collected

into individual graduated cylinders from each drip point assigned to a plant during the same time interval that the irrigation lasted. The volume that was read from the graduated cylinder was recorded for data analysis.

The collection of leaf tissue and of the phenotypic data was performed during four time points: T0 = 24 hours before drought; T1 = 24 hours after imposing drought; T2 = 48 hours after imposing drought, and T3 = 24 hours after re-irrigation (Figure 1). In total, 46 RNA samples were isolated from young leaf tissues (Table 1). The samples represented at least two biological replicates of Beauregard and three biological replicates of Resisto. The unbalance number of samples of Beauregard was due to the RNA isolation of two samples at two-time points that were not of sufficient quality for RNA analyses. The failed 'Beauregard' RNA samples were collected at 48 hours of drought (T2) and 24 hours after re-irrigation (T3). Also, Bea33_TT1, representing the cv 'Beauregard' under 24 hours of drought, was not included in the analysis due to its gene-expression level's inconsistency with other samples under the same conditions. This resulted in RNAseq analysis with an unbalanced number of samples of Beauregard. However, since all the treatments of Beauregard had at least two valid biological replicates, it was possible to perform the analyses by comparing the means of remaining treatments. Phenotypic data were collected between 11:30 – 12:30 HRS from the same top young leaf tissue that was harvested later for the isolation of RNA.

Phenotypic Analyses

Nine phenotypic traits were evaluated during the four time points to document the phenotypic effects of the drought treatments on the plants. The dry weight of the aboveground parts and belowground parts, the number of storage roots**,** and the dry weight of the storage roots were collected one week after the re-irrigation, according to Nhanala & Yencho's (2020)

methodology. The height of the plants was also recorded by measuring the plant from the base of the stem to the apical meristem.

The stomatal conductance of the plants was recorded during the four time points, according to Nhanala & Yencho (2020). All measurements were taken during 11:30 - 12:30 HRS. The relative chlorophyll content, light intensity available for photosynthesis (Photosynthetically Active Radiation–PAR), leaf temperature differential, ambient temperature, and ambient humidity were also measured during the four time points. The measurement of the chlorophyll content and the remaining parameters were taken using a photosynthesis meter Photosynq MultispeQ v2.0 (PhotosynQ Inc., MI, USA) according to the instructions of the manufacturer.

Percentage change:

The percentage change of the several parameters were calculated by:

$$\%\ \text{of stress treatment} = \frac{\text{Mean of the stress treament}}{\text{Mean of the control treatment}} * 100;\ \text{and}$$

% Change = 100% - % of the stress treatment

Data Analysis

For the phenotypic characterization, an analysis of variance (ANOVA) of the data using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) was conducted by determining the least significant means at p-value ≤ 0.05, using the PROC GLM procedure and Tukey's HSD test.

Transcriptome Analyses

RNA isolation

Leaf tissue for RNA isolation was collected during the four time points (T0, T1, T2, and T3). The collection of young leaf tissue was performed in the greenhouse immediately after collecting the morphological and physiological data associated with drought stress at each time

point. Each young leaf was collected and immediately packaged in previously labeled, duplicated 16.5 cm x 14.9 cm Ziploc sandwich bags (SC Johnson Professional, Thailand). The Ziploc bags containing the leaf tissue were immediately flash-frozen in liquid nitrogen. The freeze-dried samples were transported to the laboratory for the isolation of RNA. The RNA was extracted on the same day the leaves were collected. Total RNA was isolated from 0.1g leaf tissue using the Spectrum^{TM} Plant Total RNA Kit (Sigma-Aldrich, St. Louis, Missouri, USA), following the manufacturer's instructions. The degradation and contamination of RNA was monitored on 1% agarose gel. The purity of the RNA was checked using a NanoPhotometer spectrophotometer (Implen, California). The integrity and quantification of the RNA was analyzed using the RNA Nano 6000 Assay Kit of the 2100 Bioanalyzer system (Agilent Technologies, California).

In total, 46 samples of RNA were isolated from young leaf tissues, with an unbalanced number of samples (at least two valid biological replicates) of 'Beauregard' (Table 1).

Complementary deoxyribonucleic acid (cDNA) library construction and sequencing

The library construction and transcriptome sequencing was done by Novogene (Sacramento, CA). The mRNA was obtained from the total RNA, with Novogene's Ribo-Zero kit used to remove rRNA while extracting high-quality mRNA. cDNA libraries were then prepared from the mRNA template. The cDNA library concentration was measured using a Qubit Fluorometer 2.0 (ThermoFisher Scientific, Wilmington, Delaware), followed by measuring the insert size using an Agilent 2100 Bioanalyzer (Agilent Technologies, California). The concentration of the libraries was measured by using NEBNext® Ultra RNA Library Prep Kit for Illumina (NEB, USA). A quantitative Polymerase Chain Reaction (qPCR) was performed with Phusion High-Fidelity DNA polymerase, Universal PCR primers and Index (X) Primer. A pooled library with the equimolar of each library was made. The pooled library was sequenced

by Novogene using the Illumina NovaSeq 6000 sequencing system to generate a 150 bp paired-end reads for each sample. The quality control of the sequenced data was done with a sequencing base quality value Q20, Q30 [(Base count of Phred value > 20 or 30) / (Total base count)]. In total, 1,933,353,160 raw reads (Table 2) were generated by Illumina sequencing.

RNA-Seq analysis

The quality control of the reads, trimming to eliminate adapter read through contamination, read mapping, and differential gene expression analysis were performed using the CLC Genomics workbench software (v. 21.0.3) (Qiagen, CA, USA). The clean reads were aligned to the hexaploid reference genome of *I. batatas* available on *Ipomoea* Genome Hub (https://ipomoea-genome.org/download_genome.html). We used this resource because of the hexaploid ploidy level of sweetpotato, and the fact that this is currently the only published cultivated sweetpotato reference genome available.

Differential Expression (DE) analysis

A trimmed mean of M values (TMM) was used to normalize the reads and measure the gene expression level (Robinson & Oshlack, 2010) and differential gene expression was analyzed by conducting whole transcriptome RNA-seq using. The results of the differential expression analysis were then filtered using a (False Discovery Rate (FDR) = < 5%, p-value < 0.05, and at least a two-fold change threshold) to determine the differentially expressed genes (DEGs) (Table 2).

A heatmap for the DEG was generated using the parameters cluster, based on Manhattan distance and complete linkage. A biological sample (Bea33_TT1) was eliminated due to its inconsistency in the gene clustering revealed by the heatmap. The genome annotation file (gff) file was used for structural and functional annotation of the DEGs.

The molecular function, cellular component, and biological process of the genes were consulted in the UniProt Knowledgebase (https://www.uniprot.org/uniprot/), and the physiological and molecular adaptations of the genes were consulted in the Drought Stress Gene Database (https://pgsb.helmholtz-muenchen.de/droughtdb/drought_db.html).

Venn Diagram

Venn Diagrams for the RNA seq were generated by comparing each group before water stress was imposed, and after the plants were exposed to drought at the different time points (T0, T1, T2, and T3) to their respective control groups (Figures 5a, 5b, 5c, 5d). The Venn Diagrams were generating in Jvenn (http://jvenn.toulouse.inra.fr/app/example.html), according to Bardou et al. (2014).

Identification of genes potentially involved in drought tolerance

The DEG analysis allowed us to identify thousands of genes that were differentially expressed between 'Beauregard' and 'Resisto'. The DEGs were sorted based on the descending order of fold change and p-values. The selection of candidate genes that were potentially involved in drought tolerance was based on the genes with the highest/significant fold changes and if their function was determined by functional analysis and reported in the literature.

RESULTS

Morphological assessment at harvest time

The drought treatment affected the plants' growth and development; however, the differences were not always significant. The results of the morphological assessment for control plants, and plants that were subjected to drought are presented in Table 3. The results showed

that the applied drought treatments were sufficient to cause an effect in the studied plants. In addition, ‘Resisto’ appeared to be more sensitive to drought than ‘Beauregard’.

Physiological parameters evaluated during the four time-points

Status of the plants. ‘Beauregard’ and ‘Resisto’ started to wilt 24 hours after the drought was imposed, and the wilting status of the plants became severe during the time points when the plants were under drought (Figure 1). Twenty-four hours after re-irrigating, both cultivars started to recover their normal appearance (Figure 1). However, as the plant began to recover from a wilted status to a normal appearance, some leaves had already begun the abscission process, and many of the leaves that had started to turn yellow never recovered to become green again.

Stomatal conductance. In plants that were exposed to drought, stomatal conductance was reduced during the four-time points for the two cultivars (Figure 2a). The decrease in the stomatal conductance occurred within 24 hours after drought in both ‘Beauregard’ and ‘Resisto’. However, ‘Resisto’, which had a greater ambient level of stomatal conductance than ‘Beauregard’, had a higher drop in the stomatal conductance than ‘Beauregard’.

Relative chlorophyll content. The relative chlorophyll content of both cultivars increased with drought events, followed by a decrease when the plants were re-irrigated (Figure 2b). The rise in the chlorophyll content increased with more extended periods of drought.

Leaf temperature differential (LTD). The LTD of ‘Resisto’ and ‘Beauregard’ increased 24 hours after drought (Figure 2c). However, in ‘Beauregard’, the LTD continued to increase during the period when the plants were under drought, and this parameter decreased when the plants were re-irrigated, while for ‘Resisto’, the LTD dropped after 24 hours of drought, and the LTD continued to drop when the plants were re-irrigated. Both cultivars had an LTD below the control group when 24 hours passed after the re-irrigation.

Transcriptome analysis

Differentially expressed genes (DEGs)

A total of 1,933,353,160 raw reads (~266 Gb) were generated by Illumina sequencing. After trimming for adapters and cleaning, a total of 1,932,075,734 reads were retained. The number of reads per sample are presented in Table 2. To identify the number of captured unigenes a transcriptome assembly of all clean reads was constructed to generate 64,295 unigenes using the pooled reads of all tissue types and treatments. However, the transcriptome assembly was not used for DEG analysis. Here the mapping of clean reads to the annotated sweetpotato genome is reported. The results are described below and are shown in Table 4 and Figure 3.

At T0 the 'Beauregard' control was compared to 'Beauregard' treated (drought). In this comparison 74 genes were up-regulated and 68 were down-regulated. The number of DEGs increased 24 hours after the drought treatment, with 2651 up-regulated and 4742 down-regulated genes. At 48 hours after drought, the number of DEG increased to 3648 up-regulated and 4972 down-regulated genes. At 24 hours after re-irrigation the number of DEGs decreased for 1802 up-regulated and 2822 down-regulated genes.

For the 'Resisto' control compared to 'Resisto' treated at T0, a total of 8 genes were upregulated and 78 down-regulated. The number of DEGs increased at 24 hours after the drought treatment, with 3105 up-regulated and 3007 down-regulated. At 48 hours after drought, the number of DEG increased for 4754 up-regulated and 5952 down-regulated genes. At 24 hours after re-irrigation the number of DEG decreased for 1910 up-regulated and 2718 down-regulated genes.

When the compared the control and "treated" data of 'Beauregard' and 'Resisto' before drought was imposed , it was observed two down-regulated genes. 'Beauregard' had 74 up-regulated genes and 66 down-regulated genes. 'Resisto' had 8 up-regulated and 76 down-regulated genes. Two genes were down-regulated in both 'Beauregard' and 'Resisto' 24 hours after drought was imposed (Figure 5a). These number of genes were due to the genotype. Twenty-four hours after drought was imposed, 'Beauregard' had 2500 up-regulated and 4676 down-regulated gens due to drought. 'Resisto' had 3097 up-regulated and 2941 down-regulated genes due to drought. The number of up-regulated and down-regulated gens due to drought at 48 hours after drought increased in both varieties.

Heatmap of the DEGs

The heatmap of the 6,341 DEGs revealed a cluster pattern that could be organized into three main groups according to their expression values (Figure 4). These clusters were as follows: cluster i) untreated plants at 24 hours before the drought, during 24 and 48 hours after drought, and 24 hours after re-irrigation; cluster ii) plants under drought for 24 hours and 48 hours; and cluster iii) plants at 24 hours after re-irrigation (recovery).

Comparisons of the number of DEG between and among genotypes/treatments

Two- and multiple-way comparisons of the number of DEG between and among genotypes/treatments were conducted, and the results are depicted in Figure 5.

The number of up-regulated and down-regulated DEGs was compared among/treatments before drought was imposed (Figure 5a). These DEGs were due to the differences between the genotypes. 'Beauregard' had 74 up-regulated genes, and 68 down-regulated genes. 'Resisto' had eight upregulated genes, and 78 downregulated genes. Two down-regulated genes were common for the two genotypes.

The number of up-regulated DEGs was compared among/treatments at 24 and 48 hours after drought (Figure 5b). 'Resisto' had more up-regulated DEGs than 'Beauregard' at both time points. A total of 727 DEGs were up-regulated in both cultivars at 24 and 48 hours after drought. At 48 hours after drought treatment, 'Beauregard' and 'Resisto' had 821 DEGs, the highest number of overlapped genes. The smallest overlap of DEGs, was between 'Resisto' after 24 hours of drought and 'Beauregard' after 48 hours of drought.

At 24 hours after the drought treatment, the overlap of down-regulated DEGs between 'Resisto' and 'Beauregard' was 125 (Figure 5c). At 48 hours after drought, the number of DEGs increased to 2061, the highest number of DEGs for all the possible comparisons. For 'Resisto' when comparing both treatments, 67 DEGs were down-regulated, while for 'Beauregard', 445 DEGs were down-regulated.

At 24 hours after re-irrigation, the number of up-regulated genes between the two genotypes was 1041, while 1643 genes were down-regulated. (Figure 5d). The remaining pairwise comparisons of gene expression changes (up-regulation and down-regulation) occurring 24 hours after re-irrigation, exhibited less than 10 DEG. Further experiments are required to determine the exact time.

Candidate genes involved in drought tolerance

Several other investigators have previously reported candidate genes associated with drought tolerance in rice, tomato, tobacco, wheat, and potato (Seo et al., 2011; Muñoz-Mayor et al., 2012; Li et al., 2017; Chen et al., 2016; 2019). Here we report on the expression patterns of these genes in our study. In total, we identified six genes that were potentially involved in drought tolerance and two genes that were plausibly involved in recovery from drought (Table 5). For the 24HR drought treatment the following DEG's were identified for additional analyses:

the abscisic acid and environmental stress-inducible protein TAS14-like (TAS14); an 18.8 kDa class II heat shock protein-like gene; a E3 ubiquitin-protein ligase RING1-like gene; the basic form of pathogenesis-related protein 1 (PRP 1); and expansin A15 – like (EXLA 15). While for the 48 HR after drought treatment the following DEG's were identified for additional analyses: abscisic acid and environmental stress-inducible protein TAS14-like (TAS14), basic form of pathogenesis-related protein 1 (PRP 1), expansin A15 – like (EXLA 15), and the desiccation-related protein PCC13-62-like. For the 24 HR after re-irrigation/rehydration treatments we studied further*:* Cell wall-associated receptor kinase-like 1, and Receptor-like serine/threonine-protein kinase SD1-7-like. The fold change and the differences of expression of these genes between the two cultivars are discussed in the section below. In addition, we also review the molecular function, cellular component, and biological process of each gene.

DISCUSSION

Phenotypic assessment

In the current study, 'Resisto' appeared to be less tolerant to drought than 'Beauregard'. The dry weight of the belowground part biomass was the only trait in which 'Resisto' was less affected by drought than 'Beauregard'. For the remaining eight traits, 'Beauregard' was less affected by drought than 'Resisto' (Table 3).

Leaf wilting is visually one of the first signs of drought stress in a plant. It is associated with the stomatal opening and increased water loss via transpiration (Ache et al., 2010; Waterland et al., 2010). Thus, under drought stress, stomatal conductance decreases to prevent water loss (and this loss is reflected in the wilted appearance). Likewise, slow wilting is a trait associated with drought tolerance (Waterland et al., 2010; Devi & Sinclair, 2013; Prince et al.,

2015; Ye et al., 2020). ‘Beauregard’ and ‘Resisto’, started to wilt 24 hours after drought was imposed. At 48 hours after drought being subjected, the leaves of the two varieties appeared to be even more wilted (Figure 1).

Leaf wilting is associated with the stomatal opening (Ache et al., 2010; Waterland et al., 2010). Stomatal closure is induced by abscisic acid to prevent water loss (Horton, 1971; Downton et al., 1988; McAinsh et al., 1990; Tanaka et al., 2005). Stomatal closure leads to the inhibition of photosynthesis (Downton et al., 1988). In both cultivars, stomatal conductance decreased when drought was imposed. The decrease in the stomatal conductance due to drought was also observed in soybeans (Liu et al., 2003; Makbul et al., 2011), beans (Pimentel et al., 1999; Miyashita et al., 2005), and melon (Kusvuran et al., 2012). However, 24 hours after re-irrigation, none of the cultivars had started to recover. At 24 hours after imposing drought, ‘Resisto’ had a higher reduction in stomatal conductance than ‘Beauregard’ (Figure 2a), and it appeared that ‘Resisto’ was more sensitive to drought than ‘Beauregard’. In melon, the stomatal conductance was lower in drought-sensitive genotypes than in the tolerant ones (Kusvuran, 2012). This data suggests that ‘Resisto’ may sense drought faster than ‘Beauregard’ and decreased stomatal conductance faster than ‘Beauregard’, and therefore started to prevent water loss first by its rapid response. In contrast, this data may suggest that the effects of drought on ‘Beauregard’ were less than in ‘Resisto’, and therefore, the decrease of the stomatal closure was gradual (i.e., the decrease of stomatal conductance was slower in ‘Beauregard’ than in ‘Resisto’). This perspective may support the hypothesis of ‘Beauregard’ is less sensitive to drought than ‘Resisto’ and that ‘Beauregard’ is a slow wilting cultivar.

The relative chlorophyll content increased in both varieties during the drought period. An increase in the chlorophyll content was also observed in wheat plants that were exposed to five

days of drought (Nikolaeva et al., 2010). The data (Figure 2b) shows that the increase in the chlorophyll content has a linear relationship with the time of exposure to drought. Yet, 'Resisto' had a higher increase in the chlorophyll content than 'Beauregard'. Re-irrigation led to a decrease in the chlorophyll content. However, chlorophyll content decreased with drought in other studies on wheat, barley (Li et al., 2006) and soybean (Makbul et al., 2011). The effects of drought on chlorophyll content were not however, well understand due to variability of plant response regarding the effects of drought on chlorophyll content.

Drought can lead to changes in the temperature differential between a plant and the environment. When 'Beauregard' and 'Resisto' were exposed to drought, both clones exhibited an increase in the leaf temperature after 24 hours of drought (Figure 2c). Drought also caused an increase in the leaf temperature in wheat (Siddique et al., 2000), tomato (Vermeulen et al., 2007), and melon (Kusvuran, 2012). The leaf temperature differential increased in 'Beauregard' during the two-time points when plants were under drought stress, and it decreased after the plants were re-irrigated. In 'Resisto', however, the increase of the leaf temperature differential occurred during the first 24 hours after imposing drought, followed by a decrease of this temperature differential.

Candidate genes involved in drought tolerance

Different intensities of drought affect plants in different ways. The genetic background is one of the factors that defines how a plant responds to a drought event. At the molecular level, the effects of the intensity of limited water stress will be reflected by the different drought tolerant genes that are being differentially expressed at different time points of the water scarcity. Additionally, the differences in gene expression will vary with the genetic background of the variety. In this section, we discuss the genes that we identified as potentially involved in drought

tolerance based on prior research. The discussion includes the time point when those genes were expressed, and the fold change that occurred in each cultivar for each specific gene. We also compare the ratio of the fold change between 'Beauregard' and 'Resisto' for each DEG. In addition, we also discussed two DEGs that we identified as being involved with the recovery of the plant after a period of drought.

Drought tolerance pathway

As shown by the phenotypic data in this study and other studies, drought tolerance is negatively correlated with the stomatal closure (Schroeder et al., 2001; Cominelli et al., 2005; Bartlett et al., 2016). Stomatal closure occurs due to the increasing abscisic acid (ABA), and therefore, the ABA pathway is often involved in plants' response to drought. As discussed in the phenotypic assessment, ABA induces stomatal closure. Thus, the regulation of a gene that results in stomatal closure when a plant is under drought stress can be understood as a drought tolerance sign. Genes/gene families that have been identified with potential drought tolerance activity are often associated with the biosynthesis of abscisic acid. However, it should be noted that there are other pathways that are associated with drought tolerance that do not necessarily need to be associated with ABA and stomatal closure regulation. The biosynthesis of ethylene (Manavella et al., 2006; Pan et al., 2012; Yu et al., 2017) and jasmonic acid (Seo et al., 2011; Fang et al., 2016; Ruan et al., 2019) for example, are other pathways that have been reported to be involved in drought tolerance in plants.

i) Significant DEGs at 24 hours after the drought was imposed

Abscisic acid and environmental stress-inducible protein TAS14-like (TAS14-like). TAS14 is an ABA inducible gene that encodes late embryogenesis abundant (LEA) proteins (Roychoudhury & Paul, 2012). Late-embryogenesis abundant (LEA)/dehydrins are hydrophilic

proteins involved in drought tolerance (Battaglia & Covarrubias et al., 2013; Magwanga et al., 2018). The gene is active in the cytosol, and it is associated with a response to abscisic acid and response to water deprivation. The TAS14 physiological adaptation of the gene is associated with detoxification that results as a protection factor. TAS14 is a protein that acts as a protection factor, as a response to dehydration and to drought, and it is related to drought tolerance. In sweetpotato, a TAS14-like gene was identified by Lau et al. (2018). Similarly, TAS14 was also identified as a drought-tolerant gene in tomato (Muñoz-Mayor et al., 2012), and potato (van Muijen et al., 2016). In the current study, the TAS14 had a greater fold change in 'Beauregard' than in 'Resisto', with the ratio of the 'Beauregard'/'Resisto' fold change at 1.21 (Table 5, Figure 6a). The phenotypic assessment revealed that 'Resisto' had a higher decrease of stomatal conductance than 'Beauregard' (Figure 2a). This fact may be associated with the observed differential expression of 'Beauregard'/'Resisto'.

18.8 kDa class II heat shock protein-like. Heat-shock proteins (HSP's) have been associated with drought tolerance in plants such as in Arabidopsis, and rice (Huang et al., 2016; Xiang et al., 2018). In a review, Haq et al. (2019), also attributes the ability of HSP's to increase drought tolerance to detoxification by positively regulating the antioxidant enzyme system, resulting in enhanced membrane stability. Therefore, it can be inferred that HSP's act as reactive oxygen species (ROS) scavenging-related genes. Heat-shock proteins have been associated with the induction of proteins associated with biotic stresses (Park & Seo, 2015; Haq et al., 2019), such as the pathogenesis-related family of proteins. Heat shock is associated to the reaction of the plant due to the increase of internal temperature when drought is imposed. The impact of the changes in temperature in the two cultivars is shown in Figure 2c. HSP's are active in the cytoplasm, the molecular functions are protein self-association and unfolded protein binding, and

the biological processes are responses to salt stress and to heat, hydrogen peroxide, protein folding, and protein complex oligomerization. The exact biological function of the 18.8 KDa heat shock protein-like gene is the response to osmotic stress. Several heat shock genes were DEG's; however, we observed that the "18.8 KDa" gene had the most significant fold changes with the ratio of the 'Beauregard'/'Resisto' fold change being 122.04 (Table 5). The HSP 18.8 KDa gene was upregulated 24 hours after drought; however, the differential expression between 'Beauregard' and 'Resisto' was very significant, and the 'Beauregard'/'Resisto' fold-change ratio was 122.04.

E3 ubiquitin-protein ligase RING1-like. The ubiquitin E3 ligase gene positively regulates drought tolerance through stomatal regulation via ABA signaling (Liu et al, 2013; Ding et al., 2015; Lim et al., 2017; He et al., 2018). The Ubiquitin E3 ligase is a regulatory protein, active in the cytoplasm. It's molecular function is associated with ubiquitin-protein ligase activity, while its biological process is protein ubiquitination. In Arabidopsis, the overexpression of E3 ubiquitin ligase RING-type has been recognized as playing a role in drought tolerance (Ding et al., 2015; Joo et al., 2018; Yang et al., 2018). In pepper, the E3 ubiquitin ligase gene drought tolerance RING1 has been associated with response to ABA and dehydration, and therefore, a positive regulator of drought response (Joo et al., 2016). Likewise, in tobacco, the overexpression of E3 ubiquitin ligase gene enhanced drought tolerance by regulating stomatal movement (Liu et al., 2013). The physiological adaptation of RING-finger protein is associated with ion and osmotic homeostasis which regulates stomatal activity. The phenotypic assessment showed the differences in stomatal movement during a drought event (Figure 2a), and this gene had a higher fold change in 'Beauregard' than in 'Resisto' with DEG ratio of 'Beauregard'/'Resisto' 30.6.

Basic forms of pathogenesis-related protein 1 (PRP-1). The PRP-1 genes have also been associated to drought tolerance (Seo et al., 2008; Ali et al., 2018; Akbudak et al., 2020). The pathogenesis-related protein-1 (PR-1) activates the defense signaling pathway and the accumulation of salicylic acid (Seo et al., 2008; Ali et al., 2018a; 2018b). The PRP-1 genes are pathogenesis-related proteins, with biological functions in plant defense and responses to a biotic stimuli, and they can be found in the extracellular regions of the cell. The ratio of the fold change 'Beauregard'/'Resisto' was 44.3 (Table 5, Figure 6c), when the plants were exposed to drought. The upregulation of the PR-1 has been shown to be induced by the accumulation of salicylic acid, and it has been associated with drought tolerance in tomato (Akbudak et al., 2020) and osmotic and salinity stresses in wheat (Wang et al., 2019).

Expansin-like A15 (EXLA15). Expansins (expansin-like proteins) are proteins whose function is associated with cell wall expansion due to the loosening of plant cell walls (Sampedro & Cosgrove, 2005; Choi et al., 2006; Marowa et al., 2016). Expansin-A15-like (EXLA15) is located (cellular component) in the nucleus, and its molecular function is to enable DNA binding, and it is important for the regulation of cellular transcription (transcriptional control), and it is related to the regulation of gene-specific transcription. The Expansin-A15, has a cell wall and cellular membrane components, with a molecular function of causing loosening and extension of the plant cell walls, and the biological process is cell wall organization. In our studies, the ratio of the fold change 'Beauregard'/'Resisto' was 2.94 (Table 5, Figure 6b), when the plants were exposed to drought. EXLA15 has been associated with drought tolerance due to leaf cell wall loosening in Arabidopsis (Clauw et al., 2015). We hypothesize that the expansin-A15-like gene is probably involved in drought tolerance by contributing to the stomatal movement through the loosing and extension of the cell wall of the guard cells. Expansins have

been associated with drought tolerance due to their regulation of stomatal activity by loosening the structure of the cell walls of the guard cells of stomata when plants are exposed to dehydration (Li et al., 2011; Wei et al., 2011a, 2011b; Zhang et al., 2011; Zhao et al., 2012). Arabidopsis, tobacco, and wheat (Li et al., 2001; Zhao et al., 2011; Clauw et al., 2015; Zhou et al., 2015; Chen et al.,2016) have been engineered to overexpress expansins in response to drought. Expansins are induced by abscisic acid (Zhao et al., 2012), which is involved in stomatal aperture regulation in plants under drought stress.

ii) Significant DEGs at 48 hours after the drought was imposed

Depending on the duration and intensity of drought, different genes for drought tolerance may be expressed. The expression of E3 ubiquitin-protein ligase RING1-like, and heat shock genes (18.8 kDa class II heat shock protein-like) was not significant at 48 hours after the drought was imposed. Instead, desiccation-related protein PCC13-62-like, dehydration-responsive element-binding protein 1D-like, and NAC domain-containing protein 21/22-like genes were significantly expressed at 48 hours after the drought was imposed. The DEG: abscisic acid and environmental stress-inducible protein TAS14, expansin-A15-like (EXLA15), and Basic form of pathogenesis-related protein-1, were significantly expressed during the two-time points (24 and 48 hours after drought was imposed). These observations support the hypothesis that genotypes and extent of drought, combined, are critical factors in the determining the number of DEGs expressed during a stress period.

The *desiccation-related protein PCC13-62* was identified in *Craterostigma plantagineum* (Bartels et al., 1990; Piatkowski et al, 1990; Rodriguez et al., 2010; Giarola et al., 2018), a species also known as “resurrection plant” due to its capacity of recovery from drought after extended periods of water stress (Giarola et al., 2017). The PCC13-62, a late-embryogenesis

abundant (LEA) family gene**,** was induced by leaf desiccation in leaves and by ABA, and it appears to be involved in the cellular response to desiccation and high salt concentrations. In our studies, the desiccation-related protein PCC13-62 was significantly upregulated when the plant was exposed to 48 hours for drought; however, it was not significantly upregulated during the first 24 hours of drought. This suggests that the expression of PCC13-62 may increase the longer drought progresses. The name of the gene itself (desiccation), instead of dehydration, suggests that this gene may increase its fold change the more the plant loses water content. At 48 hours after drought, 'Beauregard' had a greater fold change than 'Resisto', with a 'Beauregard'/'Resisto' fold change ratio of 3.92 (Table 5).

iii) Significant DEGs at 24 hours after re-irrigation (rehydration) – Genes associated with plant recovery

Wall-associated receptor kinase-like 1 (WAK1). WAK1 was upregulated in 'Beauregard' and 'Resisto', with 'Beauregard' showing higher levels of fold change than 'Resisto' for all the WAK1s that were identified during the DEG analysis. Wall-associated receptor kinase-like 1 proteins were affected significantly after rehydration, with a 'Beauregard'/'Resisto' fold-change ratio of 47.39 (Table 5). The upregulation of WAK1 after a rehydration episode was also observed by Giarola et al. (2016). *Craterostigma plantagineum* cell wall-associated protein kinase 1 (CpWAK1), a gene from a "resurrection plant" was downregulated when leaves were desiccated and was upregulated when they were rehydrated (Giarola et al., 2016). This result shows that for plant recovery after a period of drought, the capacity of expansion of the cell wall plays a critical role. The wall-associated receptor kinase is an integral component of the membrane, and its molecular function is ATP binding and protein serine/threonine kinase activity, and its involvement in biological process includes protein phosphorylation. WAK-like

genes (WAKL) are wall-associated proteins and they are required for cell expansion (Kohorn & Kohorn, 2012). Wall receptor-like kinases (RLKs) are transmembrane proteins (Shiu &Bleecker, 2001), meaning that their function in response to rehydration is associated with wall extensibility. Wall-associated receptor kinase has been identified as a candidate gene for drought tolerance in barley (Abou-Elwafa, 2018).

Receptor-like serine/threonine-protein kinase SD1-7-like. The Receptor-like serine/threonine-protein kinase has been associated with activities related to plant recovery from stress in Arabidopsis (Lease et al., 2001; Sun et al., 2013). They are located in the membrane and, therefore, responsible for signaling different processes in response to stress (Walker, 1994). In our studies, the 'Beauregard'/'Resisto' fold change ratio was 28.27 (Table 5). The biological processes of these proteins are associated with the ABA-activated signaling pathway, cellular response to ABA stimulus, protein phosphorylation, and pollen recognition; this means that the biological process is associated with the ABA signaling pathway. The molecular functions are ATP binding, carbohydrate-binding, protein kinase activity, protein serine/threonine kinase activity, protein serine kinase activity, protein threonine kinase activity, and ubiquitin protein ligase binding, and this means that the molecular function is associated with kinase, receptor, serine/threonine-protein kinase, and transferase.

These data suggest that the plants under drought started to return to an equilibrium point quickly after the drought event was concluded.

iv) Significant DEGs at 24 hours and 48 hours after the drought was imposed

Abscisic acid and environmental stress-inducible protein TAS14-like. TAS14 was expressed 24 hours after the drought was imposed with a ratio of the fold change of 'Beauregard'/'Resisto' of 1.21. Forty-eight hours after the drought was imposed, the ratio of the

fold change of ‘Beauregard’/’Resisto’ increased by 1.66 (Figure 6a). This increase of TAS14 is in accordance with the slight decrease of stomatal conductance in both cultivars from 24 hours to 48 hours after the drought was imposed (Figure 2a).

The *expansin-A15-like (EXLA15)* also was significantly differentially expressed at 48 hours after drought, with the ratio of ‘Beauregard’/’Resisto’ fold change increasing from 2.94 at 24 hours after drought to 5.72 at 48 hours.

Basic form of pathogenesis-related protein 1 (PRP-1). At 24 hours after the drought, the ratio of fold change ‘Beauregard’/’Resisto’ was 44.3. However, this ratio decreased 48 hours after drought, with the ‘Beauregard’/’Resisto’ fold change being 7.49. Both varieties went through a decrease in the fold change and ‘Beauregard’ suffered the most significant decrease from 24 to 48 hours after the drought event.

CONCLUSIONS

In the current study, we did not evaluate the survival rate of the cultivars. However, Nhanala & Yencho (2020) observed survival rates of 89% (‘Beauregard’), and 33% (‘Resisto’) when the plants were exposed to 50 days of drought. Our phenotypic data were in accordance with what was observed before by Nhanala & Yencho (2020), which was that ‘Beauregard’ tended to perform better than ‘Resisto’ in a greenhouse-based study during severe drought.

We identified six DEG’s [abscisic acid and environmental stress-inducible protein TAS14-like (TAS14), 18.8 kDa class II heat shock protein-like, E3 ubiquitin-protein ligase RING1-like, pathogenesis-related protein 1 (PRP-1), expansin -A15 (EXLA15), and desiccation-related protein PCC13-62] that have been associated with drought tolerance. All those genes had a greater fold change in ‘Beauregard’ than in ‘Resisto’, and sometimes some of the genes could

be confirmed by phenotypic responses (e.g., the stomatal conductance response and genes that are related to ABA signaling such as TAS14). We also identified two genes that may be involved in the recovery of the plant after a period of drought (wall-associated receptor kinase-like 1, and receptor-like serine/threonine-protein kinase SD1-7-like**).**

In addition, we observed that some of the genes were expressed only during one-time point (24 or 48 hours of drought) (e.g., E3 ubiquitin-protein ligase RING1-like, and heat shock genes (18.8 kDa class II heat shock protein-like)). The effects of drought were variable depending on the duration and intensity of drought. We also concluded that differences in the duration of the drought event exhibit different damage symptoms at a phenotypic level due to the over/under expression of different genes. Finally, the current study was complementary to our previous work (Nhanala &Yencho, 2020), where a greenhouse-based study to evaluate *Ipomoea spp.* for drought tolerance was conducted. The differences in the performance of the different varieties of sweetpotato led to the current study. Further studies are needed to confirm our results, including quantitative real-time polymerase chain reaction (qRT-PCR) analysis to confirm/validate the potential role of these genes in increased drought tolerance in sweetpotato.

REFERENCES

Abou-Elwafa, S. F. (2018). Identification of genes associated with drought tolerance in barley. *Biologia Plantarum*, *62*(2), 299-306.

Ache, P., Bauer, H., Kollist, H., Al-Rasheid, K. A., Lautner, S., Hartung, W., & Hedrich, R. (2010). Stomatal action directly feeds back on leaf turgor: new insights into the regulation of the plant water status from non-invasive pressure probe measurements. *The Plant Journal*, *62*(6), 1072-1082.

Akbudak, M. A., Yildiz, S., & Filiz, E. (2020). Pathogenesis related protein-1 (PR-1) genes in tomato (*Solanum lycopersicum* L.): Bioinformatics analyses and expression profiles in response to drought stress. *Genomics*, *112*(6), 4089-4099.

Aleem, M., Raza, M. M., Haider, M. S., Atif, R. M., Ali, Z., Bhat, J. A., & Zhao, T. (2020). Comprehensive RNA-seq analysis revealed molecular pathways and genes associated with drought tolerance in wild soybean (Glycine soja Sieb. and Zucc.). *Physiologia Plantarum*.

Ali, S., Ganai, B. A., Kamili, A. N., Bhat, A. A., Mir, Z. A., Bhat, J. A., Tyagi, A., Islam, S.T., Mushtaq, M., Yadav, P., & Grover, A. (2018a). Pathogenesis-related proteins and peptides as promising tools for engineering plants with multiple stress tolerance. *Microbiological Research*, *212*, 29-37.

Ali, S., Mir, Z. A., Bhat, J. A., Tyagi, A., Chandrashekar, N., Yadav, P., Rawat, S., Sultana, M. & Grover, A. (2018b). Isolation and characterization of systemic acquired resistance marker gene PR1 and its promoter from *Brassica juncea*. *3 Biotech*, *8*(1), 1-14.

Allagulova, C. R., Gimalov, F. R., Shakirova, F. M., & Vakhitov, V. A. (2003). The plant dehydrins: structure and putative functions. *Biochemistry (Moscow)*, *68*(9), 945-951.

Andrade, M. I., Naico, A., Ricardo, J., Eyzaguirre, R., Makunde, G. S., Ortiz, R., & Grüneberg, W. J. (2016). Genotype× environment interaction and selection for drought adaptation in sweetpotato (*Ipomoea batatas* (L.) Lam) in Mozambique. *Euphytica*, *209*(1), 261–280. https://doi.org/10.1007/s10681-016-1684-4

Arisha, M. H., Ahmad, M. Q., Tang, W., Liu, Y., Yan, H., Kou, M., Zhang, Y., & Li, Q. (2020). RNA-sequencing analysis revealed genes associated drought stress responses of different durations in hexaploid sweet potato. *Scientific reports*, *10*(1), 1-17.

Bardou, P., Mariette, J., Escudié, F., Djemiel, C., & Klopp, C. (2014). jvenn: an interactive Venn diagram viewer. *BMC bioinformatics*, *15*(1), 1-7.

Bartels, D., Schneider, K., Terstappen, G., Piatkowski, D., & Salamini, F. (1990). Molecular cloning of abscisic acid-modulated genes which are induced during desiccation of the resurrection plant *Craterostigma plantagineum*. *Planta*, *181*(1), 27-34.

Bartlett, M. K., Klein, T., Jansen, S., Choat, B., & Sack, L. (2016). The correlations and sequence of plant stomatal, hydraulic, and wilting responses to drought. *Proceedings of the National Academy of Sciences*, *113*(46), 13098-13103.

Battaglia, M., & Covarrubias, A. A. (2013). Late embryogenesis abundant (LEA) proteins in legumes. *Frontiers in plant science*, *4*, 190.

Blum, A. (2011). Drought resistance – is it really a complex trait?. *Functional Plant Biology*, *38*(10), 753-757.

Cellier, F., Conéjéro, G., Breitler, J. C., & Casse, F. (1998). Molecular and physiological responses to water deficit in drought-tolerant and drought-sensitive lines of sunflower: accumulation of dehydrin transcripts correlates with tolerance. *Plant physiology*, *116*(1), 319-328.

Charfeddine, S., Charfeddine, M., Saïdi, M. N., Jbir, R., & Bouzid, R. G. (2017). Potato dehydrins present high intrinsic disorder and are differentially expressed under ABA and abiotic stresses. *Plant Cell, Tissue and Organ Culture (PCTOC)*, *128*(2), 423-435.

Chen, Y., Han, Y., Zhang, M., Zhou, S., Kong, X., & Wang, W. (2016). Overexpression of the wheat expansin gene TaEXPA2 improved seed production and drought tolerance in transgenic tobacco plants. *PLoS One*, *11*(4), e0153494.

Chen, Y., Li, C., Zhang, B., Yi, J., Yang, Y., Kong, C., Lei, C., & Gong, M. (2019). The role of the late embryogenesis-abundant (LEA) protein family in development and the abiotic stress response: a comprehensive expression analysis of potato (*Solanum tuberosum*). *Genes*, *10*(2), 148.

Chen, Z., Hong, X., Zhang, H., Wang, Y., Li, X., Zhu, J. K., & Gong, Z. (2005). Disruption of the cellulose synthase gene, AtCesA8/IRX1, enhances drought and osmotic stress tolerance in Arabidopsis. *The Plant Journal*, *43*(2), 273-283.

Choi, D., Cho, H. T., & Lee, Y. (2006). Expansins: expanding importance in plant growth and development. *Physiologia plantarum*, *126*(4), 511-518.

Chung, P. J., Jung, H., Do Choi, Y., & Kim, J. K. (2018). Genome-wide analyses of direct target genes of four rice NAC-domain transcription factors involved in drought tolerance. *BMC genomics*, *19*(1), 1-17.

Clauw, P., Coppens, F., De Beuf, K., Dhondt, S., Van Daele, T., Maleux, K., K., Storme, V., Clement, L., Gonzalez, N., & Inzé, D. (2015). Leaf responses to mild drought stress in natural variants of Arabidopsis. *Plant physiology*, *167*(3), 800-816.

Close, T. J. (1997). Dehydrins: a commonalty in the response of plants to dehydration and low temperature. *Physiologia Plantarum*, *100*(2), 291-296.

Cominelli, E., Galbiati, M., Vavasseur, A., Conti, L., Sala, T., Vuylsteke, M., M., Leonhardt, N., Dellaporta, S.L., & Tonelli, C. (2005). A guard-cell-specific MYB transcription factor regulates stomatal movements and plant drought tolerance. *Current biology*, *15*(13), 1196-1200.

Deshmukh, R., Singh, A., Jain, N., Anand, S., Gacche, R., Singh, A., & Singh, N. (2010). Identification of candidate genes for grain number in rice (*Oryza sativa* L.). *Functional & integrative genomics*, *10*(3), 339-347.

Devi, M. J., & Sinclair, T. R. (2013). Fixation drought tolerance of the slow-wilting soybean PI 471938. *Crop Science*, *53*(5), 2072-2078.

Downton, W. J. S., Loveys, B. R., & Grant, W. J. R. (1988). Stomatal closure fully accounts for the inhibition of photosynthesis by abscisic acid. *New Phytologist*, *108*(3), 263-266.

Drought Stress Gene Database (http://pgsb.helmholtz-muenchen.de/droughtdb/drought_db.html, accessed in January 2021)

Egea, I., Albaladejo, I., Meco, V., Morales, B., Sevilla, A., Bolarin, M. C., & Flores, F. B. (2018). The drought-tolerant *Solanum pennellii* regulates leaf water loss and induces genes involved in amino acid and ethylene/jasmonate metabolism under dehydration. *Scientific reports*, *8*(1), 1-14.

Fang, L., Su, L., Sun, X., Li, X., Sun, M., Karungo, S. K., Fang, S., Chu, J., Li, S., & Xin, H. (2016). Expression of *Vitis amurensis* NAC26 in Arabidopsis enhances drought tolerance by modulating jasmonic acid synthesis. *Journal of experimental botany*, *67*(9), 2829-2845.

FAOSTAT (http://www.fao.org/faostat/en/#data/QC, accessed on June 28, 2021)

Firon, N., LaBonte, D., Villordon, A., Kfir, Y., Solis, J., Lapis, E., Perlman, T.S., Doron-Faigenboim, A., Hetzroni, A., Althan, L., & Nadir, L. A. (2013). Transcriptional profiling of sweetpotato (Ipomoea batatas) roots indicates down-regulation of lignin biosynthesis and up-regulation of starch biosynthesis at an early stage of storage root formation. *BMC genomics*, *14*(1), 1-25.

Fischer, R. A., & Maurer, R. (1978). Drought resistance in spring wheat cultivars. I. Grain yield responses. *Australian Journal of Agricultural Research*, *29*(5), 897-912.

Fujita, M., Fujita, Y., Maruyama, K., Seki, M., Hiratsu, K., Ohme-Takagi, M., Tran, L.S.P., Yamaguchi-Shinozaki, K., & Shinozaki, K. (2004). A dehydration-induced NAC protein, RD26, is involved in a novel ABA-dependent stress-signaling pathway. *The Plant Journal*, *39*(6), 863-876.

Gao, F., Xiong, A., Peng, R., Jin, X., Xu, J., Zhu, B., Chen, J., & Yao, Q. (2010). OsNAC52, a rice NAC transcription factor, potentially responds to ABA and confers drought tolerance in transgenic plants. *Plant Cell, Tissue and Organ Culture (PCTOC)*, *100*(3), 255-262.

Garay-Arroyo, A., Colmenero-Flores, J. M., Garciarrubio, A., & Covarrubias, A. A. (2000). Highly hydrophilic proteins in prokaryotes and eukaryotes are common during conditions of water deficit. *Journal of Biological Chemistry*, *275*(8), 5668-5674.

Giarola, V., Hou, Q., & Bartels, D. (2017). Angiosperm plant desiccation tolerance: hints from transcriptomics and genome sequencing. *Trends in Plant Science*, *22*(8), 705-717.

Giarola, V., Jung, N. U., Singh, A., Satpathy, P., & Bartels, D. (2018). Analysis of pcC13-62 promoters predicts a link between cis-element variations and desiccation tolerance in Linderniaceae. *Journal of experimental botany*, *69*(15), 3773-3784.

Goel, D., Singh, A. K., Yadav, V., Babbar, S. B., & Bansal, K. C. (2010). Overexpression of osmotin gene confers tolerance to salt and drought stresses in transgenic tomato (*Solanum lycopersicum* L.). *Protoplasma*, *245*(1-4), 133-141.

Golldack, D., Li, C., Mohan, H., & Probst, N. (2014). Tolerance to drought and salt stress in plants: unraveling the signaling networks. *Frontiers in plant science*, *5*, 151.

Gong, Lei, Hongxia Zhang, Xiaoyan Gan, Li Zhang, Yuchao Chen, Fengjie Nie, Lei Shi et al. "Transcriptome profiling of the potato (*Solanum tuberosum* L.) plant under drought stress and water-stimulus conditions." *PLoS One* 10, no. 5 (2015): e0128041.

Hanin, M., Brini, F., Ebel, C., Toda, Y., Takeda, S., & Masmoudi, K. (2011). Plant dehydrins and stress tolerance: versatile proteins for complex mechanisms. *Plant signaling & behavior*, *6*(10), 1503-1509.

Hong, Z. H. U., ZHOU, Y. Y., Hong, Z. H. A. I., HE, S. Z., Ning, Z. H. A. O., & LIU, Q. C. (2019). Transcriptome profiling reveals insights into the molecular mechanism of drought tolerance in sweetpotato. *Journal of integrative agriculture*, *18*(1), 9-23.

Horton, R. F. (1971). Stomatal opening: the role of abscisic acid. *Canadian Journal of Botany*, *49*(4), 583-585.

Hu, H., Dai, M., Yao, J., Xiao, B., Li, X., Zhang, Q., & Xiong, L. (2006). Overexpressing a NAM, ATAF, and CUC (NAC) transcription factor enhances drought resistance and salt tolerance in rice. *Proceedings of the National Academy of Sciences*, *103*(35), 12987-12992.

Huang, Q., Wang, Y., Li, B., Chang, J., Chen, M., Li, K., ... & He, G. (2015). TaNAC29, a NAC transcription factor from wheat, enhances salt and drought tolerance in transgenic Arabidopsis. *BMC plant biology*, *15*(1), 1-15.

Huang, Y. C., Niu, C. Y., Yang, C. R., & Jinn, T. L. (2016). The heat stress factor HSFA6b connects ABA signaling and ABA-mediated heat responses. *Plant physiology*, *172*(2), 1182-1199.

Ipomoea Genome Hub (https://ipomoea-genome.org/, accessed in December 2020)

Jeong, J. S., Kim, Y. S., Baek, K. H., Jung, H., Ha, S. H., Do Choi, Y., Kim, M., Reuzeau, C., & Kim, J. K. (2010). Root-specific expression of OsNAC10 improves drought tolerance and grain yield in rice under field drought conditions. *Plant physiology*, *153*(1), 185-197.

Joshi, R., Wani, S. H., Singh, B., Bohra, A., Dar, Z. A., Lone, A. A., Pareek, A., & Singla-Pareek, S. L. (2016). Transcription factors and plants response to drought stress: current understanding and future directions. *Frontiers in Plant Science*, *7*, 1029.

Khan, A., Ali, M., Khattak, A. M., Gai, W. X., Zhang, H. X., Wei, A. M., & Gong, Z. H. (2019). Heat shock proteins: dynamic biomolecules to counter plant biotic and abiotic stresses. *International journal of molecular sciences*, *20*(21), 5321.

Khan, M. S., Ahmad, D., & Khan, M. A. (2015). Utilization of genes encoding osmoprotectants in transgenic plants for enhanced abiotic stress tolerance. *Electronic Journal of Biotechnology*, *18*(4), 257-266.

Kivuva, B. M., Githiri, S.M., Yencho, G. C.,&Sibiya, J. (2015). Screening sweetpotato genotypes for tolerance to drought stress. *Field Crops Research*, *171*, 11–22. https://doi.org/10.1016/j.fcr.2014.10.018

Kosma, D. K., & Jenks, M. A. (2007). Eco-physiological and molecular-genetic determinants of plant cuticle function in drought and salt stress tolerance. In *Advances in molecular breeding toward drought and salt tolerant crops* (pp. 91-120). Springer, Dordrecht.

Krannich, C. T., Maletzki, L., Kurowsky, C., & Horn, R. (2015). Network candidate genes in breeding for drought tolerant crops. *International journal of molecular sciences*, *16*(7), 16378-16400.

Kumar, S. A., Kumari, P. H., Jawahar, G., Prashanth, S., Suravajhala, P., Katam, R., Sivan, P., Rao, K.S., Kirti, P.B., & Kishor, P. K. (2016). Beyond just being foot soldiers–osmotin like protein (OLP) and chitinase (Chi11) genes act as sentinels to confront salt, drought, and fungal stress tolerance in tomato. *Environmental and Experimental Botany*, *132*, 53-65.

Kumar, S., Dwivedi, S. K., Singh, S. S., Jha, S. K., Lekshmy, S., Elanchezhian, R., , Singh, O.N., & Bhatt, B. P. (2014). Identification of drought tolerant rice genotypes by analysing drought tolerance indices and morpho-physiological traits. *SABRAO Journal of Breeding & Genetics*, *46*(2).

Kusvuran, S. (2012). Effects of drought and salt stresses on growth, stomatal conductance, leaf water and osmotic potentials of melon genotypes (Cucumis melo L.). *African Journal of Agricultural Research*, *7*(5), 775-781.

Kyoto Encyclopedia of Genes and Genomes (https://www.genome.jp/kegg/pathway.html, accessed in January 2021)

Lau, K. H., del Rosario Herrera, M., Crisovan, E., Wu, S., Fei, Z., Khan, M. A., Buell, C.R., & Gemenet, D. C. (2018). Transcriptomic analysis of sweet potato under dehydration stress identifies candidate genes for drought tolerance. *Plant direct*, *2*(10), e00092.

Laurie, R. N., Laurie, S. M., Du Plooy, C. P., Finnie, J. F., & Van Staden, J. (2015). Yield of drought-stressed sweet potato in relation to canopy cover, stem length and stomatal conductance. *Journal of Agricultural Science*, *7*(1), 201

Lease, K. A., Lau, N. Y., Schuster, R. A., Torii, K. U., & Walker, J. C. (2001). Receptor serine/threonine protein kinases in signalling: analysis of the erecta receptor-like kinase of Arabidopsis thaliana. *New Phytologist*, *151*(1), 133-143.

Lenka, S. K., Katiyar, A., Chinnusamy, V., & Bansal, K. C. (2011). Comparative analysis of drought-responsive transcriptome in Indica rice genotypes with contrasting drought tolerance. *Plant biotechnology journal*, *9*(3), 315-327.

Li, F., Xing, S., Guo, Q., Zhao, M., Zhang, J., Gao, Q., Wang, G., & Wang, W. (2011). Drought tolerance through over-expression of the expansin gene TaEXPB23 in transgenic tobacco. *Journal of plant physiology*, *168*(9), 960-966.

Li, H., Li, M., Wei, X., Zhang, X., Xue, R., Zhao, Y., & Zhao, H. (2017). Transcriptome analysis of drought-responsive genes regulated by hydrogen sulfide in wheat (*Triticum aestivum* L.) leaves. *Molecular Genetics and Genomics*, *292*(5), 1091-1110.

Li, R. H., Guo, P. G., Michael, B., Stefania, G., & Salvatore, C. (2006). Evaluation of chlorophyll content and fluorescence parameters as indicators of drought tolerance in barley. *Agricultural Sciences in China*, *5*(10), 751-757.

Liu, F., Jensen, C. R., & Andersen, M. N. (2003). Hydraulic and chemical signals in the control of leaf expansion and stomatal conductance in soybean exposed to drought stress. *Functional Plant Biology*, *30*(1), 65-73.

Lopez, C. G., Banowetz, G. M., Peterson, C. J., & Kronstad, W. E. (2003). Dehydrin expression and drought tolerance in seven wheat cultivars. *Crop Science*, *43*(2), 577-582.

Magwanga, R. O., Lu, P., Kirungu, J. N., Lu, H., Wang, X., Cai, X., Zhou, Z., Zhang, Z., Salih, H., Wang, K., & Liu, F. (2018). Characterization of the late embryogenesis abundant

(LEA) proteins family and their role in drought stress tolerance in upland cotton. *BMC genetics*, *19*(1), 1-31.

Makbul, S., GÜLER, N. S., DURMUŞ, N., & GÜVEN, S. (2011). Changes in anatomical and physiological parameters of soybean under drought stress. *Turkish Journal of Botany*, *35*(4), 369-377

Manavella, P. A., Arce, A. L., Dezar, C. A., Bitton, F., Renou, J. P., Crespi, M., & Chan, R. L. (2006). Cross-talk between ethylene and drought signalling pathways is mediated by the sunflower Hahb-4 transcription factor. *The Plant Journal*, *48*(1), 125-137.

Marowa, P., Ding, A., & Kong, Y. (2016). Expansins: roles in plant growth and potential applications in crop improvement. *Plant cell reports*, *35*(5), 949-965.

McAinsh, M. R., Brownlee, C., & Hetherington, A. M. (1990). Abscisic acid-induced elevation of guard cell cytosolic Ca 2+ precedes stomatal closure. *Nature*, *343*(6254), 186-188.

Miyashita, K., Tanakamaru, S., Maitani, T., & Kimura, K. (2005). Recovery responses of photosynthesis, transpiration, and stomatal conductance in kidney bean following drought stress. *Environmental and experimental botany*, *53*(2), 205-214.

Muñoz-Mayor, A., Pineda, B., Garcia-Abellán, J. O., Antón, T., Garcia-Sogo, B., Sanchez-Bel, P., Flores, F.B., Atarés, A., Angosto, T., Pintor-Toro, J.A., & Bolarin, M. C. (2012). Overexpression of dehydrin tas14 gene improves the osmotic stress imposed by drought and salinity in tomato. *Journal of plant physiology*, *169*(5), 459-468.

Muñoz-Rodriguez, P., Carruthers, T., Wood, J. R., Williams, B. R., Weitemier, K., Kronmiller, B., Ellis, D., Anglin, N.L., Longway, L., Harris, S.A. and Rausher, M.D., & Scotland, R. W. (2018). Reconciling conflicting phylogenies in the origin of sweet potato and dispersal to Polynesia. *Current Biology*, *28*(8), 1246-1256.

Negi, S., Tak, H., & Ganapathi, T. R. (2018). A banana NAC transcription factor (MusaSNAC1) impart drought tolerance by modulating stomatal closure and H 2 O 2 content. *Plant molecular biology*, *96*(4), 457-471.

Nhanala, S. E. C., & Yencho, G. C. (2020). Assessment of the Potential of Wild Ipomoea spp. for the Improvement of Drought Tolerance in Cultivated Sweetpotato *Ipomoea batatas* (L.) Lam. Crop Science. 1-16. https://doi.org/10.1002/csc2.20363

Nikolaeva, M. K., Maevskaya, S. N., Shugaev, A. G., & Bukhov, N. G. (2010). Effect of drought on chlorophyll content and antioxidant enzyme activities in leaves of three wheat cultivars varying in productivity. *Russian Journal of Plant Physiology*, *57*(1), 87-95.Pan, Y., Seymour, G. B., Lu, C., Hu, Z., Chen, X., & Chen, G. (2012). An ethylene response factor (ERF5) promoting adaptation to drought and salt tolerance in tomato. *Plant cell reports*, *31*(2), 349-360.

Park, C. J., & Seo, Y. S. (2015). Heat shock proteins: a review of the molecular chaperones for plant immunity. *The plant pathology journal*, *31*(4), 323.

Parkhi, V., Kumar, V., Sunilkumar, G., Campbell, L. M., Singh, N. K., & Rathore, K. S. (2009). Expression of apoplastically secreted tobacco osmotin in cotton confers drought tolerance. *Molecular breeding*, *23*(4), 625-639.

Piatkowski, D., Schneider, K., Salamini, F., & Bartels, D. (1990). Characterization of five abscisic acid-responsive cDNA clones isolated from the desiccation-tolerant plant *Craterostigma plantagineum* and their relationship to other water-stress genes. *Plant Physiology*, *94*(4), 1682-1688.

Pimentel, C., Hebert, G., & da Silva, J. V. (1999). Effects of drought on O2 evolution and stomatal conductance of beans at the pollination stage. *Environmental and Experimental Botany*, *42*(2), 155-162.

Podzimska-Sroka, D., O'Shea, C., Gregersen, P. L., & Skriver, K. (2015). NAC transcription factors in senescence: from molecular structure to function in crops. *Plants*, *4*(3), 412-448.

Ponniah, S. K., Thimmapuram, J., Bhide, K., Kalavacharla, V. K., & Manoharan, M. (2017). Comparative analysis of the root transcriptomes of cultivated sweetpotato (*Ipomoea batatas* [L.] Lam) and its wild ancestor (*Ipomoea trifida* [Kunth] G. Don). *BMC plant biology*, *17*(1), 1-14.

Prince, S. J., Joshi, T., Mutava, R. N., Syed, N., Vitor, M. D. S. J., Patil, G., Song, L., Wang, J., Lin, L., Chen, W., Shannon, J.G., & Nguyen, H. T. (2015). Comparative analysis of the drought-responsive transcriptome in soybean lines contrasting for canopy wilting. *Plant Science*, *240*, 65-78.

Ricardo, J. (2011). Screening sweetpotato (*Ipomoea batatas L.*) for drought tolerance and high β-carotene content in Mozambique (Master's thesis).

Ricardo, J., & Andrade, M. I. (2013). Screening sweetpotato (*Ipomoea batatas L.*) for drought tolerance and high ß-carotene content in Mozambique.

Robinson, M. D., & Oshlack, A. (2010). A scaling normalization method for differential expression analysis of RNA-seq data. *Genome biology*, *11*(3), 1-9.

Rodriguez, M. C. S., Edsgärd, D., Hussain, S. S., Alquezar, D., Rasmussen, M., Gilbert, T., Nielsen, B.H., Bartels, D., & Mundy, J. (2010). Transcriptomes of the desiccation-

tolerant resurrection plant *Craterostigma plantagineum*. *The Plant Journal*, *63*(2), 212-228.

Roth, J. A., Ciampitti, I. A., & Vyn, T. J. (2013). Physiological evaluations of recent drought-tolerant maize hybrids at varying stress levels. *Agronomy Journal*, *105*(4), 1129-1141.

Ruan, J., Zhou, Y., Zhou, M., Yan, J., Khurshid, M., Weng, W., Cheng, J., & Zhang, K. (2019). Jasmonic acid signaling pathway in plants. *International journal of molecular sciences*, *20*(10), 2479.

Saminathan, T., Alvarado, A., Lopez, C., Shinde, S., Gajanayake, B., Abburi, V. L., Vajja, V.G., Jagadeeswaran, G., Reddy, K.R., Nimmakayala, P., & Reddy, U. K. (2019). Elevated carbon dioxide and drought modulate physiology and storage-root development in sweet potato by regulating microRNAs. *Functional & integrative genomics*, *19*(1), 171-190.

Sampedro, J., & Cosgrove, D. J. (2005). The expansin superfamily. *Genome biology*, *6*(12), 1-11

Schroeder, J. I., Allen, G. J., Hugouvieux, V., Kwak, J. M., & Waner, D. (2001). Guard cell signal

Seo, J. S., Joo, J., Kim, M. J., Kim, Y. K., Nahm, B. H., Song, S. I., Cheong, J.J., Lee, J.S., Kim, J.K., & Choi, Y. D. (2011). OsbHLH148, a basic helix-loop-helix protein, interacts with OsJAZ proteins in a jasmonate signaling pathway leading to drought tolerance in rice. *The Plant Journal*, *65*(6), 907-921.

Shekhawat, U. K. S., Srinivas, L., & Ganapathi, T. R. (2011). MusaDHN-1, a novel multiple stress-inducible SK 3-type dehydrin gene, contributes affirmatively to drought-and salt-stress tolerance in banana. *Planta*, *234*(5), 915-932.

Siddique, M. R. B., Hamid, A. I. M. S., & Islam, M. S. (2000). Drought stress effects on water relations of wheat. *Botanical Bulletin of Academia Sinica*, *41*.Singh, S. K., & Reddy, K.

R. (2011). Regulation of photosynthesis, fluorescence, stomatal conductance and water-use efficiency of cowpea (Vigna unguiculata [L.] Walp.) under drought. *Journal of Photochemistry and Photobiology B: Biology*, *105*(1), 40-50.

Solis, J., Villordon, A., Baisakh, N., LaBonte, D., & Firon, N. (2014). Effect of drought on storage root development and gene expression profile of sweetpotato under greenhouse and field conditions. *Journal of the American Society for Horticultural Science*, *139*(3), 317-324.

Sun, X. L., Yu, Q. Y., Tang, L. L., Ji, W., Bai, X., Cai, H., ... & Zhu, Y. M. (2013). GsSRK, a G-type lectin S-receptor-like serine/threonine protein kinase, is a positive regulator of plant tolerance to salt stress. *Journal of plant physiology*, *170*(5), 505-515.

Sweetpotato Genomics Resource (http://sweetpotato.plantbiology.msu.edu/)

Tanaka, Y., Sano, T., Tamaoki, M., Nakajima, N., Kondo, N., & Hasezawa, S. (2005). Ethylene inhibits abscisic acid-induced stomatal closure in Arabidopsis. *Plant physiology*, *138*(4), 2337-2343.

Tao, X., Gu, Y. H., Wang, H. Y., Zheng, W., Li, X., Zhao, C. W., & Zhang, Y. Z. (2012). Digital gene expression analysis based on integrated de novo transcriptome assembly of sweet potato [*Ipomoea batatas* (L.) Lam.]. *PloS one*, *7*(4), e36234.

Turyagyenda, L. F., Kizito, E. B., Ferguson, M., Baguma, Y., Agaba, M., Harvey, J. J., & Osiru, D. S. (2013). Physiological and molecular characterization of drought responses and identification of candidate tolerance genes in cassava. *AoB plants*, *5*.

UniProt Knowledgebase (https://www.uniprot.org/uniprot/, accessed in January 2021)

Van der Mescht, A.*, De Ronde, JA** &Rossouw, F. (1999). Chlorophyll fluorescence and chlorophyll content as a measure of drought tolerance in potato. *South African journal of science*, *95*(9), 407-412.

Van Heerden, P. D. R., & Laurie, R. (2008). Effects of prolonged restriction in water supply on photosynthesis, shoot development and storage root yield in sweet potato. *Physiologia Plantarum*, *134*(1), 99-109.

Vaseva, I. I., Grigorova, B. S., Simova-Stoilova, L. P., Demirevska, K. N., & Feller, U. (2010). Abscisic acid and late embryogenesis abundant protein profile changes in winter wheat under progressive drought stress. *Plant Biology*, *12*(5), 698-707.

Vermeulen, K., Steppe, K., Linh, N. S., Lemeur, R., De Backer, L., Bleyaert, P., P., Dekock, J., Aerts, J.M., & Berckmans, D. (2007, October). Simultaneous response of stem diameter, sap flow rate and leaf temperature of tomato plants to drought stress. In *International Symposium on High Technology for Greenhouse System Management: Greensys2007 801* (pp. 1259-1266).

Walker, J. C. (1994). Structure and function of the receptor-like protein kinases of higher plants. *Plant molecular biology*, *26*(5), 1599-1609.

Wang, J., Mao, X., Wang, R., Li, A., Zhao, G., Zhao, J., & Jing, R. (2019). Identification of wheat stress-responding genes and TaPR-1-1 function by screening a cDNA yeast library prepared following abiotic stress. *Scientific reports*, *9*(1), 1-12.

Wang, Z., Fang, B., Chen, J., Zhang, X., Luo, Z., Huang, L., Chen X., & Li, Y. (2010). De novo assembly and characterization of root transcriptome using Illumina paired-end sequencing and development of cSSR markers in sweetpotato (*Ipomoea batatas*). *BMC genomics*, *11*(1), 1-14.

Waterland, N. L., Finer, J. J., & Jones, M. L. (2010). Abscisic acid applications decrease stomatal conductance and delay wilting in drought-stressed chrysanthemums. *HortTechnology*, *20*(5), 896-901.

Weber, R. L. M., Wiebke-Strohm, B., Bredemeier, C., Margis-Pinheiro, M., de Brito, G. G., Rechenmacher, C., Bertagnolli, P.F., de Sá, M.E.L., de Araújo Campos, M., de Amorim, R.M.S., & Bodanese-Zanettini, M. H. (2014). Expression of an osmotin-like protein from *Solanum nigrum* confers drought tolerance in transgenic soybean. *BMC Plant Biology*, *14*(1), 1-9.

Wei, P. C., Zhang, X. Q., Zhao, P., & Wang, X. C. (2011a). Regulation of stomatal opening by the guard cell expansin AtEXPA1. *Plant signaling & behavior*, *6*(5), 740-742.

Wei, P., Chen, S., Zhang, X., Zhao, P., Xiong, Y., Wang, W., Chen, J., & Wang, X. (2011b). An α-expansin, VfEXPA1, is involved in regulation of stomatal movement in *Vicia faba* L. *Chinese Science Bulletin*, *56*(33), 3531-3537.

Wu, J., Jiang, Y., Liang, Y., Chen, L., Chen, W., & Cheng, B. (2019). Expression of the maize MYB transcription factor ZmMYB3R enhances drought and salt stress tolerance in transgenic plants. *Plant physiology and biochemistry*, *137*, 179-188.

Wu, J., Wang, L., Li, L., & Wang, S. (2014). De novo assembly of the common bean transcriptome using short reads for the discovery of drought-responsive genes. *PloS one*, *9*(10), e109262.

Wu, S., Lau, K. H., Cao, Q., Hamilton, J. P., Sun, H., Zhou, C., Eserman, L., Gemenet, D.C., Olukolu, B.A., Wang, H., & Fei, Z. (2018). Genome sequences of two diploid wild relatives of cultivated sweetpotato reveal targets for genetic improvement. *Nature communications*, *9*(1), 1-12.

Xiang, J., Chen, X., Hu, W., Xiang, Y., Yan, M., & Wang, J. (2018). Overexpressing heat-shock protein OsHSP50. 2 improves drought tolerance in rice. *Plant cell reports*, *37*(11), 1585-1595.

Xie, F., Burklew, C. E., Yang, Y., Liu, M., Xiao, P., Zhang, B., & Qiu, D. (2012). De novo sequencing and a comprehensive analysis of purple sweet potato (*Ipomoea batatas* L.) transcriptome. *Planta*, *236*(1), 101-113.

Xu, J., Yuan, Y., Xu, Y., Zhang, G., Guo, X., Wu, F., Wang, Q., Rong, T., Pan, G., Cao, M., & Tang, Q. (2014). Identification of candidate genes for drought tolerance by whole-genome resequencing in maize. *BMC Plant Biology*, *14*(1), 83.

Yang, X., Liu, J., Xu, J., Duan, S., Wang, Q., Li, G., & Jin, L. (2019). Transcriptome profiling reveals effects of drought stress on gene expression in diploid potato genotype P3-198. *International journal of molecular sciences*, *20*(4), 852.

Ye, H., Song, L., Schapaugh, W. T., Ali, M. L., Sinclair, T. R., Riar, M. K., Mutava, R.N., Li, Y., Vuong, T., Valliyodan, B., Pizolato Neto, A., & Nguyen, H. T. (2020). The importance of slow canopy wilting in drought tolerance in soybean. *Journal of experimental botany*, *71*(2), 642-652.

Yu, Y., Yang, D., Zhou, S., Gu, J., Wang, F., Dong, J., & Huang, R. (2017). The ethylene response factor OsERF109 negatively affects ethylene biosynthesis and drought tolerance in rice. *Protoplasma*, *254*(1), 401-408.

Zhang, H., Zhang, Q., Zhai, H., Li, Y., Wang, X., Liu, Q., & He, S. (2017). Transcript profile analysis reveals important roles of jasmonic acid signalling pathway in the response of sweet potato to salt stress. *Scientific reports*, *7*(1), 1-12.

Zhang, N., Liu, B., Ma, C., Zhang, G., Chang, J., Si, H., & Wang, D. (2014). Transcriptome characterization and sequencing-based identification of drought-responsive genes in potato. *Molecular Biology Reports*, *41*(1), 505-517.

Zhang, X. Q., Wei, P. C., Xiong, Y. M., Yang, Y., Chen, J., & Wang, X. C. (2011). Overexpression of the Arabidopsis α-expansin gene AtEXPA1 accelerates stomatal opening by decreasing the volumetric elastic modulus. *Plant cell reports*, *30*(1), 27-36.

Zhang, X., Liu, S., & Takano, T. (2008). Two cysteine proteinase inhibitors from Arabidopsis thaliana, AtCYSa and AtCYSb, increasing the salt, drought, oxidation and cold tolerance. *Plant molecular biology*, *68*(1-2), 131-143.

Zhao, M. R., Han, Y. Y., Feng, Y. N., Li, F., & Wang, W. (2012). Expansins are involved in cell growth mediated by abscisic acid and indole-3-acetic acid under drought stress in wheat. *Plant cell reports*, *31*(4), 671-685.

Zhao, M. R., Li, F., Fang, Y., Gao, Q., & Wang, W. (2011). Expansin-regulated cell elongation is involved in the drought tolerance in wheat. *Protoplasma*, *248*(2), 313-323.

Zhou, S., Han, Y. Y., Chen, Y., Kong, X., & Wang, W. (2015). The involvement of expansins in response to water stress during leaf development in wheat. *Journal of plant physiology*, *183*, 64-74.

Zhu, B., Chen, T. H., & Li, P. H. (1995). Activation of two osmotin-like protein genes by abiotic stimuli and fungal pathogen in transgenic potato plants. *Plant Physiology*, *108*(3), 929-937.

Tables

Table 1- Sample identifications of RNA, respective group of study, time-points when the leaf tissues were collected, when the treatment that was applied in each plant, and the identification of the plant from where the leaf samples were collected.

Order	RNA sampleID	Group	Time Point	Treatment	PlantID
1	Bea1_CT0	Control	T0	24H Before D	Bea1
2	Bea2_CT0	Control	T0	24H Before D	Bea2
3	Bea3_CT0	Control	T0	24H Before D	Bea3
4	Res4_CT0	Control	T0	24H Before D	Res4
5	Res5_CT0	Control	T0	24H Before D	Res5
6	Res6_CT0	Control	T0	24H Before D	Res6
7	Bea7_TT0	Treated	T0	24H Before D	Bea7
8	Bea8_TT0	Treated	T0	24H Before D	Bea8
9	Bea9_TT0	Treated	T0	24H Before D	Bea9
10	Res10_TT0	Treated	T0	24H Before D	Res10
11	Res11_TT0	Treated	T0	24H Before D	Res11
12	Res12_TT0	Treated	T0	24H Before D	Res12
13	Bea25_CT1	Control	T1	24H After D	Bea1
14	Bea26_CT1	Control	T1	24H After D	Bea2
15	Bea27_CT1	Control	T1	24H After D	Bea3
16	Res28_CT1	Control	T1	24H After D	Res4
17	Res29_CT1	Control	T1	24H After D	Res5
18	Res30_CT1	Control	T1	24H After D	Res6
19	Bea31_TT1	Treated	T1	24H After D	Bea7

Table 1 (continued).

20	Bea32_TT1	Treated	T1	24H After D	Bea8
21	Bea33_TT1	Treated	T1	24H After D	Bea9
22	Res34_TT1	Treated	T1	24H After D	Res10
23	Res35_TT1	Treated	T1	24H After D	Res11
24	Res36_TT1	Treated	T1	24H After D	Res12
25	Bea37_CT2	Control	T2	48H After D	Bea1
26	Bea38_CT2	Control	T2	48H After D	Bea2
27	Bea39_CT2	Control	T2	48H After D	Bea3
28	Res40_CT2	Control	T2	48H After D	Res4
29	Res41_CT2	Control	T2	48H After D	Res5
30	Res42_CT2	Control	T2	48H After D	Res6
-	-	Treated	T2	48H After D	Bea7
31	Bea44_TT2	Treated	T2	48H After D	Bea8
32	Bea45_TT2	Treated	T2	48H After D	Bea9
33	Res46_TT2	Treated	T2	48H After D	Res10
34	Res47_TT2	Treated	T2	48H After D	Res11
35	Res48_TT2	Treated	T2	48H After D	Res12
36	Bea49_CT3	Control	T3	24H After RI	Bea1
37	Bea50_CT3	Control	T3	24H After RI	Bea2
-	-	Control	T3	24H After RI	Bea3
38	Res52_CT3	Control	T3	24H After RI	Res4
39	Res53_CT3	Control	T3	24H After RI	Res5

Table 1 (continued).

40	Res54_CT3	Control	T3	24H After RI	Res6
41	Bea55_TT3	Treated	T3	24H After RI	Bea7
42	Bea56_TT3	Treated	T3	24H After RI	Bea8
43	Bea57_TT3	Treated	T3	24H After RI	Bea9
44	Res58_TT3	Treated	T3	24H After RI	Res10
45	Res59_TT3	Treated	T3	24H After RI	Res11
46	Res60_TT3	Treated	T3	24H After RI	Res12

*RNA sampleID = Identification of the sample of RNA

*PlantID = Plant from where the leaf samples were collected

Bea = cultivar ‘Beauregard’s, Res = cultivar ‘Resisto’

Treatments

T0 = 24H Before D = 24 hours before imposing drought

T1 = 24H After D = 24 hours after imposing drought

T2 = 48H After D = 48 hours after imposing drought

T3= 24H After RI = 24 hours after re-irrigation of drought treatments

Table 2 - List of the size of raw reads and trimmed reads per sample, including number of reads generated after sequencing and the number of the reads used for the RNA sequencing after these samples were trimmed.

Sample	Number of reads	Avg. length	Number of reads after trim	Percentage trimmed (%)	Avg. length after trim
Bea1_CT0	42,743,546	150	42,719,000	99.94	136.23
Bea2_CT0	41,625,286	150	41,594,743	99.93	136.35
Bea3_CT0	40,789,742	150	40,766,320	99.94	135.83
Res4_CT0	40,839,496	150	40,810,565	99.93	136.17
Res5_CT0	41,372,690	150	41,341,187	99.92	135.44
Res6_CT0	43,679,452	150	43,650,875	99.93	136.16
Bea7_TT0	43,577,212	150	43,550,598	99.94	136.08
Bea8_TT0	41,806,128	150	41,781,691	99.94	136.2
Bea9_TT0	41,821,870	150	41,795,059	99.94	136.12
Res10_TT0	41,479,364	150	41,446,077	99.92	136.19
Res11_TT0	43,153,098	150	43,120,184	99.92	136.13
Res12_TT0	42,303,760	150	42,278,348	99.94	136.25
Bea25_CT1	41,409,228	150	41,379,971	99.93	135.94
Bea26_CT1	43,530,982	150	43,500,697	99.93	136.31
Bea27_CT1	40,688,550	150	40,656,754	99.92	136.23
Res28_CT1	41,570,502	150	41,543,171	99.93	136.22
Res29_CT1	40,659,626	150	40,625,744	99.92	136.26
Res30_CT1	43,996,700	150	43,965,964	99.93	136.27
Bea31_TT1	44,239,094	150	44,208,933	99.93	136.19
Bea32_TT1	43,383,570	150	43,352,559	99.93	136.27
Bea33_TT1	41,788,770	150	41,758,119	99.93	136.16
Res34_TT1	41,418,908	150	41,392,736	99.94	136.22
Res35_TT1	40,782,722	150	40,756,429	99.94	136.13
Res36_TT1	43,981,660	150	43,956,196	99.94	136.23
Bea37_CT2	42,582,584	150	42,562,908	99.95	136.37
Bea38_CT2	42,893,082	150	42,866,390	99.94	136.33
Bea39_CT2	40,688,584	150	40,660,525	99.93	136.27
Res40_CT2	42,729,080	150	42,701,780	99.94	136.31
Res41_CT2	40,796,108	150	40,767,004	99.93	136.25
Res42_CT2	42,553,156	150	42,525,300	99.93	136.17
Bea44_TT2	41,243,812	150	41,217,021	99.94	136.44
Bea45_TT2	40,859,556	150	40,828,703	99.92	136.36
Res46_TT2	40,696,864	150	40,674,039	99.94	136.3
Res47_TT2	42,907,172	150	42,882,212	99.94	136.37
Res48_TT2	41,097,826	150	41,074,553	99.94	136.29
Bea49_CT3	43,155,004	150	43,133,852	99.95	136.17
Bea50_CT3	42,434,422	150	42,407,536	99.94	136.26

Table 2 (continued).

Res52_CT3	40,700,306	150	40,677,873	99.94	136.35
Res53_CT3	40,367,582	150	40,340,699	99.93	136.22
Res54_CT3	42,940,720	150	42,914,357	99.94	136.25
Bea55_TT3	42,044,888	150	42,018,830	99.94	136.21
Bea56_TT3	42,815,142	150	42,783,424	99.93	136.42
Bea57_TT3	41,741,978	150	41,717,522	99.94	136.27
Res58_TT3	43,714,764	150	43,677,423	99.91	136.26
Res59_TT3	41,489,630	150	41,462,127	99.93	136.29
Res60_TT3	40,258,944	150	40,229,736	99.93	136.14

Table 3 – Morphological traits evaluated at plant harvest. The table shows the results of the morphological assessment at plant harvest for treatments that received daily non-interrupted irrigation (control treatment) and plants where drought was imposed (drought treatment). The following traits were evaluated at the harvest time: height of the plant, dry weight of aboveground biomass, dry weight of belowground biomass, number of storage roots, and dry weight of the storage roots. Data analysis: Tukey test; alpha = 0.05.

	Height (cm)	**%H**	**ADry (g)**	**%A**	**BDry (g)**	**%B**	**nSR**	**%n**	**DrySR (g)**	**%DSR**
Bea_Cont	243.87 ± 70.94a	-	13.6 ± 5.1bc	-	3.8 ± 1.70ab	-	6.67 ± 2.52a	-	28.4 ± 13.35 a	-
Bea_Drou	167.30 ± 70.94a	31	10.2 ± 5.1c	25	1.6 ± 1.70b	58	4.33 ± 2.52ab	36	19.93 ± 13.35a	30
Res_Cont	190.37 ± 70.94a	-	23.0 ± 5.1a	-	4.83 ± 1.70a	-	2.67 ± 2.52ab	-	1.67 ± 13.35b	-
Res_Drou	116.67 ± 70.94a	39	14.37 ± 5.1b	38	2.13 ± 1.70ab	56	1.33 ± 2.52b	51	1 ± 13.35b	40

Bea_Cont = cultivar ‘Beauregard’ under control treatment;
Bea_Drou = cultivar ‘Beauregard’ under drought treatment;
Res_Cont = cultivar ‘Resisto’ under control treatment;
Res_Drou = cultivar ‘Resisto’ under drought treatment;
Height (cm) = Height of the plant;
%H= percentage change of the height after drought was imposed;
ADry (g) = Dry weight of aboveground biomass;
%A = percentage change of the dry weight of the aboveground after drought was imposed;
BDry (g) = Dry weight of belowground biomass;
%B = percentage change of the dry weight belowground after drought was imposed;
nSR = number of storage roots;
%n = percentage change in the number of the storage roots;
DrySR = Dry weight of the storage roots;
%DSR = Percentage change in the dry weight of the storage roots after drought is imposed.

Table 4 – Differential gene expression when comparing control and treated plants during the four time-points. The parameters for the gene expression were set for FDR p-value 0.05, and two-fold change. The comparisons were performed for three groups: i) 'Beauregard' control vs. 'Beauregard' treated; ii) 'Resisto' control vs. 'Beauregard' control; iii) 'Beauregard' and 'Resisto' control plants combined vs. 'Beauregard' and 'Resisto' treated.

Compared DE genes	Time point	Up-regulation	Down-regulation
'Beauregard': control vs treated	24hBeforeD	74	68
'Beauregard': control vs treated	24hAfterD	2,651	4,742
'Beauregard': control vs treated	48hAfterD	3,648	4,972
'Beauregard': control vs treated	24hAfterRI	1,802	2,822
'Resisto': control vs treated	24hBeforeD	8	78
'Resisto': control vs treated	24hAfterD	3,105	3,007
'Resisto': control vs treated	48hAfterD	4,754	5,951
'Resisto': control vs treated	24hAfterRI	1,910	2,718
BeaRes control vs BeaRes treated	24hBeforeD	8	23
BeaRes control vs BeaRes treated	24hAfterD	2,200	3,309
BeaRes control vs BeaRes treated	48hAfterD	3,103	5,066
BeaRes control vs BeaRes treated	24hAfterRI	1,721	2,937

Treatments: 24hBeforeD = 24 hours before imposing drought; 24hAfterD = 24h after imposing drought; 48hAfterD = 48h after imposing drought; 24hAfterRI = 24 hours after re-irrigation. BeaRes = combined data of 'Beauregard' and 'Resisto'.

Table 5 – Table shows the fold changes of the genes identified as potentially involved in drought tolerance in sweetpotato. The genes were named based on their identification in the reference genome. Selected genes include those that were potentially involved in drought tolerance after 24 and 48 hours of drought, and those involved in the recovery of the plant 24 hours after re-irrigation.

Treatment	Gene name	Bea F-change	FDR p-value	p-value	Res F-change	FDR p-value	p-value
24H AD	PREDICTED: expansin-A15-like [*Nicotiana tomentosiformis*]	5,910.73	3.43E-06	2.08E-07	2,013.66	5.91E-05	4.21E-06
24H AD	PREDICTED: abscisic acid and environmental stress-inducible protein TAS14-like [*N. tomentosiformis*]	290.74	2.27E-40	3.49E-43	239.47	4.39E-82	1.25E-85
24H AD	PREDICTED: basic form of pathogenesis-related protein 1 [*N. sylvestris*]	5,618.79	3.17E-06	1.91E-07	126.76	7.45E-07	3.46E-08
24H AD	PREDICTED: 18.8 kDa class II heat shock protein-like [*N. tomentosiformis*]	2,704.45	4.39E-16	5.96E-18	22.16	6.21E-11	1.43E-12
24H AD	PREDICTED: E3 ubiquitin-protein ligase RING1-like [*N. sylvestris*]	129.65	2.00E-02	4.23E-03	4.24	4.00E-02	8.58E-03
48H AD	PREDICTED: expansin-A15-like [*N. tomentosiformis*]	1,961.95	2.19E-14	4.93E-16	343.25	3.55E-09	1.29E-10
48H AD	PREDICTED: abscisic acid and environmental stress-inducible protein TAS14-like [*N. tomentosiformis*]	2,094.64	4.80E-82	7.09E-86	1,261.89	1.20E-81	1.67E-85
48H AD	PREDICTED: basic form of pathogenesis-related protein 1 [*N. sylvestris*]	452.46	1.82E-35	4.47E-38	60.44	2.02E-08	8.45E-10
48H AD	PREDICTED: desiccation-related protein PCC13-62-like [*N. tomentosiformis*]	167.91	4.84E-16	8.85E-18	42.8	1.74E-09	5.89E-11
24H ARi	PREDICTED: wall-associated receptor kinase-like 1 [*Solanum lycopersicum*]	1,008.56	6.46E-03	5.94E-04	21.28	8.64E-06	2.28E-07
24H ARi	PREDICTED: receptor-like serine/threonine-protein kinase SD1-7-like [*S. tuberosum*]	218.26	5.00E-02	7.83E-03	7.72	9.03E-03	9.64E-04

24H AD = 24 hours after drought; 48H AD = 48 hours after drought; 24H ARi = 24 hours after Re-irrigation; Bea F-change = fold change in 'Beauregard'; Res F-change = fold change in 'Resisto.

Figures

Figure 1 – Status of the plants during three time points: T1 (24 hours after drought), T2 (48 hours after drought) and T3 (24 hours after re-irrigation). Figure shows the visual appearance of the plants of the cultivars A) 'Beauregard' and B) 'Resisto' of both groups (control and treated) 24 hours after drought was imposed, a treated plant 48 hours after drought, and both group of plants (control and treated) 24 hours after the plants were re-irrigated.

A) Status of the cultivar 'Beauregard' plants during T1, T2, and T3 time points.

Figure 1 (continued).

A) Status of the cultivar 'Beauregard' plants during T1, T2, and T3 time points.

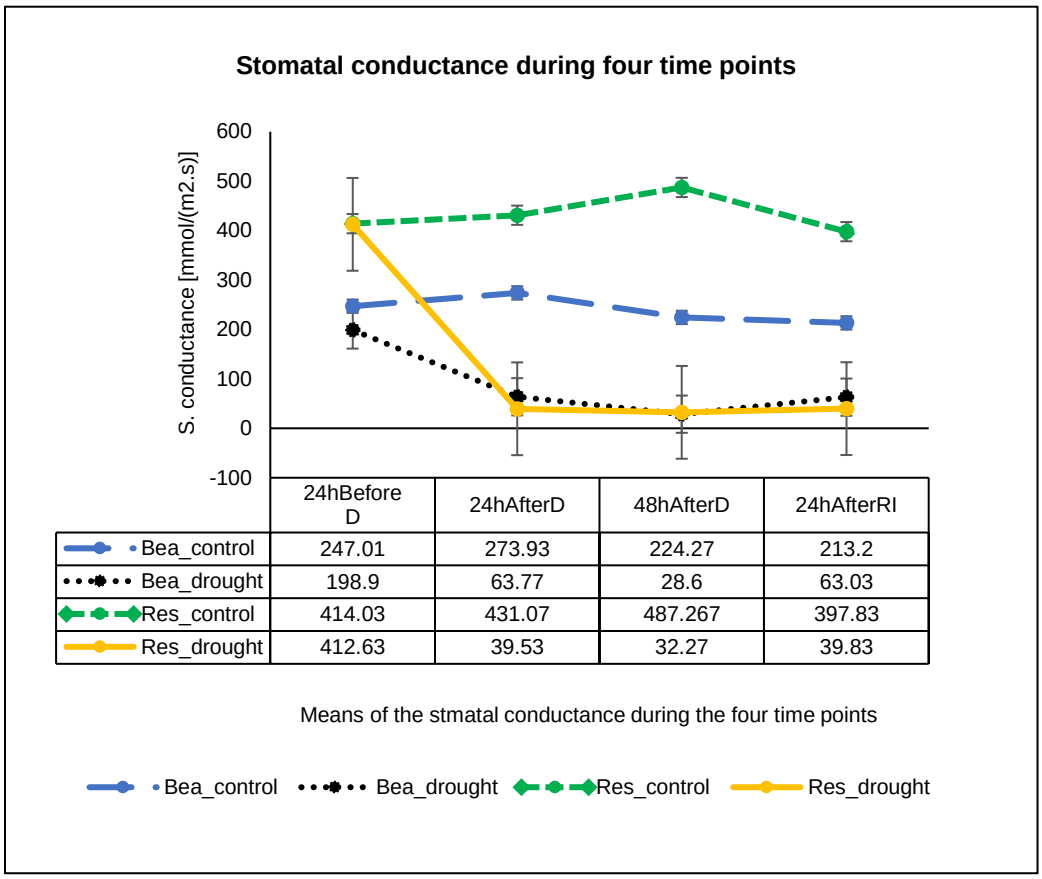
Figure 1 (continued).

B) Status of the cultivar ‘Resisto’ plants during T1, T2, and T3 time points.

Figure 1 (continued).

B) Status of the cultivar ‘Resisto’ plants during T1, T2, and T3 time points.

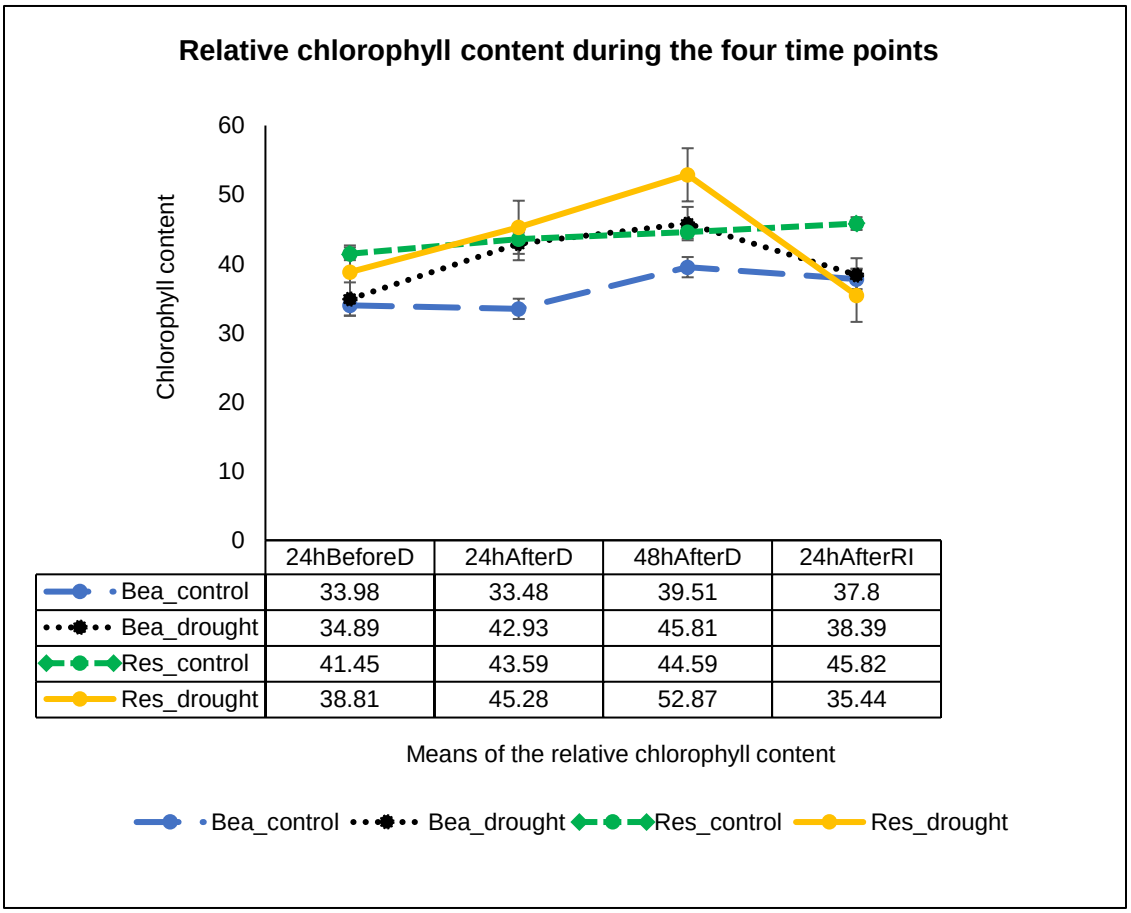
Figure 2a – Stomatal conductance of the plants during the four time points when the leaf tissues were collected for RNA isolation. 'Resisto' was negatively affected by drought, with a decrease in the stomatal conductance 24 hours after drought was imposed. The stomatal conductance of 'Beauregard' was also negatively affected by drought, however, that response occurred only 48 hours after drought was imposed.



	24hBefore D	24hAfterD	48hAfterD	24hAfterRI
Bea_control	247.01	273.93	224.27	213.2
Bea_drought	198.9	63.77	28.6	63.03
Res_control	414.03	431.07	487.267	397.83
Res_drought	412.63	39.53	32.27	39.83

Bea_control = 'Beauregard' control treatment; Bea_drought = 'Beauregard' drought treatment; Res_control = 'Resisto' control treatment; Res_drought = 'Resisto' drought treatment; 24hBeforeD = 24 hours before drought was imposed; 24hAAfterD = 24 hours after drought was imposed; 48hAfterD = 48 hours after drought was imposed; 24hAfterRI = 24 hours after re-irrigation.

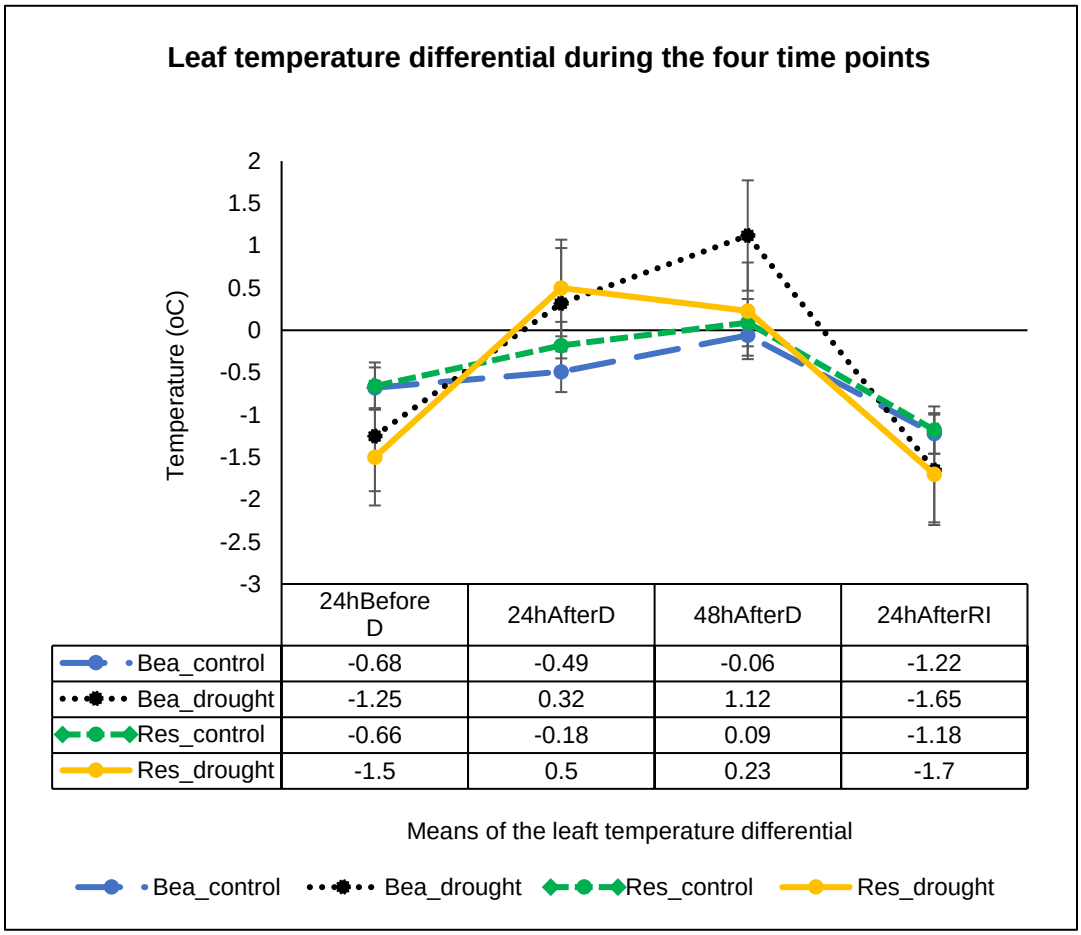
Figure 2b – Relative chlorophyll content of the plants during the four time points when the leaf tissues were collected for RNA isolation. The relative chlorophyll content in 'Resisto' increased with drought and decreased when the plants were re-irrigated. The relative chlorophyll content of 'Beauregard' increased with drought and decreased after the re-irrigation of the plants.



	24hBeforeD	24hAfterD	48hAfterD	24hAfterRI
Bea_control	33.98	33.48	39.51	37.8
Bea_drought	34.89	42.93	45.81	38.39
Res_control	41.45	43.59	44.59	45.82
Res_drought	38.81	45.28	52.87	35.44

Bea_control = 'Beauregard' control treatment; Bea_drought = 'Beauregard' drought treatment; Res_control = 'Resisto' control treatment; Res_drought = 'Resisto' drought treatment; 24hBeforeD = 24 hours before drought was imposed; 24hAAfterD = 24 hours after drought was imposed; 48hAfterD = 48 hours after drought was imposed; 24hAfterRI = 24 hours after re-irrigation.

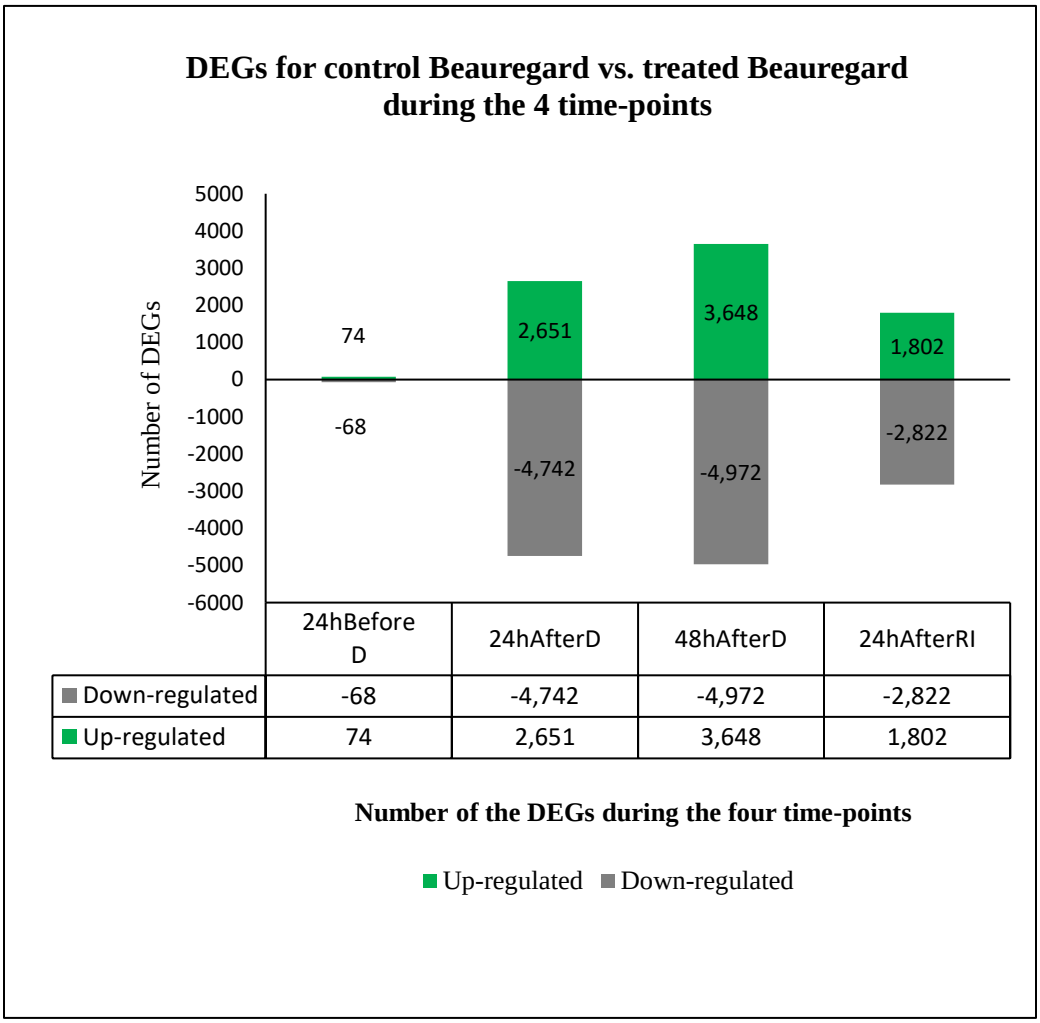
Figure 2c – Leaf temperature of the plants during the four time points when leaf tissue samples were collected for the RNA analysis. Figure shows changes in the internal temperature of the plant as a response to drought events, with an increase of temperature occurring the first 24 hours for both cultivars. At 48 hours of drought 'Beauregard' still shows a temperature increase, while 'Resisto' had a temperature drop after 24 hours of drought.



	24hBefore D	24hAfterD	48hAfterD	24hAfterRI
Bea_control	-0.68	-0.49	-0.06	-1.22
Bea_drought	-1.25	0.32	1.12	-1.65
Res_control	-0.66	-0.18	0.09	-1.18
Res_drought	-1.5	0.5	0.23	-1.7

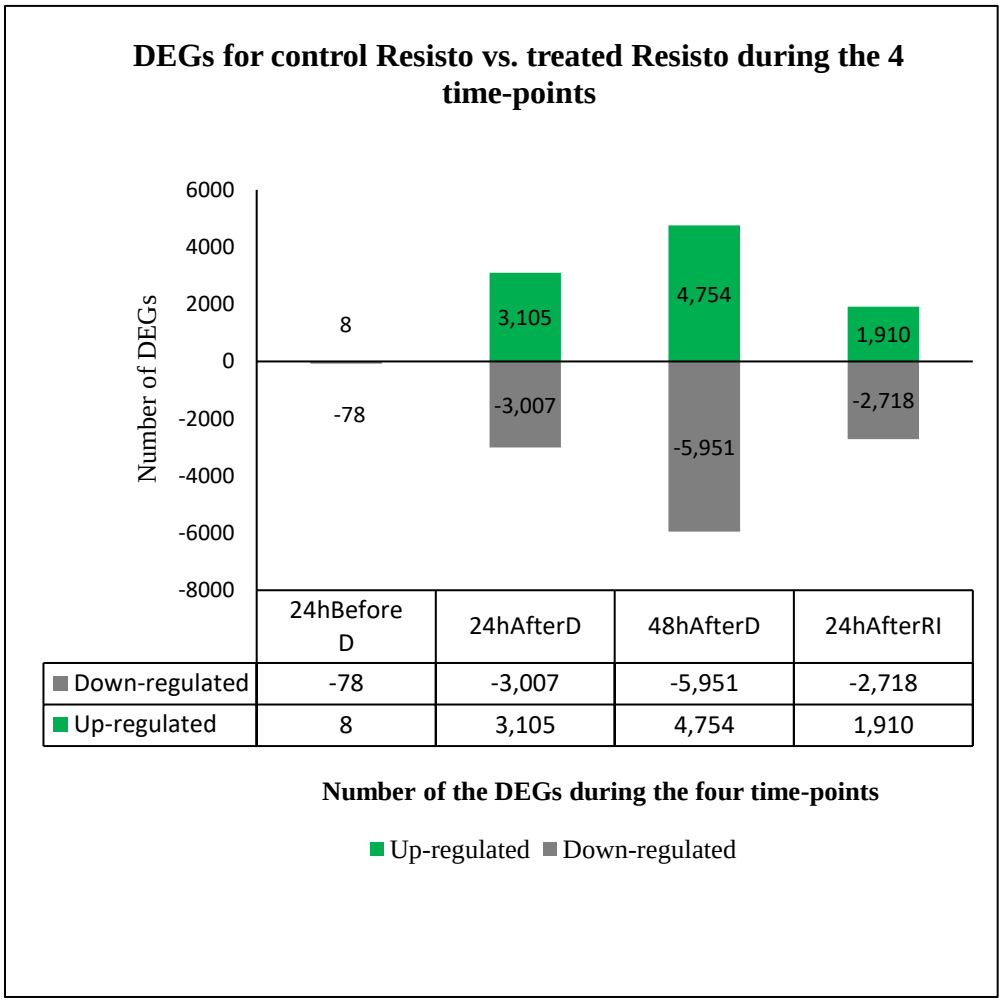
Bea_control = 'Beauregard' control treatment; Bea_drought = 'Beauregard' drought treatment; Res_control = 'Resisto' control treatment; Res_drought = 'Resisto' drought treatment; 24hBeforeD = 24 hours before drought was imposed; 24hAAfterD = 24 hours after drought was imposed; 48hAfterD = 48 hours after drought was imposed; 24hAfterRI = 24 hours after re-irrigation.

Figure 3a – Differentially expressed genes (DEGs) for 'Beauregard' and 'Resisto'. The figure shows the differentially expressed genes (DEG) for the filtering parameters FDR p-value < 0.05, fold ≥ 2. The DEG are representing different comparisons: control 'Beauregard' vs. treated 'Beauregard', at 24 hours, and 48 hours after drought, and 24 hours after re-irrigation.



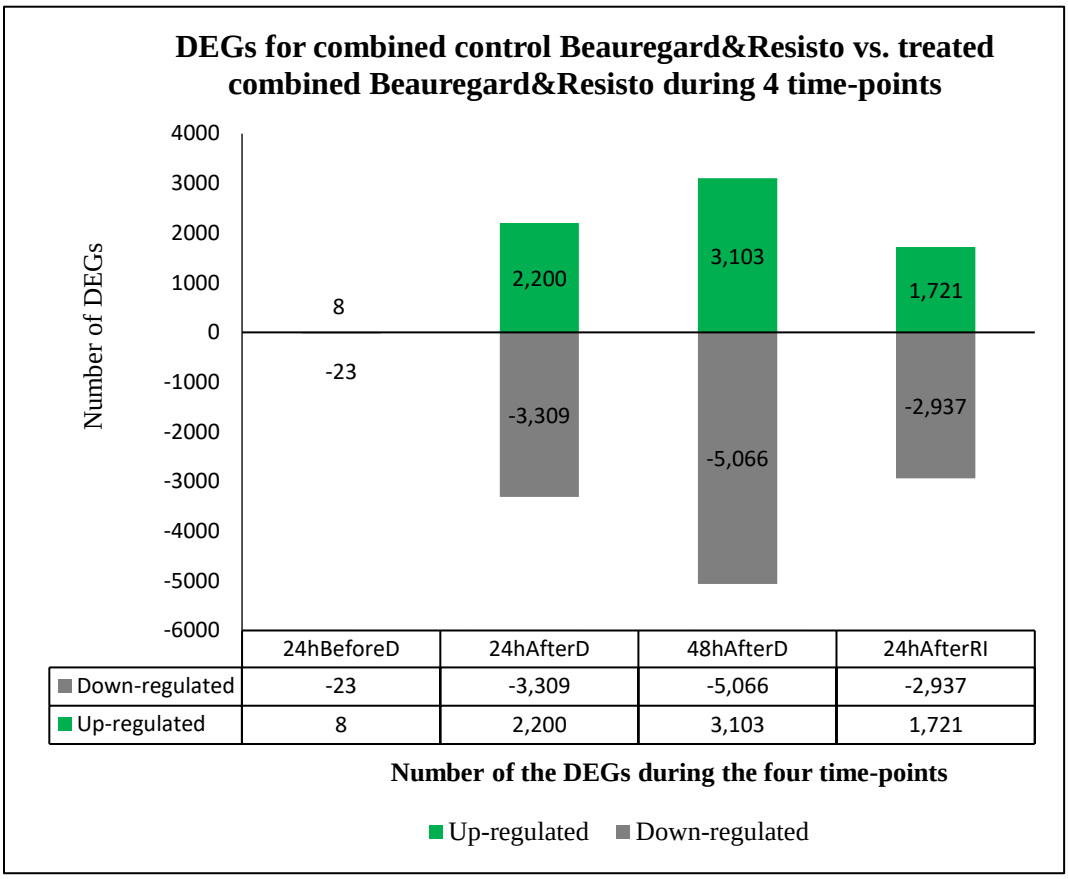
Treatments: 24hBeforeD = 24 hours before imposing drought; 24hAfterD = 24h after imposing drought; 48hAfterD = 48h after imposing drought; 24hAfterRI = 24 hours after re-irrigation.

Figure 3b – Differentially expressed genes (DEGs) for 'Beauregard' and 'Resisto'. The figure shows the differentially expressed genes (DEG) for the filtering parameters FDR p-value < 0.05, fold ≥ 2. The DEG are representing different comparisons: control 'Resisto' vs. treated 'Resisto', at 24 hours, and 48 hours after drought, and 24 hours after re-irrigation.



Treatments: 24hBeforeD = 24 hours before imposing drought; 24hAfterD = 24h after imposing drought; 48hAfterD = 48h after imposing drought; 24hAfterRI = 24 hours after re-irrigation.

Figure 3c – Differentially expressed genes (DEGs) for 'Beauregard' and 'Resisto'. The figure shows the differentially expressed genes (DEG) for the filtering parameters FDR p-value < 0.05, fold ≥ 2. The DEG are representing different comparisons: combined control 'Beauregard' & 'Resisto' vs. combined treated 'Beauregard' & 'Resisto', at 24 hours, and 48 hours after drought, and 24 hours after re-irrigation.



Treatments: 24hBeforeD = 24 hours before imposing drought; 24hAfterD = 24h after imposing drought; 48hAfterD = 48h after imposing drought; 24hAfterRI = 24 hours after re-irrigation.

Figure 4 – Heatmap of the differently expressed genes per treatment group. The heatmap was generated from calculation of the Manhattan distance with complete linkage clustering, and filtered by statistical parameters of minimum absolute fold change 1.5, FDR p-value 0.05. The heatmap reveals that 6,341 genes were differentially expressed. The upregulated genes are depicted in red, and down-regulated genes are depicted as blue. In the heatmap the following genes clusters were identified: i) control plants that were not exposed to drought in any time-point, and the group of the treated plants before drought (Untreated: 24hBeforeD, 24hAfterD, 48hAfterD, and 24hAfterRI); ii) The plants that were exposed to drought for 24 and 48 hours (Drought: 24hAfterD, 48hAfterD, and 24hAfter R); iii) treated plants, 24 hours after they were re-irrigated (Drought: 24hAfterRI).

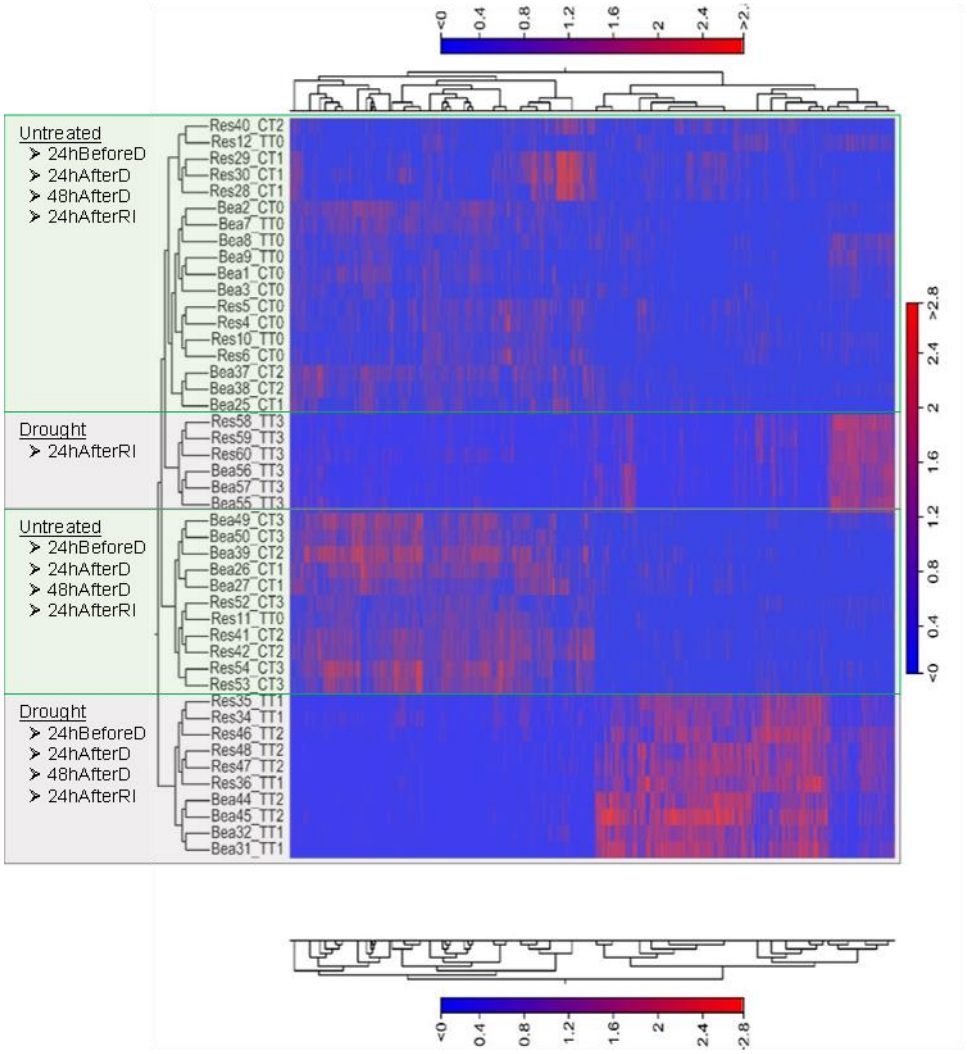
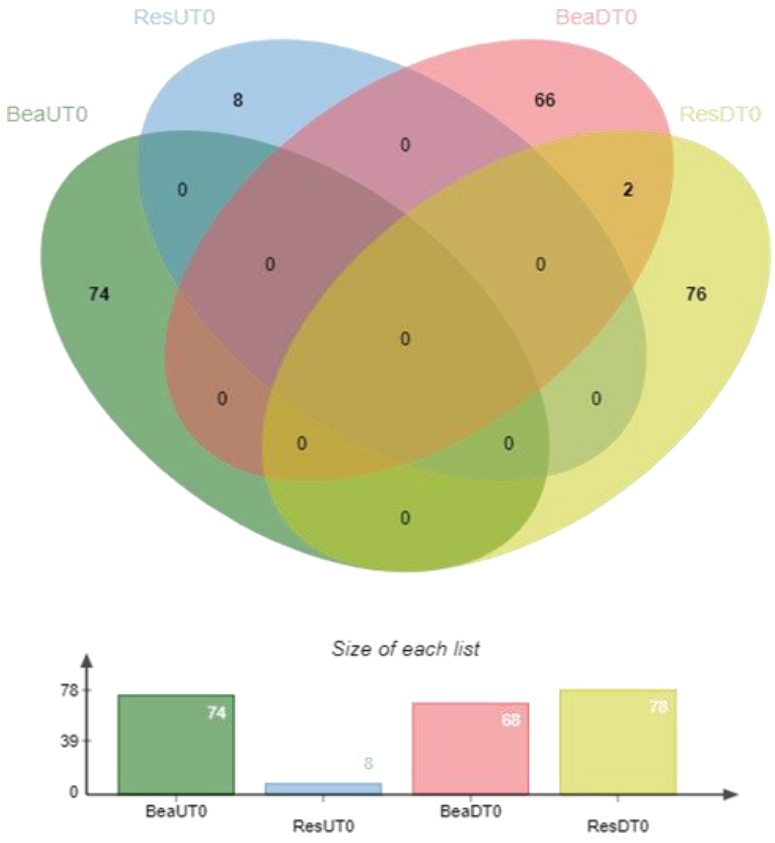
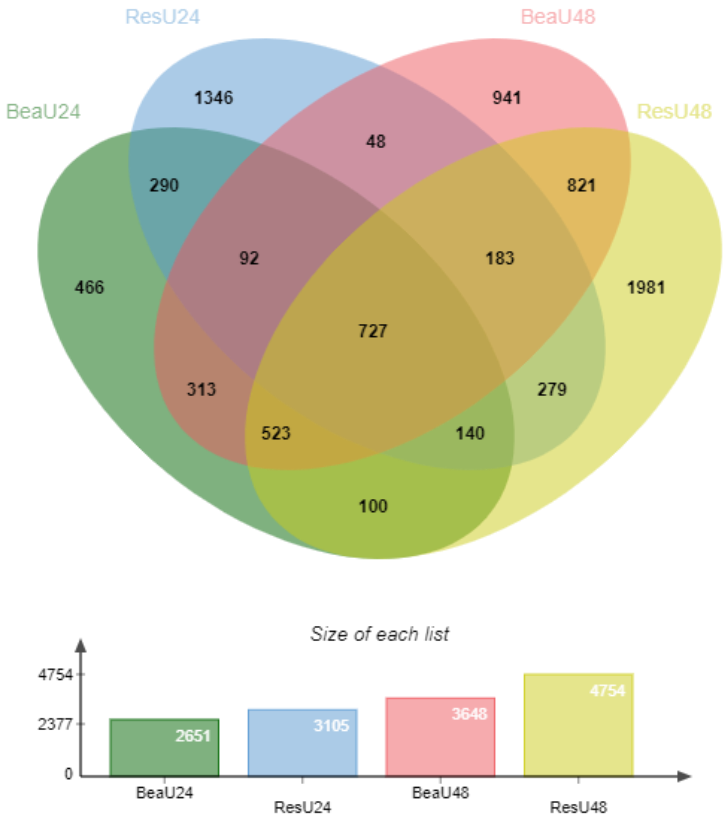


Figure 5a – Venn Diagram of the differentially expressed genes during drought events. The figure shows the number of upregulated and downregulated genes 24 hours before 'Beauregard' and 'Resisto' were exposed to drought These DEGs were due to the genotype.



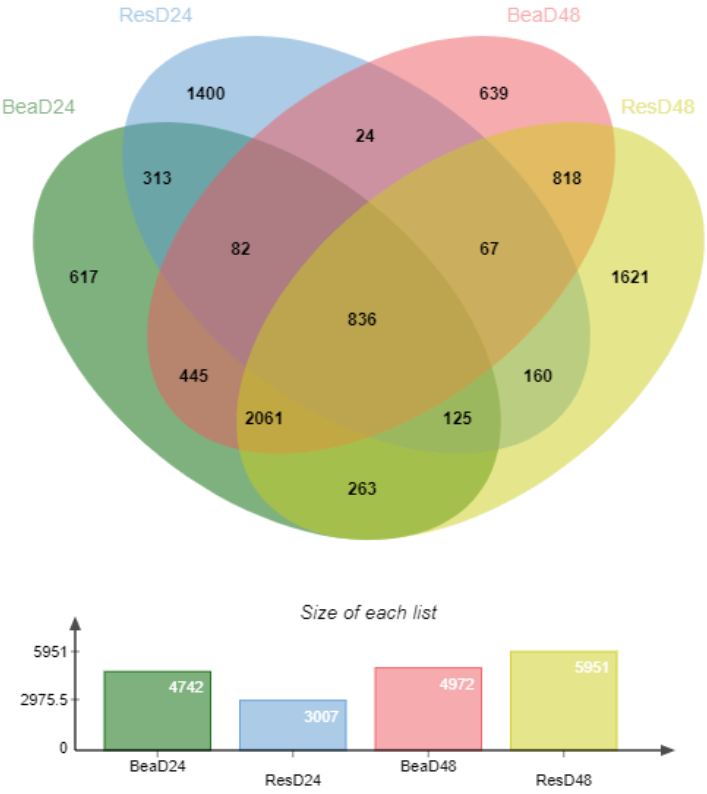
BeaUT0 = Number of upregulated genes 24 hours before 'Beauregard' was exposed to drought; ResUT0 = Number of upregulated genes 24 hours before 'Resisto' was exposed to drought; BeaDT0 = Number of downregulated genes 24 hours before 'Beauregard' was exposed to drought; ResDT0 = Number of downregulated genes 24 hours before 'Resisto' was exposed to drought.

Figure 5b – Venn Diagram of the differentially expressed genes during drought events. The figure shows the number of upregulated genes when 'Beauregard' and 'Resisto' were exposed to drought for 24 hours, and 48 hours. The figure also shows when there was an overlap of genes between the four groups that were studied. For instance. For instance, 100 genes overlap when 'Beauregard' is under drought for 24 hours after drought, and 'Resisto' is under drought for 48 hours.



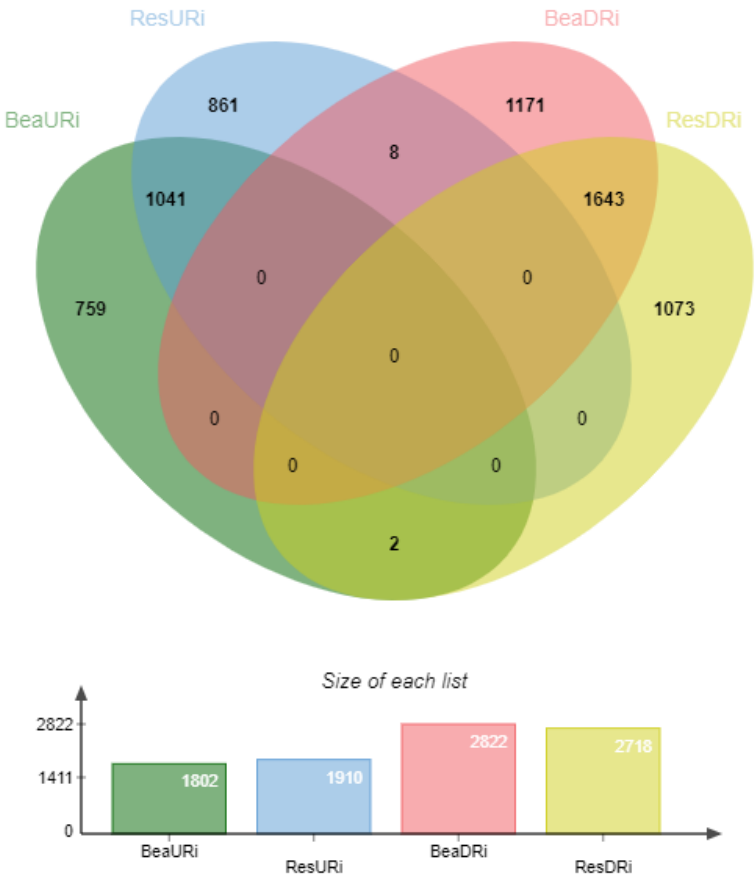
BeaU24 = Number of upregulated genes when 'Beauregard' was exposed to drought for 24 hours; ResU24 = Number of upregulated genes when 'Resisto' was exposed to drought for 24 hours; BeaU48 = Number of upregulated genes when 'Beauregard' was exposed to drought for 48 hours; ResU48 = Number of upregulated genes when 'Resisto' was exposed to drought for 48 hours.

Figure 5c – Venn Diagram of the differentially expressed genes during drought events. The figure shows the number of downregulated genes when 'Beauregard' and 'Resisto' were exposed to drought for 24 hours, and 48 hours. The figure also shows when there was an overlap of genes between the four groups that were studied. For instance, 263 genes overlap when 'Beauregard' is under drought for 24 hours after drought, and 'Resisto' is under drought for 48 hours.



BeaD24 = Number of downregulated genes when 'Beauregard' was exposed to drought for 24 hours; ResD24 = Number of downregulated genes when 'Resisto' was exposed to drought for 24 hours; BeaD48 = Number of downregulated genes when 'Beauregard' was exposed to drought for 48 hours; ResD48 = Number of downregulated genes when 'Resisto' was exposed to drought for 48 hours.

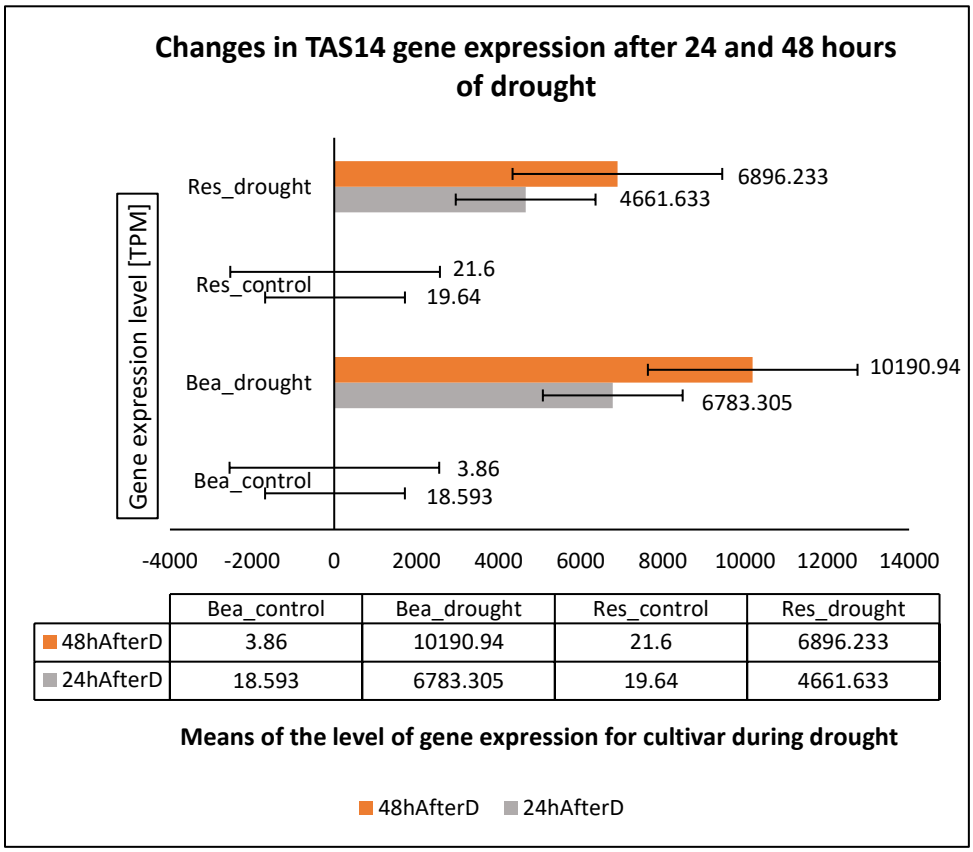
Figure 5d – Venn Diagram of the differentially expressed genes 24 hours after the plants were re-irrigated. The figure shows the number of upregulated and downregulated genes 24 hours after the re-irrigation of 'Beauregard' and 'Resisto'. The figure also shows when there was an overlap of genes between the four groups that were studied. For instance, 2 genes overlap when 'Beauregard' upregulated due to drought for 24 hours after drought, and 'Resisto' is downregulated as a response to 48 hours of drought.



BeaURi = Number of upregulated genes 24 hours after the re-irrigation of 'Beauregard'; ResURi = Number of upregulated genes 24 hours after the re-irrigation of 'Resisto'; BeaDRi = Number of downregulated genes 24 hours after the re-irrigation of 'Beauregard'; ResDRi = Number of downregulated genes 24 hours after the re-irrigation of 'Resisto'.

Figure 6a- Differential in gene expression when plants were exposed to drought for 24 hours and 48hours. The changes in the levels of the abscisic acid and environmental stress-inducible protein TAS14-like when plants were exposed to drought for 24 and 48 hours are shown.

Levels of abscisic acid and environmental stress-inducible protein TAS14 under drought

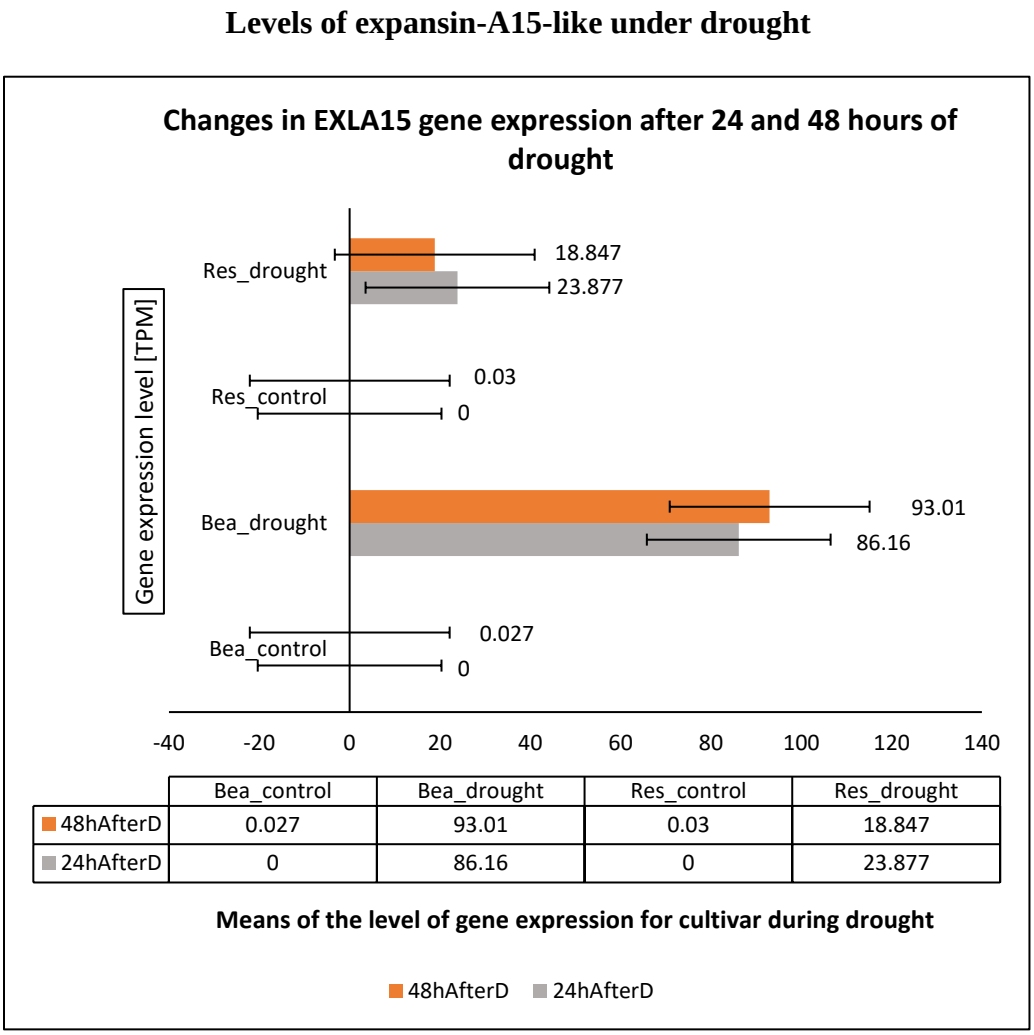


	Bea_control	Bea_drought	Res_control	Res_drought
48hAfterD	3.86	10190.94	21.6	6896.233
24hAfterD	18.593	6783.305	19.64	4661.633

Treatments: 24hAfterD = 24h after imposing drought; 48hAfterD = 48h after imposing drought.

Cultivars: Bea = ‘Beauregard’; Res = ‘Resisto’.

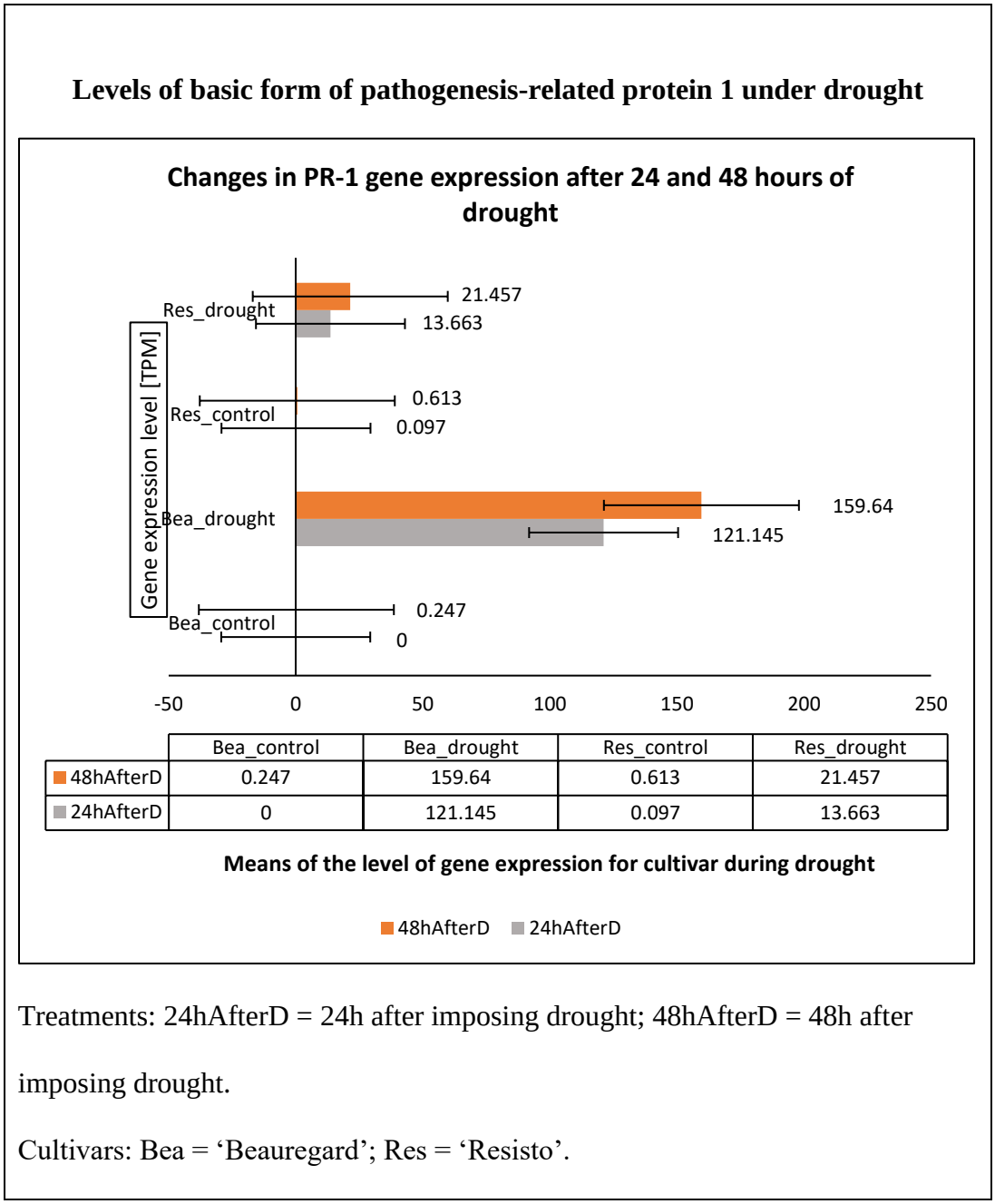
Figure 6b- Differential in gene expression when plants were exposed to drought for 24 hours and 48hours. Figure shows the changes in the levels of expansin-A15-like (EXLA15) when plants were exposed to drought for 24 and 48 hours.



Treatments: 24hAfterD = 24h after imposing drought; 48hAfterD = 48h after imposing drought.

Cultivars: Bea = 'Beauregard'; Res = 'Resisto'.

Figure 6c- Differential in gene expression when plants were exposed to drought for 24 hours and 48hours. Figure shows the changes in the levels basic form of pathogenesis-related protein 1(PR-1) when plants were exposed to drought for 24 and 48 hours.



Treatments: 24hAfterD = 24h after imposing drought; 48hAfterD = 48h after imposing drought.

Cultivars: Bea = ‘Beauregard’; Res = ‘Resisto’.

CHAPTER 5

Conclusions and recommendations

Conclusions and recommendations

I conducted this research with the primary goal of assessing the possibility of using wild *Ipomoea spp.* germplasm as a source of genes to improve drought tolerance in sweetpotato. The rationale behind this goal was that with a changing climate characterized by frequent and extended periods of drought, there is a need for the improvement of drought tolerance in cultivated sweetpotato. The conclusions and recommendations of my research studies are discussed below.

In chapter 2, I used 9223 SNPs to study the relatedness of the 52 *Ipomoea spp.* accessions of the *Batatas* complex, and I found that *I. trifida* is the closest relative of *I. batatas*, a result that agreed with what was found in previous studies (Roullier et al., 2013; Muñoz-Rodriguez et al., 2018; Wu et al., 2018; Rodriguez, 2019). I studied the phylogenetic relationship of 52 *Ipomoea spp*. accessions and identified three major clusters in the phylogenetic tree. A PCA also revealed the presence of three major groups. I also conducted a structure analysis, and I found that there are at least 6 sub-groups within the 52 *Ipomoea spp*. The results of the analyses raised the possibility of *I. trifida* being considered as a bridge species to be used in the improvement of sweetpotato.

In the third chapter of this dissertation, I screened five *Ipomoea spp*. in the *Batatas* complex (*I. batatas, I leucantha*, *I. cynanchifolia, I. trifida,* and *I. triloba*) for drought tolerance in a greenhouse-based study. In this study, I observed that the wild species were more sensitive to drought than sweetpotato. I speculated that the presence of storage roots in *I. batatas* could have an effect on their tolerance to drought because the wild species did not produce storage roots. This research was reported by Nhanala & Yencho (2020) where it is pointed out that there is a need for further studies to evaluate drought tolerance in sweetpotato and wild *Ipomoea spp.*

that produce storage roots. Therefore, I recommend a follow-up study for the assessment of drought tolerance in *Ipomoea spp.* that produce storage roots. However, it would be important to access the crossability of *I. batatas* and those wild *Ipomoea* spp. with storage roots. If conventional hybridization is not possible, other methods such as somatic hybridization should be considered.

In chapter 4, I conducted a comparative transcriptomics study for drought tolerance in 'Beauregard' and 'Resisto' as a follow-up study of the chapter 3. I identified six genes that were differentially expressed in the two cultivars when these varieties were subjected to drought. The candidate genes for drought tolerance were abscisic acid and environmental stress-inducible protein-like (TAS14), E3 ubiquitin-protein ligase RING1-like, expansin-A15-like (EXLA15), basic form of pathogenesis-related protein 1 (PRP-1), 18.8 kDa class II heat shock protein-like, and the desiccation inducible PCC13-62. I observed that the candidate genes had higher fold changes in 'Beauregard' than in 'Resisto'. The phenotypic assessment of the plants showed that 'Beauregard' was less sensitive to drought than 'Resisto'. The detection of the drought tolerant genes in these two cultivars that protect the plant for a shorter or longer period of drought could be helpful to identify drought tolerant cultivars that can tolerate extended periods of drought. The identification of varieties with genes that can tolerate extended periods of drought, could be an initial step to select for cultivars that could cope with long periods of drought. Based on the results of the transcriptomic analyses, I recommend 'Beauregard' for further studies on drought tolerance, specially to evaluation in multi-locations experiments.

The integration of phenotypic and genomics tools was applied in this research. The present dissertation included morpho-physiological assessments for drought tolerance, comparative transcriptomic analysis, and application of qRRS approach to perform a relatedness

study. In these studies, I demonstrated that the integration of phenomics and genomic tools can provide comprehensive results that can be applied in a breeding program. Different categories of data were generated from the three components of this research. None of the components could have replaced another as they generated different types of data. This dissertation demonstrates that these distinct studies are complementary to each other. A recommendation for further studies in the *Batatas* complex, would be to put in place a genome wide association study (GWAS) for traits that may be of interest to the improvement of sweetpotato.

In this research, I also observed unexpected results (e.g., the cultivated sweetpotato varieties less sensitive to drought than the wild relatives species) suggesting that there are several factors behind drought tolerance. Drought tolerance is a polygenic trait, and so, there is already a certain complexity associated with studying this trait. The level of difficulty can be increased when other factors such as storage roots and ploidy level are part of the equation. In addition to all these considerations, the interaction between the genotype and the environment (G x E) should be considered when this trait is evaluated. In a context of climate change, all the factors, discussed above, in particular G x E, will play a major role in the results. The environment where the experiment is conducted can influence in the response of the genotype. A final recommendation of this dissertation is to first review the previous information regarding each genotype and always conduct a study in the specific location of interest.

REFERENCES

Muñoz-Rodriguez, P., Carruthers, T., Wood, J. R., Williams, B. R., Weitemier, K., Kronmiller, B., Ellis, D., Anglin, N.L., Longway, L., Harris, S.A. and Rausher, M.D., & Scotland, R. W. (2018). Reconciling conflicting phylogenies in the origin of sweet potato and dispersal to Polynesia. *Current Biology*, *28*(8), 1246-1256.

Nhanala, S. E. C., & Yencho, G. C. (2020). Assessment of the Potential of Wild *Ipomoea spp.* for the Improvement of Drought Tolerance in Cultivated Sweetpotato (*Ipomoea batatas* (L.) Lam.). Crop Science. 1-16. https://doi.org/10.1002/csc2.20363

Rodriguez, P. M. (2019). Systematic Studies of the Sweet Potato and its Wild Relatives (Doctoral dissertation, University of Oxford).

Roullier, C., Duputié, A., Wennekes, P., Benoit, L., Bringas, V. M. F., Rossel, G., Tay, D., McKey, D., & Lebot, V. (2013). Disentangling the origins of cultivated sweet potato (*Ipomoea batatas* (L.) Lam.). *PLoS One*, *8*(5), e62707.

Wu, S., Lau, K. H., Cao, Q., Hamilton, J. P., Sun, H., Zhou, C., Eserman, L., Gemenet, D.C., Olukolu, B.A., Wang, H. and Crisovan, E., & Fei, Z. (2018). Genome sequences of two diploid wild relatives of cultivated sweetpotato reveal targets for genetic improvement. *Nature communications*, *9*(1), 1-12.