GENETIC STUDIES AND QTL MAPPING OF DROUGHT RELATED TRAITS IN A SWEETPOTATO (*Ipomea batatas*(L.) BI-PARENTAL MAPPING POPULATION

By

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DECLARATION

I hereby declare that, except for references to works of other researchers, which have been duly cited, this work is my original research and that neither part nor whole has been presented elsewhere for the award of a degree.

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ABSTRACT

The study was carried out to understand the genetic basis for yield under drought environments, and to map quantitative trait loci associated with yield and yield related components under drought stress in sweetpotato. The sweetpotato BxT mapping population, generated from a cross between sweetpotato varieties Beauregard and Tanzania was used for this study. Genotypes were evaluated in irrigated and drought environments to evaluate the effect of drought on yield and yield related parameters as well as genetic variability under drought conditions. Drought affected root yield, foliage yield, biomass and harvest index at varying degrees, with highest relative yield reduction in root yield. Drought tolerance indices were estimated based on root yield under drought and irrigated conditions. Suitable drought tolerance indices identified were geometric mean productivity, mean productivity and drought tolerance index. Genotypes were grouped as drought tolerant and high yielding (DTHY), drought tolerant and low yielding (DTLY), drought susceptible and high yielding (DSHY) and drought susceptible and low yielding (DSLY) based on their root yield means. Drought also reduced chlorophyll content, leaf area, normalized difference vegetation index (NDVI), photosynthetically active radiation, and increased canopy temperature. Observed low heritability and non-significant variation among genotypes for physiological traits indicates inefficiency of these traits for selection of drought tolerant genotypes in the BxT mapping population. However, morphological traits had significant variability among genotypes, high to moderate heritability under drought stress indicating they could be considered as potential secondary traits for selection of drought tolerant genotypes. To understand the genetic basis of drought tolerance in sweetpotato, QTLs associated with yield and yield related components under irrigated, drought and rainfed conditions were mapped using the genotype by sequencing method, GBSpoly. The occurrence of four QTLs on linkage group 9 in single environments and on linkage

groups 11 and 6 for combined environments under only drought stressed conditions is indicative of drought specific QTLs. With further studies, confirmation and validation of these QTLs may be useful for drought tolerance-oriented breeding programmes in sweetpotato.

DEDICATION

For the wind beneath my sail….

Mr.Douglas and Mrs. Gladys Utoblo

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVATIONS

Biom-Biomass (fresh)

CT-Canopy temperature

FY- Foliage yield (t/ha)

DBDI- Days before drought imposition

DADI- Days after drought imposition

HI- Harvest index

LAI- Leaf Area Index

%PAR- Photosynthetically active radiation

NDVI- Normalized difference vegetation index

DTI- Drought tolerance index

GMP- Geometric mean productivity

RY- Root yield (t/ha)

RYR- Relative yield reduction

RDD- Reduction due to drought

RSA- Root system architecture

QTL-Quantitative trait loci

SSA- Sub-saharan Africa

CHAPTER ONE

1. GENERAL INTRODUCTION

Despite ongoing efforts to end hunger, many people still do not have sufficient food to maintain a healthy life (FAO, IFAD and WFP, 2015). Approximately one out of every nine people in the world (about 821 million people) are undernourished. The increasing global hunger and food insecurity is attributed to population increase and demand for use of crops for animal feeds and fuels. The changing climate has little effect on demand for food and not much effect on production. Temperature variability, severe drought, irregular rainfall patterns and over dependence on agriculture predispose countries to severe hunger (FAO, IFAD, UNICEF, WFP and WHO, 2017).

Drought causes more than 80 percent of the total losses in agriculture, especially for the livestock and crop production subsectors (FAO, IFAD, UNICEF, WFP and WHO, 2018). It negatively impacts agri-food systems in diverse ways such as reduction in crop productivity and increased food prices. Reynolds and Oritz (2010) project that by 2030 developing countries will be most vulnerable to drought due to rapid population growth and reliance of over 50% of its work force on agriculture.

Among the various environmental factors that limit crop yields, heat and water stresses are the most important (Prasad *et al.*, 2008). In assessing climate impacts on food security in Sub-Saharan Africa, Ringler *et al.* (2010) reported that wheat will be most affected by climate change in Sub-sahara Africa, followed closely by Sweetpotato while millet and sorghum are likely to have slightly higher yields.

Cereals, legumes, roots and tubers remain the major suppliers of food for humans (Cordain, 1999). Roots and tuber crops, including sweetpotato, are considered the pillar of food security in the

humid Tropical parts of Sub-saharan Africa (SSA), some parts of Asia and Latin America. They are a notable food source in Sub-Saharan Africa (SSA), providing about 25% of total calorie consumption in Nigeria and possibly 60% in parts of Africa like Congo and Rwanda (Alexandratos and Bruinsma, 2012; Thiele *et al*., 2017). Besides their vital role in food security, they play a major role in nutrition and income generation through engagement in value chains for about 300 million people in developing countries (Thiele *et al.*, 2017).

In Ghana, sweetpotato is the fourth most important root and tuber crop after yam (Dioscorea spp.), cassava (*Manihot esculenta*. Crantz) and Taro (*Colocasia* spp) (Sugri *et al*., 2017). It is a vital source of food, raw material for industry with great potential for income generation. It is often consumed boiled, fried, or roasted. Shoots supplement livestock feeds, while roots are potential sources of starch and flour for industrial uses. The Orange-Flesh Sweetpotato (OFSP) varieties which are rich in beta-carotene are beneficial for combating vitamin A deficiency among children and pregnant women. Leaves are also widely used as a vegetables in parts of West Africa. (Akoroda, 2009).

Although Sweetpotato is moderately drought tolerant (Saraswati *et al*., 2004), storage root yield decreases if drought conditions prevail during the storage root initiation period estimated to be between 10 and 30 days after planting (Nair, 2000). Yield loss due to drought can be up to 50-80% depending upon the timing, duration, and intensity of drought (Solis, 2012).

Considering the important role of sweetpotato and other root and tuber crops, a scientific approach is pertinent in tackling hunger and reducing food prices despite prevailing climate change conditions (Thiele *et al.*, 2017). With unpredictable rainfall distribution across most agro-ecological environments, breeding has been very effective at targeting varieties adapted to areas with varying degrees of moisture availability (Braun *et al.*, 2010) thus, providing farmers with

access to varieties suitable for their environments (Langridge and Reynolds, 2015). Thiele *et al*. (2017) proposed a six-step framework for climate smart breeding: (1) downscaling climate change models and crop modeling; (2) identifying and understanding key climate change responsive traits; (3) breeding and varietal selection; (4) phenotyping and genomic research to accelerate gains; (5) developing management options for climate-smart varieties; and (6) deployment (seed systems).

Improving the drought tolerance capacity of crops for drought prone environments is a critical task for plant breeders. Achieving this requires genetic improvement of crop varieties for moisture-stress tolerant attributes and stable crop yields despite moisture stress. Genetic improvement of sweetpotato is challenging owing to the polyploid nature of its genome. More so, inherent self-incompatibility impedes the transfer of genes from materials with the desired traits to those needing improvement (Fajardo *et al*., 2002).

In order to strengthen breeding for improved drought tolerance, this research was conducted with the following objectives :

- estimate the effects of drought on yield and yield components in sweetpotato grown in the field under drought conditions in Ghana
- estimate the effect of drought on physiological and morphological traits of sweetpotato grown under drought conditions in Ghana, and
- Map Quantitative Trait Loci (QTLs) linked to agromorphological traits related to drought tolerance in a sweetpotato bi-parental mapping population

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Sweetpotato origin

Sweetpotato (*Ipomoea batatas*) is a polyploid and is the only hexaploid species (2n = 6x = 90) in the genus Batatas of the Convolvulaceae family (Gruneberg *et al.*, 2015). Sweetpotato is believed to be a native crop to the Americas, however when and where it was domesticated still not certain. *Ipomea trifida* and *Ipomea triloba* are the likely progenitors via a process of interspecific hybridization (Austin, 1988) in the Yucatan peninsula and the Orinoco river basin. Another hypothesis suggests that evolution of *Ipomea batatas* resulted from several ploidy level changes (diploid to hexaploid) through autoploidy of *Ipomea trifida* (Kobayashi,1984). Roullier *et al.* (2013) identified I. trifida as the closest diploid wild relative of sweetpotato based on cytogenetic and neutral-marker studies. Using nuclear and chloroplast phylogenies, Mu *et al.* (2018) support this assertion. In addition, Wu *et al*. (2018) reported a high degree of similarity between genome features of the hexaploid "Tanzania" variety and the diploid *Ipomea trifida* as well as between "Tanzania" and the diploid *Ipomea triloba* with *Ipomea trifida* contributing twice as much as *Ipomea triloba* to the *"Tanzania" genome.*

2.2 Sweetpotato production and utilization

In Sub Saharan Africa, roots and tuber crops constitute an integral component of diets due to affordability and nutritional value. Although preferences vary geographically, demand is basically for consumption as food often in combination with other foods as a means of cost reduction. Roots and tubers are an attractive food source for household security and income generation for rural poor African farmers due to their ease of adaptation in marginal soils and integration in mixed farming agricultural systems (OECD/FAO, 2016).

Sweetpotato is important in countries surrounding the Great Lakes in Eastern and Central Africa; Malawi, Angola, Mozambique, and Madagascar in Southern Africa, and Nigeria in West Africa (Woolfe, 1992). Production is expanding faster than any other major food crop in SSA. The rapid growth of sweetpotato area in sub-Saharan Africa during the past decade is attributed to changes in cropping patterns, unstable economies and the increasing commercialization of production (Low *et al.*, 2017). Low *et al*. (2009) suggested that these changes in cropping patterns by farmers is probably due to difficulty in cultivation of some traditional crops like bananas and cassava due to disease spread or cropping cycle. Frequently, sweetpotato and cassava are grown on the same farm or the same region. When cassava fails, farmers frequently switch to sweetpotato to substitute for the energy delivered by cassava.Other reasons include the rich betacarotene content of orange flesh varieties, micronutrient contents in young leaves and vines and early maturity in some varieties with high fresh and dry matter yields from roots and vines grown in fairly fertile soils.

Global sweetpotato production as in 2017 was estimated at 105,000,000 tonnes with 79,600,417 tonnes (71%) produced by Asia, 27,720,784 tonnes (25%) by Africa, 4,506,829tonnes (4%) by Americas, 85,652tonnes (0.1%) by Europe and 921,633tonnes (0.8%) by Oceania. In Africa, approximately 65% (17,907,593tonnes) of sweetpotato production comes from East Africa, 22% (6,009,817 tonnes) from West Africa, 10.98% (3,043,751 tonnes) from Middle Africa, 2.47% (683,192 tonnes) from Northern Africa and 0.23% (76,432 tonnes) from Southern Africa. In West Africa, Nigeria is the largest producer of Sweetpotato accounting for 40,137,86 tonnes (67%). Production in Ghana was estimated at 145,886 tonnes (2.4%) while Mauritania had a production rate of 4,882 tonnes (0.2%) of the total production in West Africa (FAOSTAT, 2018).

For over two decades, the percentage of area under sweetpotato production has increased more than in maize, although from a much lower base (Low *et al*., 2009). Sweetpotato is gaining

prominence in Ghana as a source of food and income (Amengor *et al.*, 2017) with a large increase in production from 107,038 tonnes in 2006 to 143,111 tonnes in 2016, area harvested from 72,495 ha in 2006 to 76,594 ha in 2016 and yield in 2006 from 14,765 hg/ha to 18,639 hg/ha in 2016 (FAOSTAT, 2018). It is produced mainly in the Eastern, Central, Northern, Upper East, and Volta Regions of Ghana (Wie *et al.*, 2017). Cultivation is done on mounds or ridges and based on seasonal variations which range from two rainy seasons in the coastal savannah and forest – savanna transitional zones to the Guinea savanna zone with one growing season. This allows for planting twice in a year in the coastal and forest zones and once in the Guinea savanna.

In SSA, Sweetpotato is consumed in diverse forms; as boiled, steamed or fried roots taken as snacks and sometimes in combination with other crops as a main meal. It also contains high amounts of ascorbic acid and amino acid and lysine, soluble fibre (Kays and Kays, 1997). The orange flesh Sweetpotato (OFSP) has higher beta carotene content and is used to combat vitamin A deficiency. It is a rich source of carbohydrates, dietary fiber, vitamins and minerals and the orange-fleshed sweetpotatoes contain high amounts of β-carotene to combat vitamin A deficiency (Low *et al*., 2017. Its leaves are edible, although consumption rate varies by region (Low *et al.*, 2017). It is often considered as "the crop that is there when the maize fails" (Low *et al*., 2009). In Ghana, besides its consumption in boiled, fried or roasted forms, sweetpotato has the potential for utilization in livestock feed and as a source of starch and flour for industrial uses. Leaves are consumed in the Guinea and coastal savanna zones and are rich sources of vitamin A in addition to theorange flesh in combating vitamin A Deficiency in Ghana (Akoroda, 2009). Bonsi *et al.* (2014) compounded a weaning food formulation usingorange flesh sweetpotato and maize to combat macro and micronutrient deficiency in Ghana and produced a puree from orange flesh sweetpotato (OFSP) for beta carotene rich bread as an alternative to 100% wheat flour breads to

supply vitamin A for lactating mothers (Awuni *et al*., 2018) and as a complementary food with broken rice for infants between the age of 6 -12 months in culturally accepted Ghanaian meals (Adisetu *et al.*, 2017).

2.3 Sweetpotato production constraints

Considering agriculture's role in confronting the challenge of hunger and improving food security, agriculture in SSA is challenged by the complexities associated with climate change, water shortage, deseterfication and pollution as factors that challenge food production. Despite the agricultural potential of Sub Saharan Africa, increases in sweetpotato production has been due primarily to area expansion. Multiple factors influence productivity gains including faster technology adoption associated with the emergence of medium-scale producers and improved integration of smallholder producers into the value chain. Notwithstanding improvements, significant yield gaps still exist in primary food products.

Major constraints to sweetpotato production include biotic and abiotic stresses. Sweetpotato productivity is often limited by challenges including declining soil fertility, drought, low yielding varieties, sweetpotato diseases predominantly sweetpotato virus disease (SPVD) and Alternaria blight, and insect pests such as the sweetpotato weevils.Other notable constraints include shortage of high quality planting materials and limited range of processing and utilization options which often result in high post-harvest losses, estimated between 30-35% (Mwanga *et al.*, 2011).

2.4 Drought

Climate change, lack of fresh water, desertification and pollution exacerbates the risks of natural hazards through changing patterns of rainfall, temperature and arability of soils over several decades and increasing occurrence and severity of drought and flood episodes (IPCC, 2014). Since

the actual timing and direction of change is unknown, it is necessary to breed for climatic conditions that are currently expressed in trials and nurseries.

Geomorphic, isotopic, and geochemical evidence from the sediments of Lake Bosumtwi, Ghana suggest that long term drought episodes were in West Africa as far back as 3000 years ago (Shanahan *et al*., 2009). Sultan and Gaetani (2016) explained that ongoing changes in climatic conditions spelt by temperature variations, increased occurrence of extreme climatic variability and a recovery of the monsoonal precipitation in West Africa are predicted to persit through the century. However, climatic expectations associated with precipitation is still probabilistic for the West African climate in regards to seasonal precipitations. Yield loss is emerging (Muller *et al*., 2010; Roudier *et al*., 2011). However, in a review Ray *et al*. (2015) suggested that among the West African countries precipitation variability alone did not account for observed yield varaiability in maize in Cameroon and northeastern Nigeria. Rainfall also contributed to nutrient loss from the soil due to leaching resulting to negative productivity. In some other west African countries, rainfall variability accounted for yield variability however, this was not a general situation as farmers have adopted different methods of managing rainfall irregularities.

2.4.1 Drought scenario in Ghana

Agriculture in Ghana is mostly rainfed and it is an integral driver of the Ghanain economy, providing employment for about 57% of its labour force(Armah *et al.*, 2011). It contributes to about 44% of its Gross Domestic Product (MoFA, 2007).The amount and pattern of rainfall influences agricultural productivity (Seini *et al*., 2004). In recent years, climate-related problems such as drought and floods have resulted in severely reduced food production (MoFA, 2007).

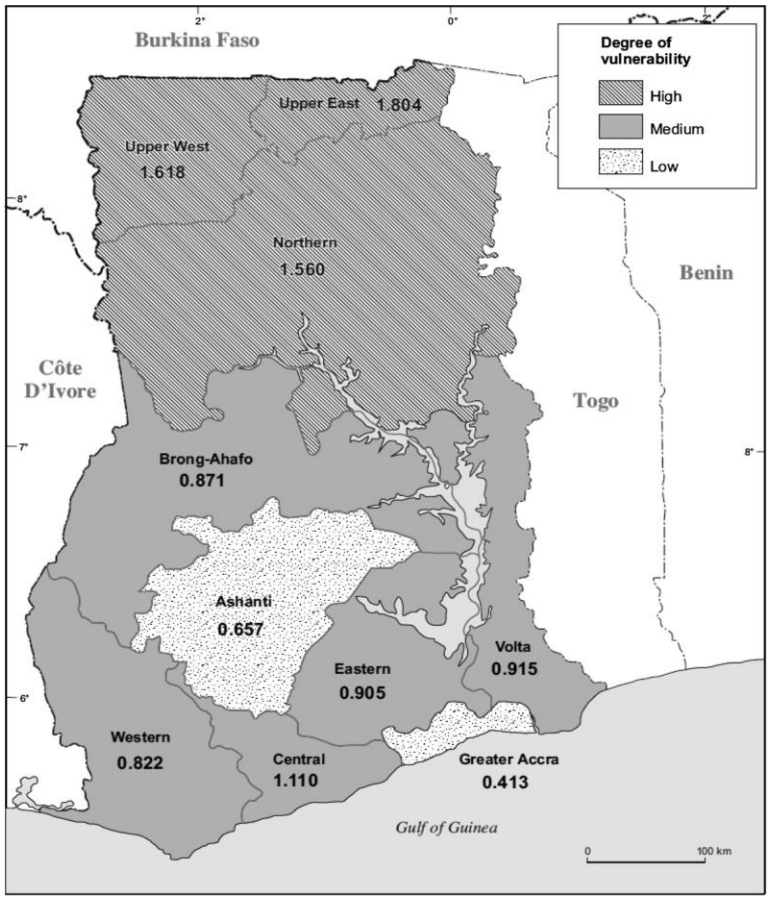


Figure 2.1: Vulnerability indices of the various regions in Ghana (Source: Mapping the vulnerability of crop production to drought in Ghana using rainfall, yield and socioeconomic data Antwi-Agyei *et al*., 2012)

Armah *et al.* (2011) have proposed that Ghana's reliance on rainfed agriculture predisposes it to to drought and desertification conditions (Fig. 2.1). Records of climatic variability over the past 40 years show increased temperature of 1 °C as well as 20% and 30% estimated reduction in rainfall and runoff, respectively (Asante, 2004). Northern Ghana is the most susceptible region in terms of water scarcity and drought (Armah *et al.*, 2011). Preceeding the drought event between 1981 and 1983was the drought which ocurred from 1975 to 1977. It was recorded as Ghana's worst drought event. Almost all parts of Ghana was affected by the drought (Ofori-Sarpong, 1980).

Futuristic climatic extrapolation predicts an annual mean temperature rise by 0.6 ^{o}C, 2.0 ^{o}C and 3.9 ^{o}C by the years 2020, 2050 and 2080 respectively in contrast to decreased amount of rainfall

by 2.8%, 10.9% and 18.6% for the same periods (GEPA, 2007). The repercussions of these trends are an expected reduction in the production of major food crops (Zougmoré *et al.*, 2018).

2.4.2 Drought effect on sweetpotato storage root yield

Plant growth, development and productivity depends on the ability to acquire and use water efficiently (Ferguson *et al.*, 2018). Green house experiments carried out by Solis *et al.* (2014) revealed a 30 to 42% reduction in root number and yield reductions of 66% at 5 and 10 days after transplanting. Further field-based experiments with soil moisture levels less than 50% field capacity supported storage root initiation but water deficit for up to 30 days reduced the number of storage roots and slowed the rate of root development. Restoration of moisture following water deficit did not compensate losses caused by moisture deficit. Lewthwaite and Triggs (2009) reported that pencil roots which are unable to store carbohydrates are formed under drought conditions due to irreparable lignification of young roots.

In estimating the effect of drought on yield of orange flesh sweetpotato, Van Heerden and Laurie (2008) observed a large reduction in marketable storage root yield in both sweetpotato varieties Resisto and A15 at water deficit conditions 30% less than the irrigated treatment. Laurie *et al.* (2009) observed a significant reduction in yield from the control due to severe stress treatments in all genotypes used in their study. Variety Blesbok had the least yield loss (74%) while Resisto experienced the highest yield loss (91%). This confirms that sweet potato experiences large yield losses under severe stress conditions.

Drought stress can occur at any time during the crop growth with varying degree of severity (Suzuki *et al.*, 2014). Development in sweetpotato entails establishment, occurring withing the first four weeks after planting, followed by the formative phase of roots 5 -8 weeks after

transplanting and lastly, storage root bulking occurring within 9 -17 weeks after planting (Stathers *et al*., 2013).

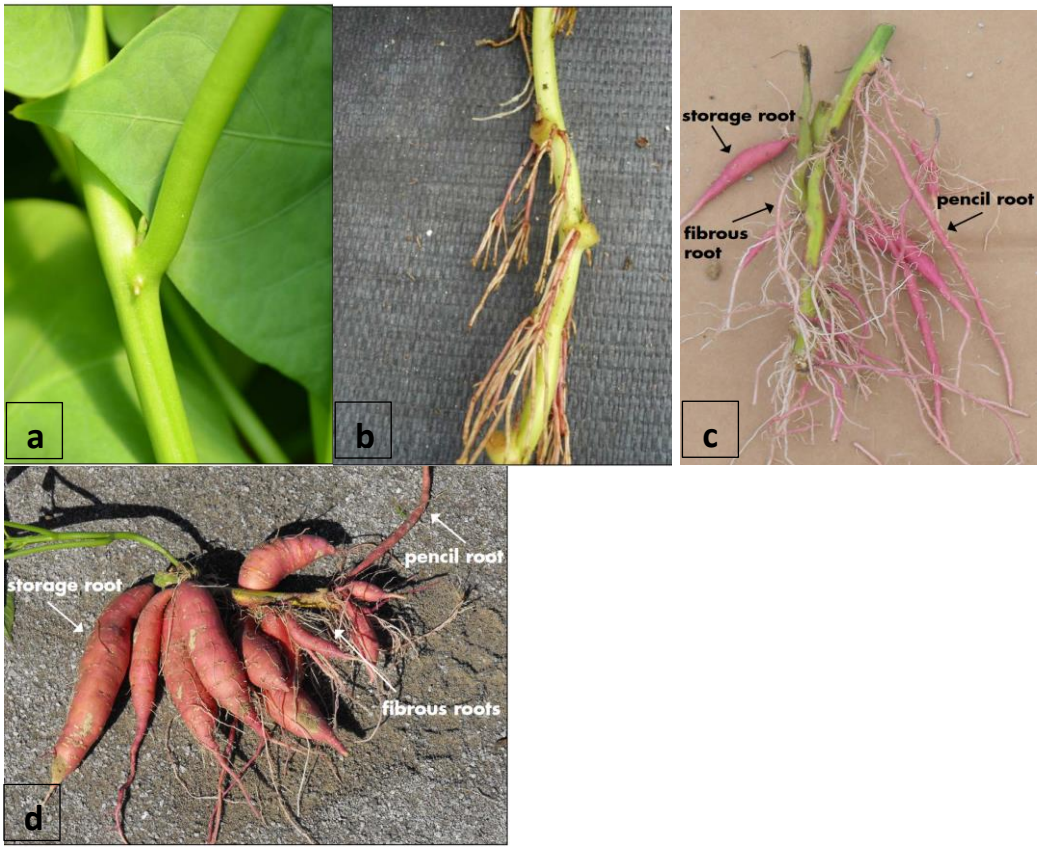
At the plant establishment phase, sweetpotato is susceptible to drought often resulting to substantial reduction in yield (Lewthwaite and Triggs, 2012). Conditions of water deficit slows sweetpotato storage root initiation. Hence, soil moisture management during the early phase of growth is desirable for sweetpotato (Gajanayake *et al.,* 2013). At the mid to late season stage,decreasing moisture levels from 100 to 20% evapotranspiration-based irrigation treatments caused about 30% reduction in storage root production efficiency (Gajanayake and Reddy, 2016). This confirms that adequate moisture is needed for optimal productivity of sweetpotato through its cycle of development.

2.4.3 Root system architecture

Roots play vital roles in adaptation to environment and crop productivity. Root features such as length, number, density, lateral roots , size and diameter comprise the the root system architecture of a plant. Roots are also relevant in symbiotic relationships with soil organisms and provide support system which holds the plant up. The diverse functions contribute to the growth and productivity in crops (Den Herder, 2010).

In sweetpotato, unlike seed propagated crops with roots emerging from the embryo, root formation in starts from preformed roots (primordia) in nodes located on each vine (shoot) (Meyers, 2014; Khan *et al.*, 2016). These primordia grow into adventitious roots and, together with hardened tissue from the cut end of the sweetpotato vine, give rise to the roots of a sweetpotato plant. Under suitable environmental conditions, adventitious roots develop into storage roots (Figure 3). When damaged, roots are unable to form storage roots and fibrous roots are formed. Unfavorable environmental conditions after planting cause the formation of pencil roots (Meyers, 2014).

Adventitious roots (ARs), lateral roots (LRs) and storage roots (SRs) make up the root system architecture (RSA) in sweetpotato (Khan *et al.*, 2016).



Meyers, S. L. (2014)

Figure 2. 2: a-Sweet potato roots formation starts as preformed root primordia, found at nodes along the stem of the sweet potato, b-roots begin to grow about 24 hours after transplanting vines given the right conditions for growth, c-At 30 days after transplanting, root tyes are visually expresed. Pencil and potential storage roots are pigmented, and storage roots have begun to enlarge. Fibrous roots remain mostly white, d-Sweet potato roots at harvest can easily be identified as either fibrous, pencil, or storage roots.

- **Effect of drought on RSA in Sweetpotato**

Drought causes more reduction in crop yield than other forms of abiotic stress. Roots provide the first form of protection of crops in drought situations. Response to stress is dependent on the degree of variation in the RSA of the plant (Giehl *et al*., 2013). These responses in turn trigger changes in the growth, development, activation of hormonal and molecular pathways linked to physiological functions and morphological traits.

Different abiotic stresses affect RSA in varied ways. In sweetpotato, moisture and nutrient availability in the soil greatly influence lateral root development (Postma *et al*., 2014). The final storage root yield depends on the capacity of a genotype to develop lateral roots on the main adventitious roots (Villordon *et al*., 2012). Drought impedes the development of adventitious and lateral roots (Pardales and Yamauchi, 2003) . Storage root yield depends on the genotype's ability to grow lateral and adventitious roots. When lateral roots fail to grow or stop growing, lignified steeles are formed inhibiting root bulking resulting in reduced yield (Villordon *et al*., 2012).

Khan *et al.* (2016) highlighted that root phenotypes suitable for drought adaptation should have deep roots, well spread branch root density from top to bottom, increased radial hydraulic conductivity at depth and less metabolic trade-offs. Root traits needed for continued growth include increased root tip diameter, steeper, ample and longer lateral roots, and vertical growth.

According to Lynch *et al.* (2018) for high-input agroecosystems, genotypes with fewer roots would be advantageous for drought resistance. Such root types promote better water uptake by growing deeper and are characterized by less developed cortical parenchyma, root cortical arenchyma, root cortical senescence and increased cortical size cell. Although some of these ideas are supported by empirical evidence, they remain largely hypothetical requiring research and development of "robust functional–structural models capable of simulating the dynamics of root–soil interactions".

2.5 Overcoming the effect of drought on sweetpotato

In coping with the adverse effect of drought, farmers adopt independent measures to mitigate yield losses including cultivation of different crops and varieties which are more drought-tolerant., planting of early maturing varieties, switching to other less drought susceptible varieties, and shifting planting date.

Other coping strategies include mulching of gardens to reduce soil temperature and lower the rate of evapotranspiration, maintaining small home gardens, planting under shade providing trees or planting in damp areas around rivers as a means of vine multiplication and preservation (Bang and Sitango, 2003).

Long term methods of combating drought effects on crops are needed to produce varieties which are adapted to drought prone environments and have yield stability. This entails improvement of the crop varieties genetically for drought adapted traits as well as water use efficiency and nutrient uptake (Tuberosa, 2011).

2.6 Breeding sweetpotato for drought tolerance

From an agricultural perspective, drought exists when available water from rains or irrigation or a combination of both is insufficient to meet transpiration requirements of a crop (Tuberosa, 2011). Sweetpotato is a clonally propagated crop. However, it is considered an open pollinated crop for the purpose of genetic improvement of varieties in order to estimate heterosis (Thiele *et al.*, 2017). Breeding varieties tolerant to drought is dependent on accumulation of desirable alleles in progeny with improved potential for drought tolerance. This requires screening of genotypes under water deficit and well-watered conditions (Kivuva, 2013). Several approaches to screening have been used in selection of drought tolerant sweetpotato including field and greenhouse-based approaches (Kivuva, 2013; Agili, 2012; Ricardo, 2011), a rapid drought screening box method (Omotobora *et al*., 2014), in-vitro based methods using high levels of Polyethylene glycol (PEG) (Agili, 2012).

Grüneberg *et al.* (2015) proposed selection of genotypes under drought conditions (during which genotypes with desired performance are selected) and subsequently under optimum conditions. Simultaneous selection can also be done using indices for selection under stress conditions.

The use of drought tolerance indices based on yield estimates from drought and irrigated conditions have also been useful in selection of drought tolerant genotypes (Ricardo, 2011; Makunde *et al.*, 2017; Agili *et al*., 2012; Andrade *et al.*, 2016). Breeders in Mozambique have developed drought tolerant varieties with good storage root yield across optimal and drought environments.

Plant breeding has been very successful in developing improved varieties using conventional tools and methodologies. Nowadays, the availability of genomic tools and resources is leading to a new revolution of plant breeding, as they facilitate the study of the genotype and its relationship with the phenotype for complex traits (Perez-de-Castro *et al.*, 2012).

Quantitative traits loci have made it possible to identify chromosome regions influencing variations associated with plant growth and development under water deficit conditions. Studies on QTLs have shown that chromosomal regions influencing drought related crops are spread through a large portion of the genome. Understanding the relationship between changes in drought related quantitative traits and their effect on yield under water deficit and optimum conditions remains a research interest. Identification of loci for specific traits under both water deficit and optimum conditions is informative with respect to the relevance of traits for improving drought tolerance and yield (Cattivelli *et al.*, 2008).

Previous efforts to map QTLs for traits in sweetpotato include works by Cervantes-Flores *et al*. (2008) using the Tanzania x Beauregard (TxB) mapping population. Tanzania is a Ugandan landrace notable for its cream flesh and high dry matter (Mwanga *et al*., 2001) served as the male parent. In contrast to Tanzania, Beauregard, a US-bred variety is characterized by orange flesh and low amounts of dry matter (Rolston *et al*., 1987) served as the female parent.Quantitative trait loci

(QTL) for root-knot nematode resistance, dry matter, starch, and β-carotene content were mapped (Cervantes-Flores *et al*., 2008; Cervantes-Flores *et al*., 2011). Ongoing efforts toward genomic assisted breeding in sweetpotato include research by the Genomic Tools for Sweet Potato Project (GT4SP; https:// sweetpotatogenomics.cals.ncsu.edu/). Using the biparental mapping population (BxT) developed from a cross between Beauregard × Tanzania and comprising 315 progeny, genotyping was done using Genotyping by Sequencing (GBSpoly). A genetic map with 15 linkage groups has been developed using new linkage mapping methods developed for polyploids (Pereira *et al*., 2019). Mapping of QTLs for numerous traits including drought is being currently carried out using new QTL mapping software for polyploids developed in-house (Monneveux and Ribaut, 2011). Leveraging on the Genome Wide Association Study (GWAS) and genomic selection models, other populations considering a family structure such as the Mwanga Diversity Panel (MDP) developed from crossings between parents from two pseudo-heterotic groups (8 parents from each group), 64 families with about 2000 genotypes is being phenotyped. Genetic maps have been developed using a cross from the diploid, *Ipomoea trifida* as parents and 212 progenies (Friedmann *et al.*, 2018). The genome of the diploid *Ipomea trifida* and *Ipomea triloba* have been sequenced and serve as references for the for hexaploid sweetpotato (Pereira *et al*., 2019)

CHAPTER THREE

3. Genotypic Variation in a Sweetpotato Mapping Population in Response to Drought Under Field Conditions and Selection of Drought Tolerant Genotypes

3.1 Introduction

Abiotic stress affects yield of major crop plants, reducing yield by 50 percent or more (Greco *et al*., 2012). Drought causes greater yield reduction in crops than other forms of abiotic stress (Hochholdinger, 2016**).** This trait causes different levels of impact in plants at different levels of severity, environments, species and developmental stages so achieving tolerance is quite challenging (Budak *et al.*, 2013).

Roots and tubers are ranked sixth in the world's most important food crops. Nonetheless, the degree of drought effects on sweetpotato production on a global scale is limited. It is unknown how drought effects vary with: 1) root and tuber crops, (2) soil texture , (3) agro-ecological regions and (4) drought timing (Daryanto *et al.*, 2016). Research centering on drought effects on production of cereals and legumes far outweigh that on roots and tubers (Daryanto *et al*., 2016).

It is often assumed that potato is drought-sensitive whereas cassava and sweet potato are resistant to drought (Daryanto *et al*., 2016) but findings indicate sweetpotato yields decrease by 14 % or more, depending on the soil type and environment (Ringler *et al.*, 2010 , Reynolds *et al.*, 2015). Daryanto *et al*. (2016) suggested that drought resistance in cassava and sweetpotato could be linked more to survival than yield. All root and tuber crops are more vulnerable to drought during periods of storage root and tuber development than at their vegetative phase of development. In sweetpotato, storage root initiation occurs usually between 2-7 weeks weeks after planting. Gajanayake and Reddy (2013) reported storage root initiation as early as 14 days after transplanting while Villordon *et al*. (2012) reported storage root initiation in variety Beauregard

19 days after transplanting of sweetpotato slips under favorable conditions. This phase determines the eventual yield of the crop (Monneveux *et al*., 2013; Okogbenin *et al.,* 2013) .

Different sweetpotato cultivars respond differently to water deficit conditions. Selection for drought tolerant cultivars is an important goal in plant breeding. Securing a reliable food supply is a top priority for drought prone environments as well as non-drought-prone environments (Solis *et al*., 2014). Because of the low heritability of drought tolerance and lack of efficient selection methods, development of drought tolerant varities is challenging (Agili *et al*., 2012). Relative yield performance in drought-stressed and non-stressed environments can be used as an indicator of drought tolerance in breeding for drought-prone environments. Several indices for comparative analysis of genotypes based on yield performance in stressed and non-stressed environments have been developed and utilized in categorizing genotypes and screening drought tolerant genotypes (Mohammadi, 2016). Indices developed by Fernandez (1992) grouped genotypes into 4 classes: Group A for genotypes with high performance under both stress and non-stress conditions, Group B for genotypes with high yield in non-stress conditions, Group C for genotypes with high yield in stress conditions while genotypes with low yield in both stress and non-stress conditions were placed in Group D. He also proposed Stress tolerance index (STI) as a useful tool for predicting yield and stress tolerance potential of genotypes as well as the Geometric Mean Productivity (GMP) for estimation of relative performance of drought stress which varies in severity in field environments over years (Fernandez, 1992). To measure yield stability of genotypes in both stress and non-stress conditions, Gavuzzi *et al*. (1997) proposed the Yield Index (YI) while Bouslama and Schapaugh (1984) proposed the Yield Stability Index (YSI). These indices are based on drought tolerance or drought susceptibility of genotypes and have been used in screening and selection of drought tolerant and susceptible *genot ypes* in many crops (Guendouz *et al*., 2014;

Tavakol and Pakniyat, 2007; El-Rawy and Hassan, 2014; Mohammadi *et al*., 2012; Meena *et al*., 2015) in wheat, (Papathanasiou *et al*., 2015; Kumar *et al*., 2016; Mohammadi *et al*., 2013 and Naghavi *et al*., 2013) in maize and flax (Asgarinia *et al*., 2016). It has also been used in cassava, (de Oliveira *et al*., 2017), in sesame (Boureima *et al*., 2016), in potato (Cabello *et al*., 2013), in sweetpotato (Andrade *et al*., 2016 ; Makunde *et al*., 2017) in Mozambique (Agili *et al*., 2012 and Kivuva, 2013) in Kenya. However, no report was found for the utilization of these indices for the selection of drought tolerant genotypes adapted to conditions in Ghana.

Given the significance of root/tuber crops for food security in various regions of the world and the uncertainties regarding the global climate, desertification, pollution and water scarcity, there is a need for greater understanding of the resilience of root/tuber species including sweetpotato to water stress and how different root/tuber species respond to drought due to timing and intensity of water stress (Daryanto *et al*., 2016).

Thus, this research work was aimed at:

1. Estimating quantitative genetic parameters for storage root yield and yield related components in a sweetpotato bi-parental mapping population (BxT) under drought and irrigated conditions
2. Estimating drought tolerance indices for genotypes and identification of suitable indices for selection of drought tolerant genotypes
3. Grouping genotypes into drought tolerant and drought susceptible genotypes for subsequent experiments

3.2 Materials and Methods

3.2.1 Location of the experiment and weather conditions

Figure 3.1 shows experimental locations on the map of Ghana. Eight field phenotyping experiments were conducted.Two experiments, N16 and F16 were carried out under rainfed conditions in Nyankpala 2016 and Fumesua, 2016. Six exeriments were carried out under conditions of water stress (drought) andnon-stress (Irrigation) after the rains had stopped in order to avoid additional water beyond what was provided via irrigation. The first experiment (rainfed denoted as environment N16) was carried out in in Nyankpala, Northern Ghana at the Savannah Agricultural Research Institute (SARI) research station located at Nyankpala (9º25'N, 0º58'W) in the Guinea Savannah Agroecological Zone of Ghana from May to September 2016. The area is characterized by an average annual rainfall of about 1100mm, lasting about 5-6 months often starting april or may and ending in October. Occassional surges of dry spells for a period of about two weeks may be experienced during the growing season. High tempreature and low precipitation is obtainable most parts of the year. Periods of dryness vary between 4-6 months from November to April.

The second experiment (denoted as environment F16) was conducted in Fumesua (Ashanti Region) at the CSIR-CIR research station from September to December 2016 under rainfed conditions. The third (denoted as N17 Irr) and fourth experiments (N17 Drgt) were carried out concurrently at the SARI research station in Nyankpala under non-stressed (irrigation) and water stressed conditions (drought) respectively from December 2016 to May 2017. The fifth (W17 Irr) was conducted using a field belonging to the Irrigation Development Authority (IDA) in Wenchi in Brong -Ahafo Region under irrigated conditions concurrently with the sixth experiment (W17 Drgt) which was carried out under water stressed (drought) conditions from January 2017 to May

2017. The Brong Ahafo region is located within longitude 0^0 15' E-3^0 W and Latitude 8^0 45' N-7^0 30' S in the west central part of Ghana. The Region shares common boundaries with five others namely, Northern Region to the north, Ashanti and Western Regions to the south, the Volta Region to the east and the Eastern Region to the south-east. Temperature in the Brong- Ahafo Region is generally high, averaging over 23.9^{0}C (75^{0}F) throughout the year. Relative humidity in the region is also quite high with an annual mean of 75% throughout the year. Humidity is high in the wet months and low in the dry months. The average annual total rainfall of the region is 1,088mm – 1,197mm. Soils in some regions are characterized by pH ranging from 3.5- 6.7, %organic matter 0.34-1.69(mg/kg), available phosphorus: 0.12-64.25 (mg/kg), available Calcium(mg/kg) 16.0-140.3 (MOFA:http://mofa.gov.gh/site/?page_id=644).

The seventh experiment (N18 Irr) was carried out under non-stressed (irrigated) conditions while the eigth experiment (N18 Drgt) was done under water stressed (drought) conditions . Both experiments were located in Nyankpala at the SARI research station from September 2017 to January 2018.

Figure 3.1 Map showing experimental Locations in Ghana

The weather conditions from for trials are presented in Table 3.1. The minimum temperature ranged between 10.10 0C and 20.0 0C and the maximum between 34.20 0C and 40.80 0C. Relative humidity had a minimum value between 10% and 90% while maximum values ranged between 90.0% to 99.0% .

Mean volumetric water content (VWC) m^3/m^3 for irrigated experiments in Nyankpala (N17 Irr) ranged from 0.13 m^3/m^3 to 0.29 m^3/m^3 while the drought experiment (N17 Drgt) had a VWC range of 0.05 m^3/m^3 to 0.21 m^3/m^3. Mean volumetric water content for environments N18 Irri was between 0.15 m^3/m^3 and 0.21 m^3/m^3 while environment N18Drgt had a lower water content

ranging from 0.13 m^3/m^3 to 0.18 m^3/m^3.Environment Wenchi 2017 (W17 Drgt- drought experiment and W17 Irr- Irrigated) had the highest levels of soil moisture and precipitation.

Soil pH values for all locations ranged from 5.21 to 6.22 indicating soils were slightly acidic and texture was loamy sand. Appendix 1 and appendix 2 represent soil information for pH, soil organic carbon %, Percentage Total Nitrogen, percentage organic matter, calcium, potassium, sodium, effective cation exchange (ECEC), Total exchangeable bases (TEB) , base saturation and soil texture for enironments studied.

Table 3.1: Weather condition and mean soil moisture content in environments studied

Environments	**N17**		**N18**		**W17**		**N16**		**F16**	
Weather Parameter	**MIN**	**MAX**	**MIN**	**MAX**	**MIN**	**MAX**	**MIN**	**MAX**	**MIN**	**MAX**
Precipitation (mm)	0	13.2	0	56.6	0	262.4	0	44.4	0	41.2
R.Humidity (%)	8	96	11	93	15	96	50	10	90	99
Temp °C	18.1	40.8	17.2	37.8	10.1	38.2	20	34.2	18.4	37.4
Solar Radiation (W/m^2)			0	1038.2	0	1045.53	0	1052.9	0	971
soil moisture (m^3/m^3 VWC)										
Irrigation	0.20		0.21		0.27					
Drought	0.12		0.16		0.24					

*environments : F16, N16, N17 , W17 and N18

3.2.2. Plant materials

Germplasm used was the BxT mapping population comprised of 317 genotypes and two parents, Beauregard (female parent) and Tanzania (male parent). Beauregard is a leading orange flesh variety from the United States while Tanzania is a cream fleshed African landrace, released in Ghana as Sauti in 1998. These materials were obtained from International Potato Center (CIP), Lima, Peru.Varying number of genotypes were used for each of the experiments conducted as only genotypes which had enough plant materials after multiplication were selected. For the first experiment, 246 genotypes were used, the second experiment was carried out using 247 genotypes

while the third, fourth, fifth and sixth experiments were carried out using 270 genotypes per experiment. The seventh and eighth experiments were carried out using 260 genotypes. Excluding the first experiment, genotypes used for all other experiments included both parents, Beauregard and Tanzania.

3.2.3 Experimental design

Vines measuring 30 cm were planted on ridges, ensuring that at least 3 nodes were buried. Plants were spaced 0.3 m apart and rows were spaced 1m apart. For the first and second experiments (rainfed), vines were planted in plots comprising two rows with a total of 20 plants and three replications. Experiments in environment Nyankpala 2017(N17 Irr and N17 Drgt) and Wenchi 2017 (W17 Irr and W17 Drgt) were carried out using 45 x 6 (45 blocks, 6 genotypes per block) alpha lattice design with two replications and two rows per plot. Each row had 8 plants spaced at 0.3m and inter row distance of 1m. A 65 x 4 (65 blocks, 4 genotypes per block) alpha lattice design was used for experiments in Nyankpala 2018 (N18 Irr and N18 Drgt). A 19 x 13 (19 blocks, 13 genotypes per block) alpha lattice was used for the N16 environment while a 41 x 6 (41 blocks, 6 genotypes per block) alpha lattice was used for the F16 experiment. Both N16 and F16 were replicated three times.Two hundred and five (205) genotypes common to all environments were used for the combined statistical analysis.

3.2.4 Watering regimes

- **Water-stress treatments**

With the aid of drip lines installed on each row of plants, watering of both control (irrigated) and drought stress treatments commenced immediately after planting and continued till the time of drought imposition to ensure establishment and development of plants. Watering of plants in both treatments was carried out at field capacity till the time of initiation of drought, 70 days after

transplanting (DAT). This was done to simulate terminal drought at the root bulking phase of the plant development. Application of water to the drought treatment was stopped to impose drought (terminal drought) till harvest in only the drought experiments while irrigated experiments received water till harvest. Survival watering was done when plants in the drought experiments showed signs of terminal wilting. Drip laterals were laid on each row per plot at an inter row spacing of one metre. Drip holes spaced at 30 cm interval on each line had a discharge capacity of 1.3 L/hr.

Soil Moisture Conditions

Data on soil moisture was collected with using a GS3 Decagon moisture sensor and ECH$_2$O 10HS connected to a data logger (a component of the Decagon Micro-Climate kit). One sensor was installed into the soil of each replication per treatment by gently pushing the probe into the soil and its position was rotated daily within each treatment. Data was collected for both drought and irrigated treatments on the soil temperature, and volumetric moisture content (VWC), precipitation (mm), humidity (RH), temperature (°C), solar radiation (W/m^2) and average soil moisture (m^3/m^3 VWC). Average readings per month were computed for each treatment throughout the duration of the experiment for each environment. Moisture content was measured using a ProCheck tensiometer by Decagon Devices to ensure that moisture content of soil for the irrigated experiment was maintained at -0.033 Mpa while the drought experiments were maintained at ≥ -1.2 MPa (Appendix 6).

3.2.5 Data collection

Data was collected for agronomic traits as described in the Sweetpotato Trait Ontology in the Sweetpotato Base (https://sweetpotatobase.org/) for agronomic traits. Data on traits were collected

with the aid of Field Book App for android (Rife and Poland, 2014) installed on Samsung Tablets model A6-2016.

To monitor plant establishment, data on number of plants planted (NOPS) per plot and number of plants established (NOPE) per plot were collected.

Harvest data collected for Yield and yield components included:

1. NOPH = Number of plants harvested.
2. NOPR = Number of plants with storage roots.
3. NOCR = Number of commercial storage roots (weighing 100g and above) per net plot
4. NONC = Number of non-commercial storage roots (weighing less than 100g) per net plot
5. CRW = Weight of commercial storage roots (weighing 100g and above) per net plot in kg
6. NCRW= Weight of non-commercial storage roots (weighing less than 100g) per net plot in kg
7. VW = Fresh weight of vines per net plot in kg

At harvest, data on damage by *Cylas* weevil, millipede and *Alcidodes* were also collected. Derived traits like root yield (t/ha), foliage yield (t/ha), biomass (root and vine yield, t/ha) and harvest index (root yield/biomass yield) were computed from the already measured traits using the R package st4gi (Statistical tools for genetic improvement R package).

3.2.6 Data analysis

Analysis was done using the Lme4 and predictmeans packages of Rstudio version R-3.3.1 for single site analysis experiments conducted under drought and irrigation (control) conditions and Best Linear Unbiased Prediction means per trait was estimated.

$$Y_{ijkl} = \mu + G_i + B_j + R_k + I_l + e_{ijkl}$$

where Y_{ijkl} is the phenotypic response, μ is the mean value, G_i is the genotype, B_j is the effect of block, R_k is the effect of replication, I_l is the $_l^{th}$ effect of interaction between block and replication and e_{ijkl} is the random residual. The random and fixed effect of all the factors were estimated.

For the combined analysis, two hundred and five (205) genotypes common to all 8 environments were selected and analyzed for root yield (RY), foliage yield (FY), biomass(biom) and harvest index (HI).

$$Y_{ijk} = \mu + G_i + E_j + + I_k + e_{ijk}$$

where Y_{ijk} is the phenotypic response, μ is the mean value, G_i is the genotype, E_j *is* environment defined by location and treatment, I_k is the k^{th} effect of interaction between genotype and environment *eijk* is the random residual. The random and fixed effect of all the factors were estimated.

Broad sense heritability (H^2) for single trials was estimated according to Phuke *et al.* (2017).

$$H^2 = \sigma^2 g/(\sigma^2 g + \frac{\sigma^2 \mathrm{e}}{r})$$

Where $\sigma^2 g$ is the genotypic variance and σ^2e is the environmental variance

Broad-sense heritability (H^2) estimates for combined analysis per trait was calculated from variance components according to International Rice Research Institute (2006):

$$H^2 = \sigma^2 g/(\sigma^2 g + \frac{\sigma^2 \mathrm{ge}}{e} + \frac{\sigma^2 \mathrm{e}}{re})$$

Where *e* and *r* are the numbers of environments and replications per environment, respectively. The genetic coefficient of variation was computed using the formula (Ogunniyan and Olakojo, 2014).

$CV_g = (\sigma^2 g)^{½}/\bar{x}$

Where $\sigma^2 g$ is the genetic variance and $\bar{x}$ is the sample mean.

Relative yield reduction (RYR) in drought versus irrigated treatment was estimated:

$RYR = \left[1 - \frac{\bar{x}\ drought}{\bar{x}\ irrigation}\right] * 100$ (Venuprasad *et al.*, 2007)

Where $\bar{x}\ drought$ and $\bar{x}\ irrigation$ refer to mean yield (RY) under drought and irrigated conditions

Selection differential:

$S = \bar{x}\ (selected\ genotypes) - \bar{x}\ (population)$

Where $= \bar{x}\ (selected\ genotypes)$ refers to the mean of the selected genotypes and $\bar{x}\ (population)$, the mean of the population (Leiser *et al.*, 2012).

Drought tolerance indices were computed for each genotype considering the relationships between total root yield in irrigated (Y_p) and drought stress (Y_s) environments. Drought Indices were calculated using the BLUP means per genotype under drought and irrigation conditions by applying the following formulae:

$Drought\ intensity\ index(DII) = 1 - \bar{Y}_s / \bar{Y}_p$ *Fisher and Maurer (1978)*

$Drought\ Susceptibility\ Index(DSI) = \frac{\left[1-\left(\frac{\bar{Y}_s}{\bar{Y}_p}\right)\right]}{DII}$, *Fisher and Maurer (1978)*

Percent reduction (PR) $= \left[\frac{\bar{Y}_p - \bar{Y}_s}{\bar{Y}_p}\right] * 100$

Geometric mean productivity (GMP)$= \sqrt{\bar{Y}_p * \bar{Y}_s}$ *(Fernandez, 1992)*

Drought Tolerance Index (DTI)= ($\bar{Y}_p$) ($\bar{Y}_s$)/sqrt ($\bar{Y}_p$)* (Fernandez, 1992)

Mean Productivity (MP)= ($\bar{Y}_p$ + $\bar{Y}_s$)/2, (Rosielle and Hamblin,1981)

Susceptibility (SUS)= ($\bar{Y}_p$ - $\bar{Y}_s$) (Hossain *et al.*, 1990)

Yield stability index (YSI)= $\bar{Y}_s$/ $\bar{Y}_p$ (Bouslama and Schapaugh, 1984)

Pearson's correlation coefficients between the BLUP means and traits and the drought tolerance indices were calculated using the R package "ggcorrplot". Principal component analysis (PCA) was performed to identify relationship between variables and patterns between the genotypes using R package factoextra (Kassambara and Mundt, 2016).

3.3 Results

3.3.1 Mean performance of genotypes for single environments

Table 3.2 represents means, coefficient of genetic variation, heritability estimates and relative yield reduction rates for root yield, foliage yield, biomass and harvest index for single environments. Under all single site trials conducted, reduction in root yield (RY), foliage yield (FY), biomass (biom) and harvest index (hi) (Table 3.2) was observed under drought conditions, except at Wenchi where biomass and foliage yield in the non-irrigated trial exceeded that in irrigated. From the drought experiments in each environment (N17, W17 and N18), root yield (RY) ranged from 0.93 t/ha in environment N17 to 2.98 t/ha in environment N18. Foliage yield varied from 1.61 t/ha in environment N17 to 11.07 t/ha in environment W17. Plants had a biomass between 2.51 t/ha in environment N17 and 11.76 t/ha in environment W17 while Harvest index ranged from 11.85 in environment W17 to 60.05 in environment N18.

Under irrigation, root yield (RY) ranged from 2.11 t/ha (W17) to 7.86 t/ha (N18). Foliage yield varied from 4.10 (N18) t/ha to 9.70 t/ha (W17). Plants had biomass ranging from 8.83 t/ha (N17) to 11.94 t/ha (N18). Harvest index ranged 14.57 (W17) to 62.26 (N18) under irrigation.

Relative Yield Reduction (RYR) ranged from 10.5% (W17)to 74.28% (N17) in root yield (RY), foliage ranged from -14.09% (W17) to 69.34% (N17). Relative Yield Reduction for biomass ranged from -9.91% (W17) to 71.62 % (N17) and 3.54% (N18) to 18.66% (W17) in Harvest Index. The highest relative yield reduction for root yield was 74.28% in environment N17, the least was 10.50% in environment W17. Foliage had the highest reduction rate (69.34%)under environment N17 and the lowest reduction rate (-14.09 %) in environment W17. Biomass had the highest reduction rate ((71.69%) in environment N17 and the least reduction rate in environment W17 (-

9.91%). The highest reduction rate in harvest index (18.66%) was found in wenchi and the least (3.54%) was found in N18.

Under rainfed conditions, average root yield was 27.19 t/ha while mean foliage yield was 18.80 t/ha. Mean biomass was 45.80 t/ha and Harvest Index had a mean of 54.88.

Table 3.2 Means, coefficient of genetic variance and heritability estimates for root yield, foliage yield, biomass and harvest index for single environment

Trait	Environment Treatment	N17 irr	N17 drgt	W17 irr	W17 drgt	N18 irr	N18 drgt	N16 rf	F16 rf	$\bar{x}$ irr	$\bar{x}$ dgrt	$\bar{x}$ rf
RY	$\bar{x}$	3.6	0.93	2.11	1.89	7.86	2.98	46.02	8.36	4.52	1.93	27.19
	CVg	60.58	71.18	97.62	98.29	42.58	21.96	41.00	60.00	66.93	63.81	50.00
	H^2	0.57	0.44	0.46	0.52	0.48	0.38	0.39	0.42	0.51	0.45	0.41
	RYR	74.28		10.5		62.06						
FY	$\bar{x}$	5.25	1.61	9.7	11.07	4.10	1.65	7.34	30.25	6.35	4.78	18.80
	CVg	34.88	45.24	43.91	50.59	25.2	27.24	57.96	16.59	34.66	41.03	25.00
	H^2	0.43	0.45	0.35	0.48	0.25	0.25	0.21	0.08	0.34	0.39	0.29
	RYR	69.34		-14.09		59.89						
Biom	$\bar{x}$	8.83	2.51	11.76	12.92	11.94	4.59	76.01	15.58	10.84	6.67	45.80
	CVg	39.99	48.43	48.74	53.94	33.42	32.83	40.51	28.76	40.71	45.07	35.00
	H^2	0.52	0.47	0.41	0.52	0.43	0.35	0.3	0.41	0.45	0.45	0.38
	RYR	71.62		-9.91		61.54						
HI	$\bar{x}$	36.62	31.64	14.57	11.85	62.26	60.05	49.98	59.78	37.82	34.51	54.88
	CVg	38.37	46.39	75.76	26.2	21.16	27.07	26.15	13.08	45.1	33.22	20.00
	H^2	0.6	0.49	0.48	0.44	0.59	0.6	0.29	0.2	0.56	0.51	0.25
	RYR	13.59		18.66		3.54						

*RY- root yield (t/ha), FY – foliage yield (t/ha), biom – biomass (t/ha) , HI- harvest index, $\bar{x}$ – mean
Irr- irrigated environments, drgt- drought environments, N17, W17, N18, N16 and F16 environments used

3.3.2 Broad sense heritability and coefficient of genetic variation for single environments

Mean broad sense heritability for single sites estimates for root yield were higher under irrigation than under drought and rainfed conditions (Table 3.2). Broad sense heritability estimates for root yield ranged from 0.38 in environment N18 to 0.52 in environment W17. Coefficient of genetic variation for root yield under drought conditions ranged from 21.96 % to 98.29% with a mean of 63.81%. For foliage yield (Table 3.2), broad sense heritability for single site experiments ranged from 0.25 to 0.45 with a mean of 39%. The coefficient of genetic variation for foliage yield ranged from 27.24 % to 50.59 % with a mean of 41.02% .Mean coefficient of genetic variation estimated for biomass under drought was between 32.83% and 53.94% with a mean of 45.07%. Broad sense heritability ranged from 0.35 in environment N18 to 0.52 in environment W17. Estimated broad sense heritability (single sites) values for harvest index was between 0.44 and 0.60 with a mean of 0.51 for drought environments. CVg values for harvest index ranged from 26.20 % and 46.39% having a mean of 33.22 % .

Mean broad sense heritability for single sites estimates for root yield were higher under irrigation than under drought and rainfed conditions (Table 3.2). Coefficient of genetic variation for root yield under irrigation was between 42.58% to 97.62% with a mean of 64.85%. For foliage yield (Table 3.2), broad sense heritability for single sites in the irrigated experiments ranged from 0.25 to 0.43 with a mean of 0.34. The coefficient of genetic variation ($CVg_{)}$ for foliage yield under irrigation was between 25.20 % to 43.91% with a mean of 34.66%. Broad sense heritability (single sites) for biomass under irrigation ranged from 0.41 to 0.52, with a mean 0.4. Mean coefficient of genetic variation estimated for biomass under irrigation was 40.71% with CVg varying from 33.42 % to 48.43%. Estimated broad sense heritability (single sites) values for harvest index under irrigated conditions ranged from 0.48 and 0.60. The coefficient of genetic variation had a mean of

45.10% under irrigation. CVg values for harvest index under irrigation was between 27.07% and 75.76%.

For root yield under rainfed conditions, mean coefficient of genetic variation was 50% while broad sense heritability 41%. Foliage yield had a mean CVg of 25% and Broad sense heritability of 29%. Biomass recorded a mean CVg of 35% and broad sense heritability of 38%. Harvest Index had a CVg of 20% while Broad sense heritability was 25%.

Table 3.3 Estimates of variance components, means(μ), broad sense heritability (h^2), coefficient of genetic variation (CVg) and correlations for combined analysis within treatments (drought and irrigation) for root yield(RY), foliage yield(FY), biomass(biom) and harvest index(HI).

Random	**RY**		**FY**		**biom**		**HI**	
term	**irrigation**	**drought**	**irrigation**	**drought**	**irrigation**	**drought**	**irrigation**	**drought**
G	1.66***	0.24**	1.72***	0.97*	5.84***	1.34+	56.90***	53.60***
G X E	1.44***	1.52***	4.58***	6.28**	0.365***	10.78***	1.67E-07ns	9.77ns
E	27.51***	4.44***	50.40***	44.49***	128.31***	67.49***	1838.788***	1620.94***
Mean	4.68	1.96	6.61	4.87	11.26	6.8	38.3	35.24
H^2	0.47	0.23	0.36	0.19	0.52	0.17	0.66	0.49
CVg	27.53	25.10	19.83	20.20	21.45	17.00	19.70	20.78
RYR%	58.17		26.38		39.64		8.00	
r_p irr vs drgt	0.68		0.29		0.61		0.72	

*,***significant at the 0.05 , 0.001probability level respectively, ns- not significant; G- Genotype, G X E- genotype x environment interaction

Table 3.4 Variance components, Best Linear Unbiased Predictor means (μ), broad sense heritability (H^2), coefficient of genetic variation (CVg) for combined analysis across treatments (drought and irrigation) for traits

Parameter	RY	FY	Biom	HI
G	0.89***	1.36***	3.52***	52.33***
G X E	1.68	5.71***	9.71***	117.67***
G x T x E	3.48ns	6.19***	11.97***	27.31ns
Mean(μ)	3.31	5.73	5.63	36.68
CVg	28.58	20.36	33.34	19.72
H^2	0.49	0.34	0.41	0.51

*,***significant at the 0.05 , 0.001probability level respectively; G- Genotype, G X E- genotype x environment interaction; G X T X E- Genotype x Treatment x Environment interaction

Table 3.5 Combined analysis of traits of traits across drought, irrigation and rainfall (8 environments)

Parameter	RY	FY	biom	HI
G	5.53 ***	4.13 ***	14.69 ***	76.21***
GxE	19.10***	10.17***	3.53**	71.99***
Mean	9.87	9.13	18.92	41.68
H^2	0.35	0.49	0.51	0.67
CVg	23.83	22.25	20.26	20.94

*,***significant at the 0.05 , 0.001probability level respectively
G- Genotype, G X E- genotype x environment interaction, G X T X E- Genotype x Treatment x Environment interaction

3.3.3 Comparism of Performance between Treatments and Mean of Traits for Combined Analysis within treatments and across treatments.

For combined analysis within treatments (drought and irrigation experiments, Table 3.3), all traits had a lower mean under drought compared to irrigation. Root yield under drought had a mean of 1.96t/ha, while mean root yield under irrigation was 4.68t/ha, accounting for a yield reduction of 58.17%. Foliage yield under irrigation had a relative yield reduction of 26.38% with a mean foliage yield of 6.61 t/ha under irrigation and 4.87 t/ha under drought. Notable reduction in biomass yield (39.64%) was observed, with a mean of 11.3 t/ha under irrigation and 6.80 t/ha under drought. Slight differences in the mean Harvest Index values under irrigation (38.30) and drought (35.24) resulted in a relatively low reduction in harvest index (8%). Moderately positive correlation(r) under drought and irrigation conditions was was observed for traits RY, biom and hi (0.68, 0.61 and 0.72) respectively. In contrast, low positive correlation (0.29) was observed between drought and irrigation conditions for FY.

Across treatments (drought and irrigation) (Table 3.4) among the traits; root yield (RY),foliage yield (FY), biom and HI, differences in mean values was larger for HI (19.14) and biom (5.63).

3.3.4 Broad sense heritability and genetic variation for Combined Analysis within treatments and across treatments.

The findings indicate lower genetic variation for each trait under drought environments compared to the irrigated environments (Table 3.3). The genetic variance estimates for root yield, foliage yield, biomass, and HI in the irrigated experiments were about 7, 2, 4, and 1 time higher, respectively, than those obtained in the drought experiments.

Under drought conditions, the lowest coefficients of genetic variation (CVg) was observed for biomass and the highest CVg was observed in RY (25.10%). Greater estimates of coefficient of genetic variation ranging from 19.70% to 27.53% were observed under irrigated conditions than drought conditions which was between 17.00% and 25.10%. Coefficient of genetic variation (CVg) for root yield and biomass under irrigation were slightly higher than CVg for drought while CVg for foliage yield and harvest index were higher under drought than irrigation. Root yield had a slightly higher coefficient of genetic variation under irrigation compared to drought. Coefficient of genetic variation CVg for biomass was also lower under drought (17.0 %) than irrigation (21.5%). The coefficient of genetic variation for foliage yield was higher under drought (20.2%) than irrigation (19.8%). Harvest index had a relatively lower CVg under irrigation (19.7%) than drought (20.8%). Although broad sense heritability was higher under irrigation for all traits compared to drought, heritablitity estimates were low to moderate under drought and medium to high under irrigation .Heritability ranged from 0.17 (biomass) to 0.49 (harvest index) under drought and from 0.36 (foliage yield) to 0.66 (harvest index) under irrigation.

Across treatments (Table 3.4), there was a wide range of variation (0.89) in root yield to (52.33) in harvest index . Broad sense heritability estimates for all traits were larger than 20%. Foliage yield recorded the least heritability (34%) and the highest was recorded for harvest index (51%) followed by root yield (49%). Coefficient of genetic variation for traits was least for harvest index (19.72%) and highest in Biom (33.34%) followed by root yield (28.58%).

Across all drought, irrigation and rainfall experiments, broad sense heritability was highest for the trait hi (66.8%) and lowest for root yield (34.7%) (Table 3.5). Root yield had the highest coefficient of genetic variation (23.83%) while biomass had the lowest (20.26%).

3.3.5 Variance components and Genotype x Environment Interaction

For combined analysis within treatments(Table 3.3), there was significant genetic variation for all traits under both drought and irrigated conditions. Significant GxE interaction was highest under drought than irrigation for all traits. Significant genotype by environment interaction was observed for all traits under irrigated and drought conditions. For combined analysis across treatment (Table 3.4), GxE estimate was lowest for root yield. Significant genotype by environment interaction (GxE) was observed for all traits. For experiments across drought, irrigated and rainfall environments, genotype by environment interaction was significant for all traits including root yield. Significant genetic variation as well as moderate heritability ranging from 35% in root yield to 67% in harvest index was noted. For combined analysis across drought, irrigated and rainfed environments (Table 3.5), there was a wide range of genetic variation between genotypes per trait (4.13 to 76.21 for root yield and harvest index respectively). GxE estimate was lowest for biomass and highest for harvest index. However, significant GxE interaction was observed for all traits.

3.3.6 Stability analysis of genotypes

3.3.6.1 Genotype stability under drought and irrigated environments

Based on root yield, the GGE biplot analysis for the top performing 20 genotypes and least performing five genotypes across six environments (3 drought and 3 irrigation experiments) as well as 8 environments (3 drought, 3 irrigation experiments and 2 rainfed experiments) are presented in Figures 3.2, 3.3, 3.4 and Figures 3.5, 3.6 and 3.8.

The biplot for the 6 environments revealed that the first two principal component axes accounted for 72% of the variation due to GxE on root yield (Figure 3.2, 3.3 and 3.4). The convex-hull drawn

on genotypes from the biplot origin identified four sectors with CIP113641.308, CIP105269.135, CIP105269.99, CIP105269.90 and CIP105269.127 as the vertex genotypes (Figure 3.3). Genotypes around the vertex of a sector are considered the best performing genotype in all the environments. The genotype CIP105269.127 was within the sector containing environments N17D (Nyankpala 2017 drought), N17C (Nyankpala 2017 irrigated), W17D (Wenchi 2017 drought) and W17C (Wenchi 2017 irrigated). Thus, it can be termed the winning vertex and considered as a mega environment. N18D and N18C fell within the vertex of the sector containing CIP113641.308, forming another mega environment.

The GGEbiplot (Figure 3.2) revealed genotype CIP105269.127 (G4) as the highest yielding genotype followed by CIP113641.280 (G18), CIP113641.308 (G11) and CIP113641.244 (G10). In contrast, Genotypes CIP105269.99, CIP113641.349, CIP105269.90, CIP105269.156, CIP105269.198, CIP113641.403 and CIP105269.135 had very low yields. The most stable genotypes across environments were CIP113641.244, CIP105269.104, CIP113641.355 and CIP113641.2.

No environments fell in sectors with CIP105269.135, CIP105269.99 and CIP105269.90 suggesting that these genotypes were unresponsive and were the poorest genotypes in some or all of the environments because they were located far from the origin of the biplot. No environment was discriminatory, however, N17D was the most representative (Figure 3.4).

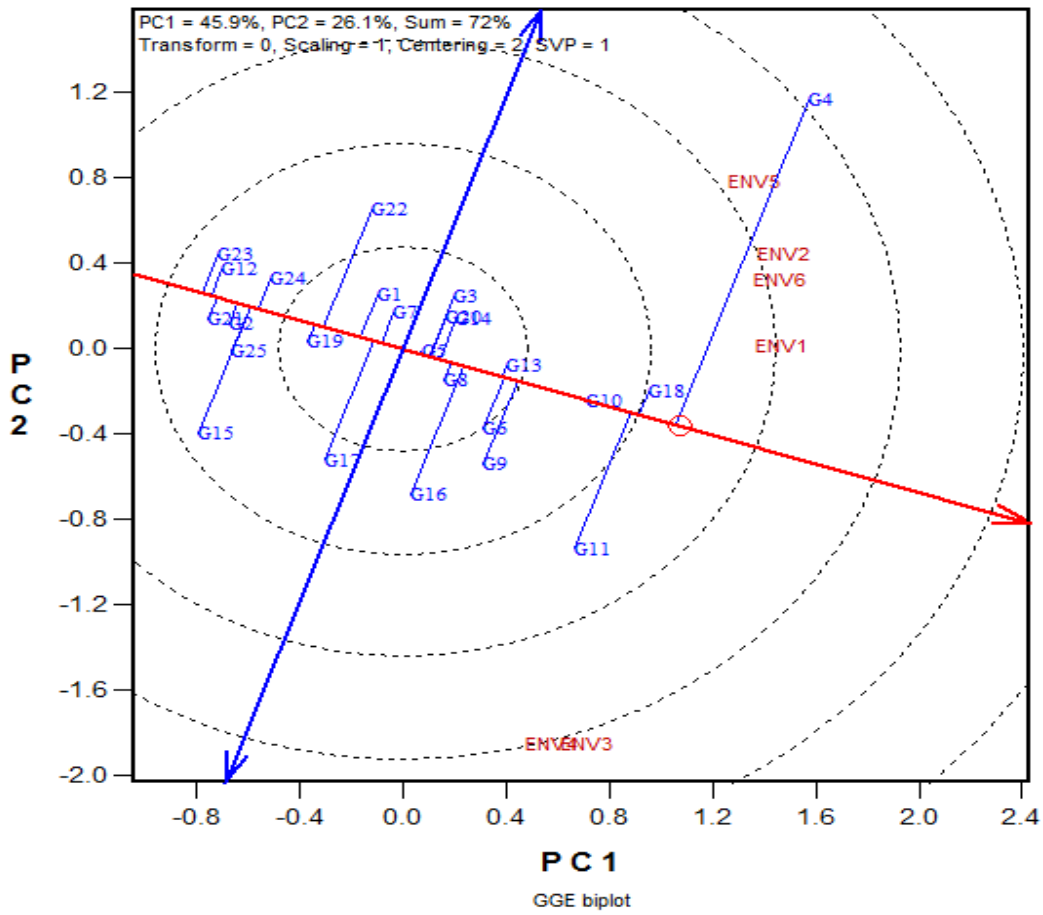


Figure 3. 2 A genotype main effect plus genotype x environment biplot of root yield for top 20 and least 5 performing selected genotypes across 6 (3 stress, 3 non-stress) environments in Ghana

Genotype and environment codes: G1- CIP113641.319, G2- CIP105269.198, G3- CIP113641.262, G4- CIP105269.127, G5- CIP105269.104, G6- CIP113641.255, G7- CIP105269.43, G8- CIP113641.2, G9- CIP113641.127, G10- CIP113641.244, G11- CIP113641.308, G12- CIP105269.90, G13- CIP113641.355, G14- CIP113641.69, G15- CIP105269.135, G16- CIP105269.115, G17- CIP113641.291, G18- CIP113641.280, G19- CIP105269.128, G20-CIP113641.242, G21-CIP105269.156, G22-CIP113641.429, G23-CIP105269.99,G24- CIP113641.349, G25-CIP113641.403
ENV1- N17D, ENV2- N17C, ENV3- N18D, ENV4- N18C, ENV5- W17D, ENV6- W17C

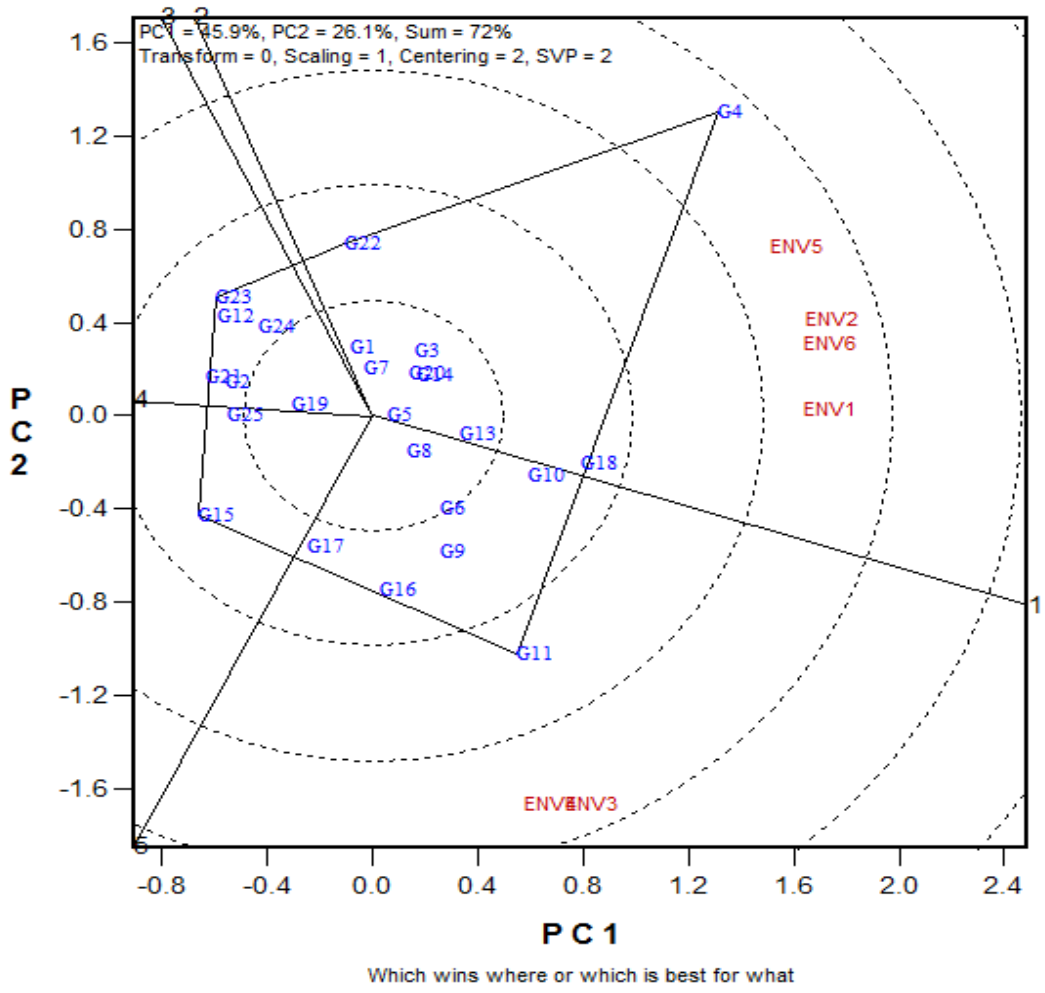


Figure 3.2 A 'which won where' GGE biplot of root yield for 25 genotypes from the BxT mapping population evaluated in 3 stress and 3 non- stress environments in Ghana

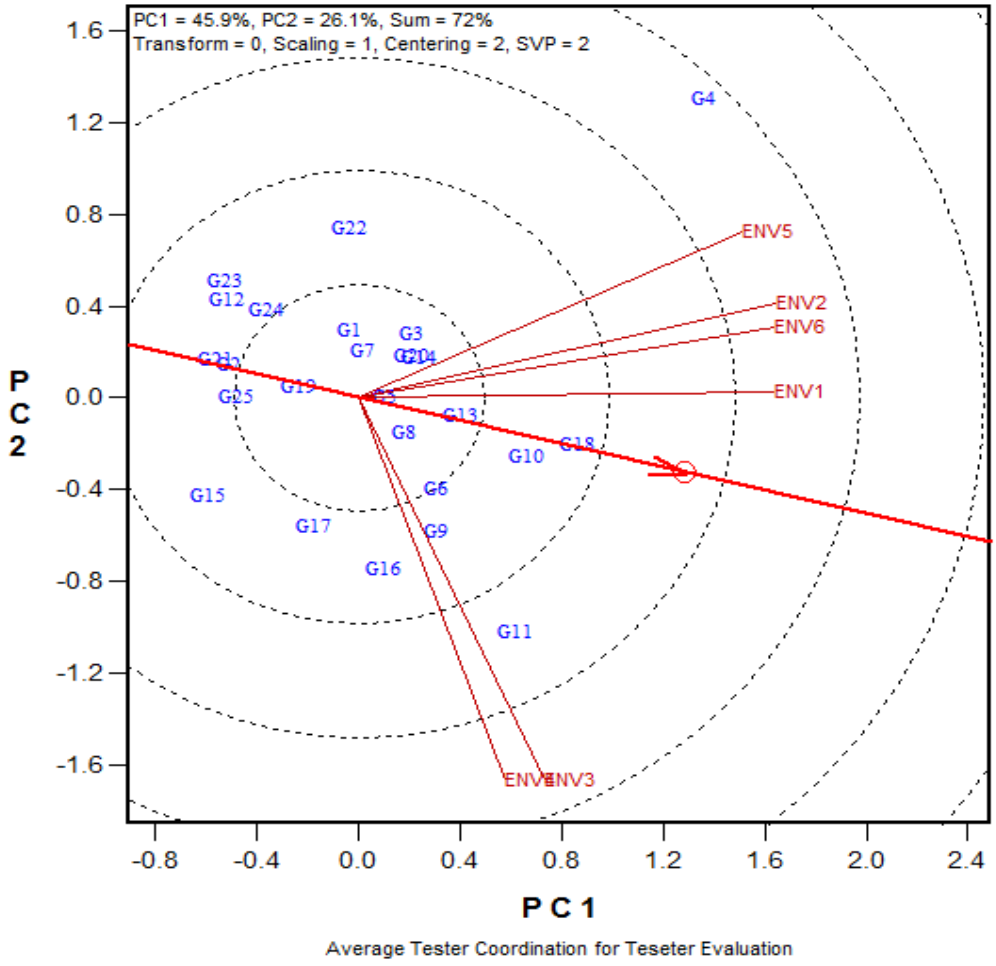


Figure 3.3 A vector view of genotype plus genotype x environment biplot showing the relationship among 3 stress and 3 non-stress test environments used for evaluating 25 genotypes from the BT mapping population in Ghana.

ENV1- N17C, ENV2-N17D, ENV3-W17C, ENV4-W17D, ENV5- N18C, ENV6- W17D

3.3.6.2 Genotype stability under drought, irrigated and rainfed environments

GGEbiplot for root yield under eight environments comprising irrigation, drought and rainfall explained 66% of the observed variation due to G X E in root yield (Figure 3.5, 3.6 and 3.7). In the GGE biplot displays (Figure 3.5) genotypes with below average means separated from those with above average means by the double-arrow line (ATC ordinate). The average yield of a

genotype is approximated by the projections of their markers on the average-tester axis while, stability of the genotypes is measured by their projection onto the average-tester coordinate y axis single-arrow line (ATC abscissa). The absolute length of the projection of a genotype determines its stability. Genotypes with shorter absolute lengths are more stable. Genotypes CIP105269.104 and CIP113641.2 were the most stable. Genotypes CIP113641.319, CIP105269.128, CIP105269.90, CIP105269.198, CIP105269.43and CIP105269.99 were stable but had low yields. Genotypes CIP113641.244, CIP113641.280, CIP113641.308 and CIP105269.127 had high yields while Genotypes CIP105269.99, CIP113641.429, CIP105269.90, CIP105269.156, CIP105269.198, CIP113641.403 and CIP105269.135 had low yields. Genotypes CIP113641.244 and CIP113641.280 were high yielding and stable while CIP113641.308 and CIP105269.127 were high yielding and less stable.

The two principal axes used to plot the biplot explained 66% of the yield variation due to GGE. The convex-hull (Figure 3.6) drawn on genotypes from the biplot origin identified five sectors with CIP113641.308, CIP105269.135, CIP105269.156, CIP105269.90, CIP113641.429 and CIP105269.127 as the vertex genotypes. High root yield variability was observed in PC1, which explained 45.4% of the total variation (Figure 3.7). It was possible to identify two principal mega-environments. The first mega environment included the environments: N18D and N18C with genotype CIP113641.308 having the highest root yield while the second mega-environment comprised N17D, N17C, F16, W17D, W17C and N16. In this mega-environment, genotype CIP105269.127 had the highest root yield.

Among all 8 environments, N18D was the most discriminatory environment while N17D was the most representative while N17C was both representative and discriminatory (Figure 3.7). The representativeness and discriminating ability of the environments is depicted in Figure 3.7. The

straight line from the origin to the coordinates where an environment falls is termed the research environment vector. The straight line with a single arrow passing through the origin and the average environment denotes the average environment axis (AEA). The length measures its discriminating power to assess genotypes under the test environments that is, the longer the vector length the more discriminating the environment. The angle between an environment and AEA measures its representativeness, therefore, the shorter the projection is from the marker of an environment, the more representative the environment. (Ifie, 2013). According to Yan *et al.*, (2010), the shorter environmental vectors indicate that the specific environments were not strongly correlated with environments having longer vectors and were probably not strongly correlated with one another. This predisposes N17C and N18D which have longer vectors as being powerful in discriminating among the genotypes. The environment N17D with small angle was the most representative of the test environments. N18D with long vector and smaller angle was most powerful in discriminating among hybrids and was as well the most representative of all environments.

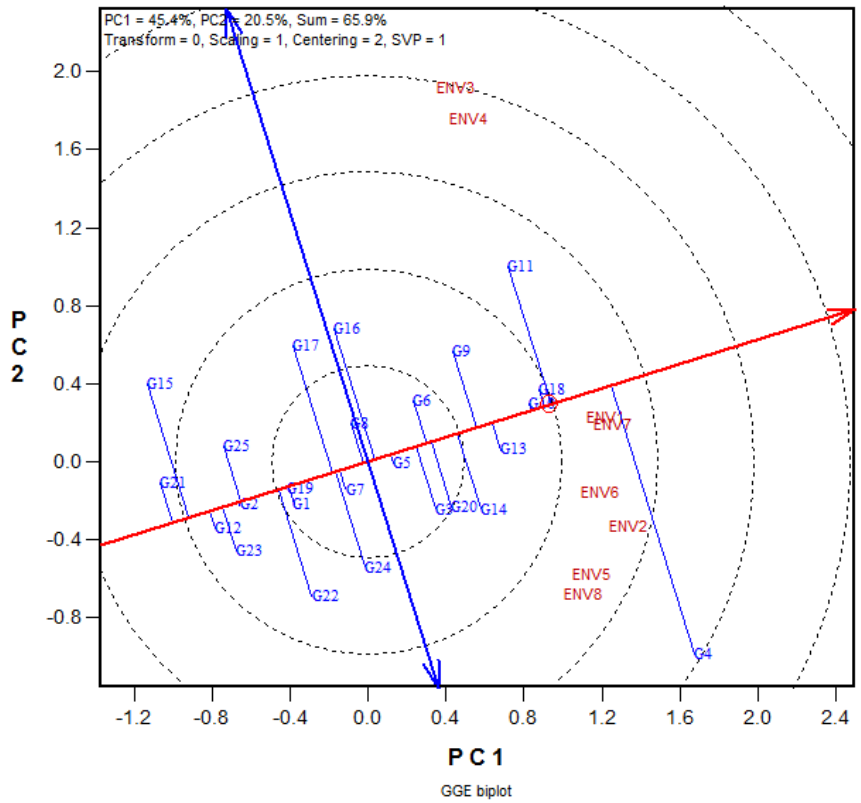


Figure 3.4 An entry/tester genotype main effect plus genotype x environment biplot of root yield of 25 selected genotypes across 8 (3 stress, 3 non-stress, 2 rainfed) environments in Ghana

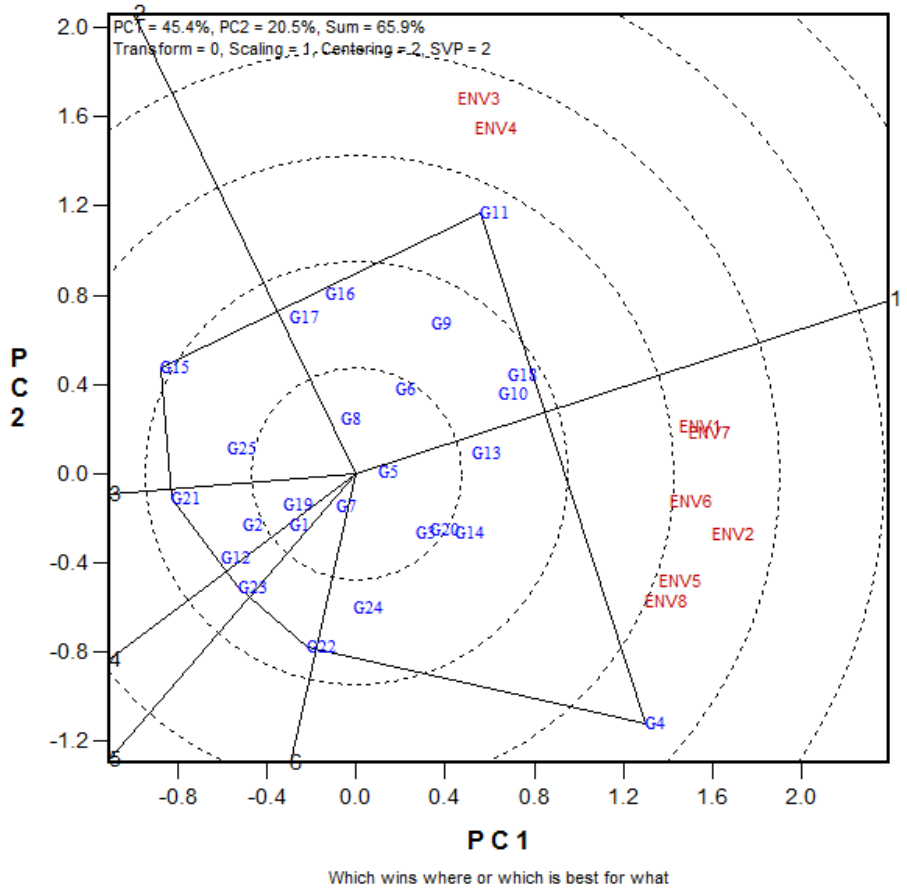


Figure 3.5 A 'which won where' GGE biplot of root yield of 25 genotypes from the BT mapping population evaluated in 3 stress and 3 non- stress and 2 rainfed environments in Ghana

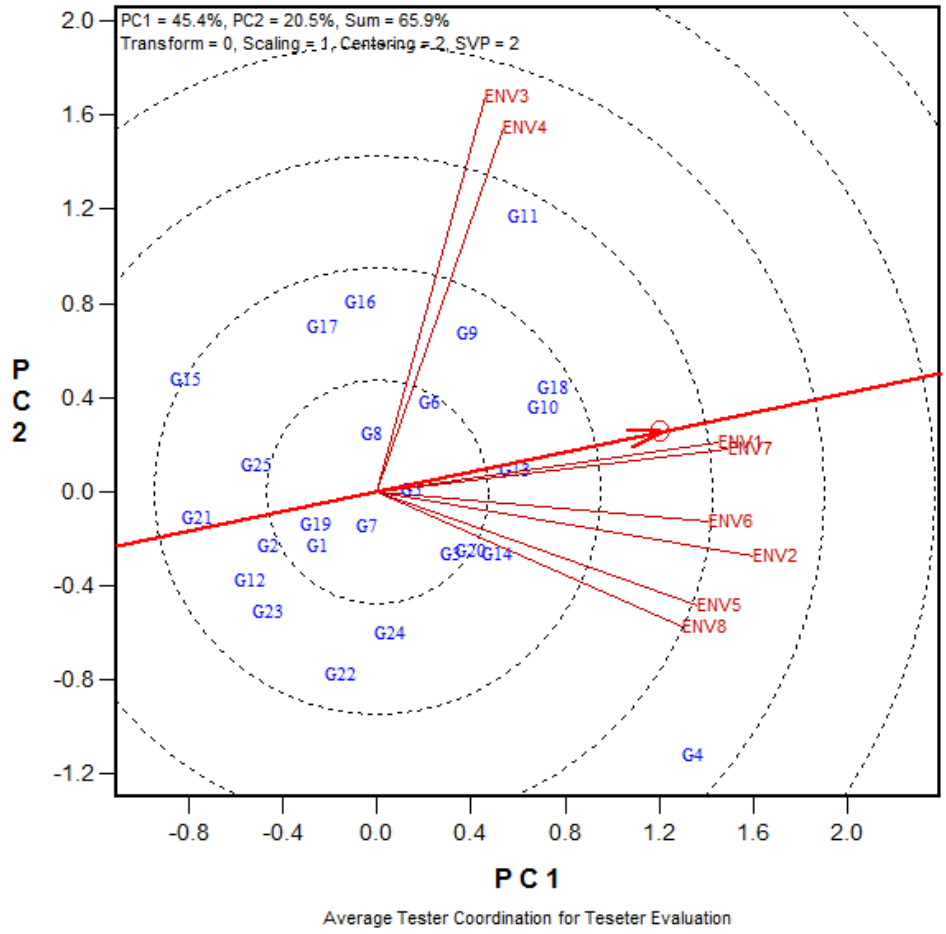


Figure 3.6 A vector view of genotype plus genotype x environment biplot showing the relationship among 3 stress ,3 non-stress and 2 rainfed test environments used for evaluating 25 genotypes from the BT mapping population in Ghana

3.3.6.3 Identifying suitable indices for selecting root yield under drought and irrigated conditions

Correlations between root yield under drought (RY_d) ,under irrigation (RY_c) and drought tolerance indices for single environments are shown in Figure 3.8 (N17), Figure 3.9 (W17), Figure 3.10 (N18) and combined enironments (Figure 3.11).

Drought tolerance indices; Geometric Mean Productivity (GMP), Mean Productivity (MP), Drought Tolerance index (DTI), Yield Stability Index (YSI), Productivity Reduction (PR),

Drought Susceptibility Index (DSI), Harmonics (HAR) and Susceptibility (SUS) were computed and correlated with root yield under drought (RY_d) and irrigated conditions (RY_c).

Correlations between drought tolerance indices and the mean BLUP values for root yield under drought (Ys) and irrigated (Yp) conditions were estimated for N17, W17 and N18 (Figure 3.8, 3.9 and 3.10). It was observed that mean BLUP root yield values under drought and irrigated conditions had a significantly high positive correlation with : Geometric Mean Productivity (GMP), Mean Productivity (MP) and Drought Tolerance index (DTI) in all locations.

In contrast, there was a negative correlation between the mean BLUP values for root yield under drought (Ys) conditions and Drought Sensitivity Index (DSI), Harmonics (HAR) and Productivity Reduction (PR) in all experiments except one, where low correlation was observed. Low correlation between root yield under drought and Drought sensitivity index (DSI) indicate that genotypes with large DSI values are likely to be more susceptile under drought conditions. Significantly low ($P>0.05$) positive correlation (0.13, 0.11 and 0.14 respectively) was noted in N17 between root yield under drought (Ys) and Drought Tolerance Efficiency (DTE), Suceptibility (SUS) and Yield Susceptibility Index (YSI). In Wenchi, low positive correlations were also found between root yield under drought (Ys) and DTE (0.24), SUS (0.10) and YSI (0.24). root yield had a positive correlation with SUS under both drought and irrigated conditions, negative correlation with DTE and YSI in N18.

Drought intensity Index (DII)for each environment: N17, W17 and N18 varied considerably. N17 had the highest intensity index (0.73) , N18 (0.62) and W17 had the lowest intensity (0.12), indicating that N17 had severe drought while W17 had a low drought intensity.

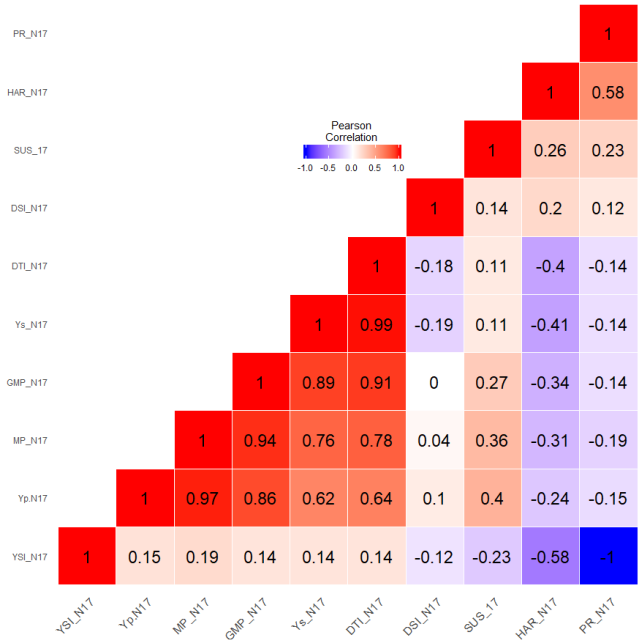


Figure 3.7 Pearson correlation between root yield under drought (Y_s) and irrigated(Y_p) conditions in Nyankpala 2017

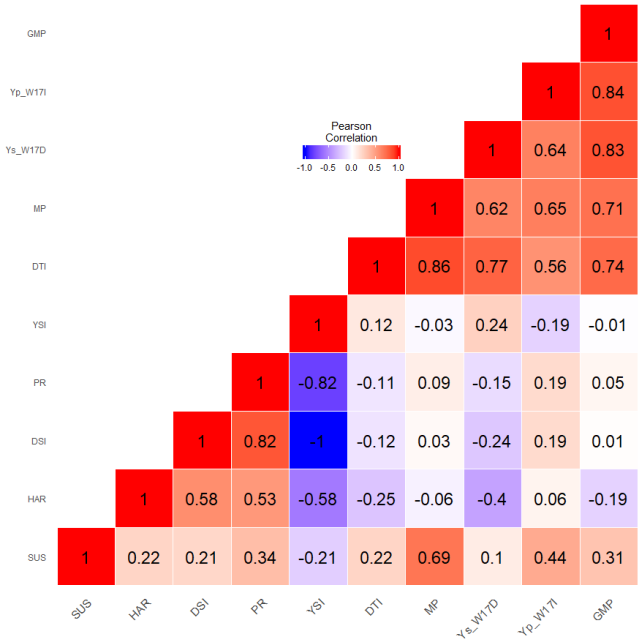


Figure 3.8 Pearson correlation between root yield under drought (Y_s) and irrigated(Y_p) conditions in Wenchi 2017

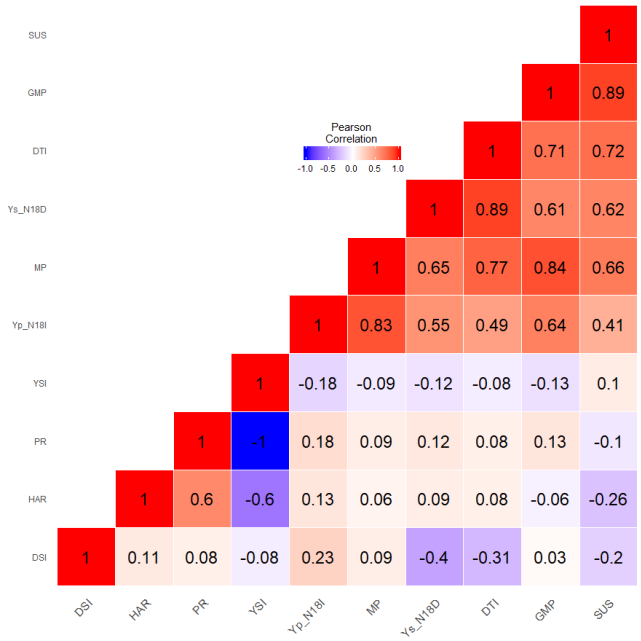


Figure 3.9 Pearson correlation between root yield under drought (Y_s) and irrigated(Y_p) conditions in Nyankpala 2018

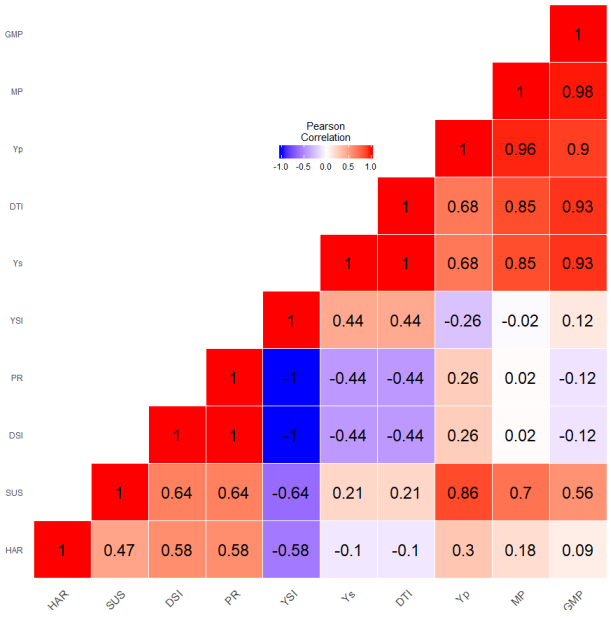


Figure 3.10 Pearson correlation between root yield under drought (Y_s) and irrigated(Y_p) conditions across six environments

Across environments, correlations among the BLUP means for root yield and DT indices were determined using 205 genotypes common to all environments(Figure 3.11). BLUP means for root yield under drought (RY_d) and root yield irrigated conditions (RY_c) across locations was estimated and used in computing drought tolerance indices.

Positive high correlations were detected between root yield and MP, GMP and DTI under both drought and irrigated conditions. Genotypes with high GMP, DTI and MP included CIP113641.280, CIP105269.127, CIP105269.186, CIP113641.308 and CIP113641.183 while DSI and PR had negative correlations with root yield under drought conditions while DTE and YSI had negative correlation with root yield under irrigated conditions. YSI values ranged from 0.09 -1.19; a higher rate indicates greater stability. Genotypes that showed higher indices include CIP105269.166, CIP113641.282, CIP113641.316, CIP105269.43 and CIP105269.38.

Positive correlation between root yield under drought and SUS (0.21) as well as YSI (0.44) were low compared to MP, GMP and DTI. On the other hand, root yield under irrigated conditions had low but positive correlation with DSI (0.26) and PR (0.26). Averagely, across environments, Drought Intensity Index (DII) of 0.58 indicated severe stress.

3.3.6.4 Comparison between selection methods based on genotype mean performance and drought tolerance indices and grouping of genotypes

Table 3.6. shows means for the top 21 geontypes (10%) under irrigation ($\bar{x}$Irri), drought conditions ($\bar{x}$drg) and using drought indices, their ranks and corresponding selection differential (S) at 10% selection intensity. Figure 3.12 shows the grouping of genotypes into four categories based genotype mean for root yield.

Based on the means of genotypes (205 genotypes) common to all locations, (the 21 top (10%) genotypes under drought and irrigated conditions were selected. Estimated selection differential (S) at 10 % selection intensity was higher under drought conditions (2.34) than irrigated conditions (1.82) (Table 3.6). On the other hand, estimated selection differential using a base index (drought tolerant indices GMP, MP and DTI) was 2.30.

Table 3.6 Means for the top 21 geontypes (10%) under irrigation ($\bar{x}$Irr) ,drought conditions ($\bar{x}$drgt) and drought indices, their ranks and corresponding selection differential (S) at 10 % selection intensity

	Selection under irrigation					**Selection under drought**					**Selection using drought indices**				
Genotype	$\bar{x}$Irr	$\bar{x}$drgt	Rank Irr	Rank drgt	Genotype	$\bar{x}$drgt	$\bar{x}$Irr	Rank drgt	Rank Irr	genotype	base index	Rank	$\bar{x}$drgt	$\bar{x}$Irr
CIP113641.308	11.29***	4.29***	1	136	CIP113641.280	7.64***	8.26***	1	119	CIP113641.280	23.5	119	7.64	8.26
CIP105269.186	11.25***	4.33***	2	33	CIP105269.127	6.70***	8.93***	2	14	CIP105269.127	22.3	14	6.70	8.93
CIP113641.183	10.65***	4.36***	3	84	CIP113641.244	5.39***	8.20***	3	107	CIP105269.186	19.1	33	4.33	11.3
CIP113641.42	10.13***	2.10***	4	183	CIP105269.43	4.80***	4.89***	4	43	CIP113641.308	19	136	4.29	11.3
CIP113641.355	9.07***	3.57***	5	154	CIP105269.104	4.48***	7.30***	5	4	CIP113641.244	18.8	107	5.39	8.20
CIP105269.127	8.93***	6.70***	6	14	CIP113641.183	4.36***	10.65***	6	84	CIP113641.183	18.7	84	4.36	10.7
CIP113641.69	8.82***	3.14***	7	195	CIP105269.186	4.33***	11.25***	7	33	CIP105269.104	16.1	4	4.48	7.30
CIP113641.14	8.71***	3.60***	8	68	CIP113641.308	4.29***	11.29***	8	136	CIP113641.108	15.7	62	3.81	8.52
CIP105269.102	8.60***	2.55***	9	3	CIP113641.113	4.10***	5.65***	9	64	CIP113641.355	15.6	154	3.57	9.07
CIP113641.108	8.52***	3.81***	10	62	CIP113641.2	4.09***	6.32***	10	90	CIP113641.14	15.4	68	3.60	8.71
CIP113641.307	8.42***	3.56***	11	135	CIP113641.242	3.98***	7.44***	11	106	CIP113641.242	15.1	106	3.98	7.44
CIP105269.125	8.32***	3.28***	12	13	CIP113641.1	3.81***	6.55***	12	60	CIP113641.307	15	135	3.56	8.42
CIP113641.251	8.27***	2.87***	13	110	CIP113641.108	3.81***	8.52***	13	62	CIP113641.278	14.9	117	3.59	8.20
CIP113641.280	8.26***	7.64***	14	119	CIP113641.414	3.75***	3.95***	14	182	CIP113641.127	14.5	66	3.44	8.16
CIP113641.244	8.20***	5.39***	15	107	CIP113641.14	3.60***	8.71***	15	68	CIP105269.43	14.5	43	4.80	4.89
CIP113641.278	8.20***	3.59***	16	117	CIP113641.278	3.59***	8.20***	16	117	CIP113641.255	14.5	111	3.52	7.87
CIP105269.197	8.19***	2.58***	17	35	CIP113641.355	3.57***	9.07***	17	154	CIP113641.69	14.4	195	3.14	8.82
CIP113641.127	8.16***	3.44***	18	66	CIP113641.307	3.56***	8.42***	18	135	CIP113641.2	14.4	90	4.09	6.32
CIP105269.115	8.05***	2.55***	19	7	CIP113641.255	3.52***	7.87***	19	111	CIP105269.125	14.3	13	3.28	8.32
CIP113641.38	7.93***	2.36***	20	168	CIP113641.127	3.44***	8.16***	20	66	CIP113641.1	14	60	3.81	6.55
CIP113641.255	7.87***	3.52***	21	111	CIP113641.39	3.40***	6.26***	21	173	CIP113641.113	13.8	64	4.10	5.65
µselect	8.85	3.77			µselect	4.3	7.9			µselect			4.26	8.23
µpopulation	4.68	1.96			µpop	1.96	4.68			µpop			1.96	4.68
S	4.17	1.82			S	2.34	3.22			S			2.3	3.55

**** = significantly different from grand mean (µ-population) at P<0.001, µ-select= the mean at 10% selection intensity, S- selection differential.

Principal component analysis (PCA) was performed based on the BLUP means for root yield under drought and irrigated conditions as well as already estimated drought tolerance indices. This was then submitted to biplot analysis to obtain the relationships between the indices and sweetpotato genotypes across all environments.Figure 3.12 represents genotypes classified based on performance under drought and irrigated conditions adapted from the method of Gerloff (1977) categorizing crops based on performance under low and high nutrient available conditions. Thus, genotypes were classified into four groups: (1) Drought tolerant and high yielding (DTHY), (2) Drought susceptible and high yielding (DSHY), (3) Drought tolerant and low yielding (DTLY) and (4) Drought susceptible and low yielding (DSLY). DTHY referred to genotypes with above average yield under drought and irrigated conditions, DTLY referred to genotypes with above average yield under drought but below average yield under irrigation conditions, DSHY represented the group of genotypes which had below average yield under drought and above average yield under irrigated conditions and DSLY represented genotypes characterized by below average yield under drought and irrigated conditions. DTHY genotypes are shown in Appendix 3.1, 3.2 and 3.3, DTLY (Appendix 3.4), DSHY (Appendix 3.5 and 3.6) and DSLY genotypes are presented in Appendix 3.7.

Two principal components accounted for 99.99% percent of the observed variation . The first component explained 83.79 % of the variation while PC2 (Principal Component Analysis 2) explained 16.21% of the variation.

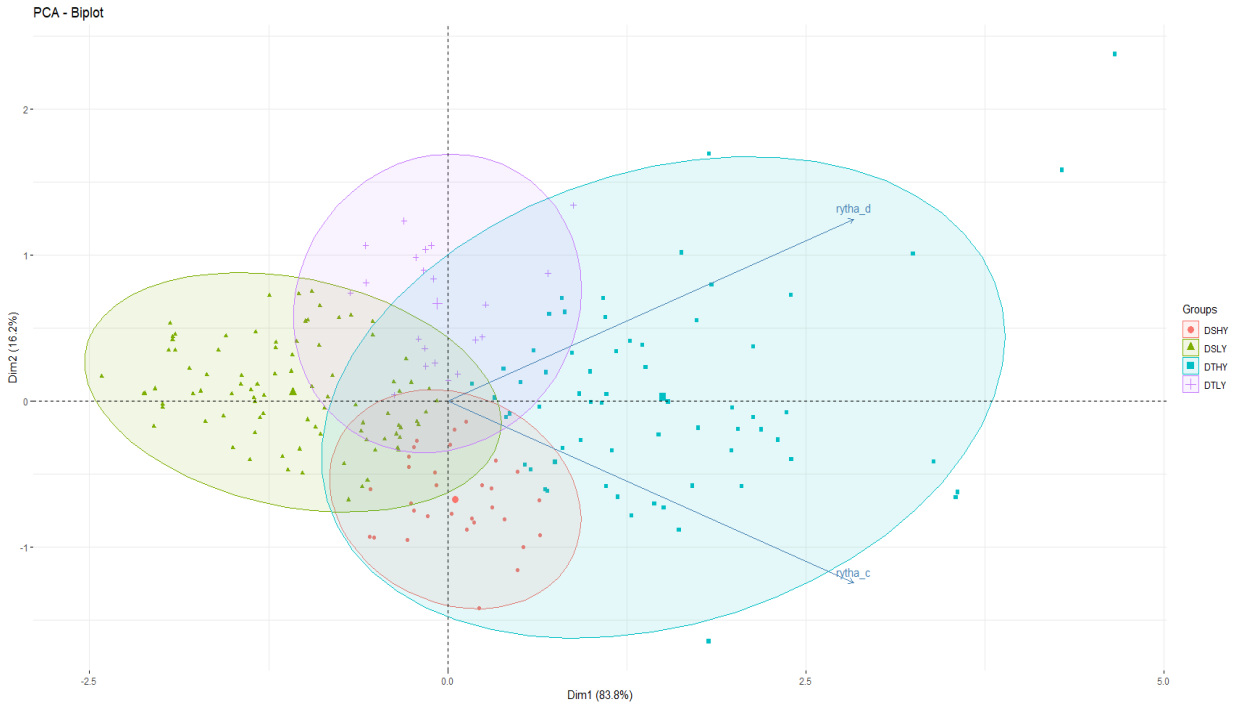


Figure 3.11 Biplot for 205 sweetpotato genotypes evaluated across environments based on principal component 1 and 2

3.4 Discussion

3.4.1 Effect of stress on yield and yield components

Drought is a global challenge to crop production because of its devastating reduction of crop yield. In this study, evaluated genotypes exhibited varying responses to stress and non-stress conditions. The ranges of yield components were greatly narrowed under drought compared to irrigated conditions. Genotypes under drought conditions had a general mean reduction in all the traits studied with root yield the most affected in terms of yield reduction. This is consistent with studies by de Oliveira *et al.* (2017) in cassava for which yield related traits suffered the greatest reduction in root yield (72.98%) and shoot yield (54.95%). Daryanto *et al.* (2016) reported that root/tuber crops generally experienced greater yield loss when droughts occur during tuber initiation (mid-season drought) and during tuber enlargement or bulking (late-season drought) than during their vegetative growth (early-season drought). Solis *et al.*(2014) reported that drought resulted in a 49% reduction in storage root yield and 43% reduction in total marketable root yield compared with the irrigated condition in the sweetpotato variety Beauregard. Andrade *et al.* (2016) reported a 25% reduction in multi-year trials for root yield under drought. A reduced storage root yield in drought environments by 35% and 26% in two different seasons was reported by Makunde *et al.* (2017). In potato, successive water stress during tuber bulking resulted in malformation of tubers including reduction in the number and size of the tubers (Monneveux *et al*., 2013). Aliche *et al.*(2018) stated that late drought reduced maximum canopy cover and tuber bulking and tuber number in potato. Storage roots across environments in this study was notably higher than yield reductions observed by Andrade *et al.* (2016), probably due to differences in drought conditions or intensity imposed on the experiments. The degree of drought imposed on the multi-year trial by

Andrade *et al.* (2016) was moderate (DII=0.25) as opposed to the severe drought (DII=0.58) imposed in this study across all drought experiments.

Besides observed reduction in root yield under drought conditions, foliage yield was also affected by drought stress though at a lesser rate. In contrast, findings by Gajanayake *et al.* (2016) maintained that at the low soil moisture deficit treatment (20% ET), shoot biomass was reduced (86%) more than root biomass (71%) compared with plants grown with irrigation to 100% ET. In cassava, Okogbenin *et al.* (2013) observed a higher reduction in shoot yield (37%) compared to root yield (22%), particularly in varieties with more vigorous vegetative growth. Many of these differences in losses caused by water stress are related to the genetic material used as well as the stress conditions imposed on the experiments (de Oliveira *et al.*, 2017). However, Kivuva (2013) reported that drought stress caused reduction in vine growth in terms of internode length, diameter and vine length. Saraswati *et al*. (2004) also observed reduction in leaf fresh weight as well as in biomass and harvest index in some cultivars of sweetpotato evaluated for drought tolerance using pot experiments in the green house. Biomass was also greatly affected by drought in my experiments. Kivuva (2013) also reported a reduction in biomass under drought stress in sweetpotato and further suggested that the observed reduction in biomass was an indication of low photosynthetic rate resulting to low vine growth and low vine biomass. Cabello *et al.* (2014) suggested that the greatly narrowed ranges of yield components under drought compared to irrigated conditions were indication of basic survival mechanisms.

The findings from this study were evidence of adequate genotypic variation in the BxT mapping population studied for traits studied under the different treatments with some genotypes performing better than others as observed from varying genotypic means for root yield and foliage yield, biomass and harvest index within treatments and across treatments.

Heritability values for root yield were comparatively lower under drought stress than irrigation for root yield across environments. In Andigena potatoes, Cabello *et al.* (2014) reported that yield heritability was drastically reduced when genotypes were subjected to drought. Cabello *et al.* (2013) also reported that estimates of heritability coefficients are from low to medium in the presence of water deficit, and medium to high in the absence of water deficit, for most agronomic traits of potato.Venuprasad *et al.* (2007) reported broad sense heritability estimates under stress conditions ranging from 0.10 to 0.43 and from 0.25 to 0.67 under the non-stress trials in rice. Mean broad sense heritability was slightly lower in stress than in non-stress trials (0.28 versus 0.42). Findings from this study revealed that root yield under drought had a moderate heritability with adequate genetic variation from which significant improvement could be achieved during selection suggesting that selection for drought tolerant genotypes could be done under drought conditions .

3.4.2 Identification of suitable drought tolerance indices, genotype grouping and genotype selection

Drought Intensity Index (DII) values for for all environments in our study agreed with storage root reduction rate for sweetpotato under drought. This corroborates similar trends reported by Makunde *et al.* (2017). In an experiment aimed at evaluating the response of 48 sweetpotato genotypes to mid-season drought in Mozambique.The magnitude of drought effects on potato production depends on the phenological timing, duration and severity of the stress. Lower Drought Intensity Index (DII) values in Wenchi as well as the lowest Relative yield reduction Rate (RYR) could be attributed to the higher levels of precipitation, humidity, and lower temperatures compared to the other experimental sites (Table 3.1). Cabello *et al.* (2013) explained that moderate

stress intensities are likely to be due to the high relative humidity of the experimental site that reduced the evaporative demand.

The correlation coefficients between the traits in the irrigated and water-deficit conditions and the drought tolerance indices can be used to determine the most suitable of them for selecting the best varieties. Generally, indices that have a high correlation between yields under stress and non-stress conditions are the best because they can separate genotypes with high yield in both conditions (Singh *et al*., 2015). This corroborates findings from the study in which Geometric Mean Productivity(GMP), Drought Tolerance Index (DTI), and Mean productivity (MP) had positive correlation with root yield under drought and irrigated environments. GMP index is often utilized in breeding programs for evaluation of genotype performance under stress and non-stressed environments while considering the variability in drought intensity across years. It is also employed in testing the performance of high yielding genotypes under stressed and optimal conditions (Fernandez, 1992). Observed high positive correlations between GMP and yield under drought and irrigated conditions are indicative of the suitability of this index to identify genotypes with high yield potential and tolerance to drought stress.

Observed positive correlations between root yield under drought and irrigated conditions with GMP, DTI and MP in all single site experiments and combined analysis from our study agrees with reports by several authors. In sweetpotato, Agili *et al.* (2012) reported that DTI, GMP and MP were strongly correlated with yield under both stressed and non-stressed conditions, signifying that these parameters are appropriate for screening drought-tolerant, high yielding genotypes in both both stressed and non-stressed conditions. Andrade *et al.* (2016), reported the same in sweetpotato, and Ali and El-Sadek (2016) in wheat and in sorghum, Menezes *et al.* (2014). This positive correlation between the BLUP means from the evaluated traits is explained by the fact

that the MP, GMP, and DTI indexes are associated with the highest value of the agronomic trait, regardless the tolerance to water stress (de Oliveira *et al.*, 2017). MP is often associated with yield under non-stressed conditions, genotypes with high yield potential have high MP values. MP may be correlated to yield under stress conditions combining high yield under stress and non-stress conditions (Cabello *et al*., 2013). Positive correlations between DTI and root yield on both stress and non-stress conditions in this study suggest that DTI was suitable for identifying genotypes with high yield in both stressed and non-stressed environments, since in general DTI is highly significantly associated with yield under both stress and non-stress conditions (de Oliveira *et al.*, 2017). The correlation coefficients between the traits in the irrigated and drought conditions and the drought tolerance indices can be used to determine the most suitable of index or indices for selecting the best varieties. Often, indices associated with a high correlation between yields under stress and non-stress conditions are the best because they can group genotypes with high yield (Singh *et al*., 2015). These indices were suitable in identification of cassava accessions with better agronomic attributes, irrespective of their drought tolerance (de Oliveira *et al.*, 2017). The correlations between the indices of DTI, GMP and MP were highly significant ($P<0.01$) in all experiments conducted, showing high similarity between these indices for genotype ranking.

On the other hand, genotypes with low DSI and SUS, and high YSI were classified as tolerant to drought stress. Conversely, Cabello *et al.* (2013) maintained that the efficiency of DSI, YSI and PR in screening genotypes was highly dependent on the nature of the test materials, thus suggesting that GMP, MP and DTI were better suited for selection of high yielding genotypes under stress and non-stress conditions.The use of PCA for the classification of genotypes based on yield and susceptibility to drought stress has been reported by Agili *et al.* (2012) in sweetpotato and de Oliveira *et al.* (2017) in cassava. It was possible to classify the sweetpotato genotypes used in the

study and identify drought tolerant genotypes in the environment studied, thus facilitating selection of drought tolerant genotypes.

3.4.3 Genotype selection approach based on yield performance

The significant correlation among the traits used in estimating the base index for selection of high yielding and drought tolerant genotypes confirms the effectiveness of the base index for identification of tolerance to drought (Ifie, 2013). Both approaches using the direct BLUP means or the base index from Drought Tolerance Indices had better response with direct selection under drought conditions than under irrigated conditions .

The question regarding what approach to take with regards breeding for drought tolerance entails varying dimensions often with the aim of selecting the approach proffering the greatest efficiency for selection of drought tolerant varieties. The moderate heritability for root yield and the higher selection differential for selection under drought suggests that direct selection under drought conditions is more effective that indirect selection under irrigated conditions. Grunerberg *et al.* (2015) opined that for sweetpotato and early breeding stages, a first step to selection should be done under stress, enabling selection of genotypes which meet set or required thresholds . A second selection could be done under non-stress conditions. Selection could also be done under both stress and non- stress conditions, being carried out concurrently using index selection methods for desired gains under conditions of stress.

3.4.4 Performance of genotypes and stability (GGE BIPLOT)

G x E was significant for root yield under drought across environments (within treatment) as well as for foliage and root yield across treatments. According to Yan and Tinker (2006) analysis of GxE data requires examination for the magnitude and nature of GxE. Checking for the presence or absence of GxE is key in determining if crossovers exist, rank changes of the genotypes in

different environments, such that different winners are picked up in different environments. The GGE biplot is a powerful statistical tool for identifying the best performing genotype in a given environment and the most suitable environment for each genotype, average yield and stability of the genotypes and the discriminating ability and representativeness of the environments (Yan *et al.,* 2000). As evidenced by indices using PCA biplots as well as groupings based on drought tolerance-indices for each environment, genotypes differed in groupings.

The PC1 and PC2 of the GGE biplot analysis explained 65.9 % of the total variation in root yield across test environments. According to Yan *et al. (*2007), an ideal genotype should have high yield and high stability. The GGE biplot identified genotype CIP113641.280 and CIP113641.244 as the most stable and high yielding.

3.5 Conclusion

Genotypes from the BxT mapping population were phenotyped under conditions of drought, irrigation as well as rainfall to examine their response to drought, identify suitable drought tolerance indices for selection, group the genotypes and select drought tolerant genotypes and f or further experiments.

Drought caused considerable reduction in root yield as well as other yield related components, however, root yield was the most affected. Drought tolerant indices, GMP, DTI and MP which correlated positively with yield under stress and non-stress conditions were suitable for the identification of high yielding and drought tolerant genotypes. Genetic variation exists within the BxT mapping population for root yield and yield related components under drought stress and irrigated conditions. Direct selection for root yield under drought conditions is expected to be a better approach for selection conventionally. Breeding for drought tolerant sweetpotato is important in Ghana as drought effects are devastating on yield and considering the benefits of

sweetpotato for health, income and nutrition especially among rural farmers and the increasing enlightening on the benefits of orange flesh sweetpotato for both rural and urban dwellers. More precise and faster methods of improvement such as genomic selection may be suitable for screening and selection of drought tolerant sweetpotato varieties in breeding programs aimed at improvement of varieties for drought tolerance.

CHAPTER FOUR

4. Genetic studies on the use of morpho-physiological parameters as secondary traits for selection under drought in sweetpotato.

4.1 Introduction

Crop productivity is influenced by diverse processes and structures which affect growth and development. Plant leaf morphology plays functional roles in radiation capture for photosynthesis and vascular systems enhance mobilization of photosynthates from source to sink (Mathan *et al.*, 2016). Drought causes changes in traits related to physiological, morphological, biochemical, and molecular processes in crops (Salehi-Lisar and Bakhshayeshan-Agdam, 2016).

To enhance crop productivity despite drought, breeding for drought tolerant varieties is pertinent. Breeding approach for drought tolerance can be done via direct selection of genotypes for yield under drought stress or indirectly (Bolaños and Edmeades, 1996) using traits which are informative about performance under specific environments (Lafitte *et al*., 2003) . However, a major concern is that genetic variance and broad sense heritability of yield declines with increasing moisture stress (Bolaños and Edmeades, 1996). In sweetpotato, selection for drought tolerance based on yield is cumbersome owning to large genotype by environment interaction and low trait heritability often associated with yield traits in drought environments (Agili *et al.*, 2012).

There exist numerous physiological, morphological, and biochemical traits which contribute towards the improvement of yield under drought-stressed environment; yet, limited number of traits have been successfully employed in breeding due to high expenses required for screening including labour (Passioura, 2007). It is important that suitable traits for drought tolerance breeding must be highly heritable, improve crop performance under drought, have high heritability, be accurate, easy to use, not laborious, inexpensive and can enhance selection under drought

conditions (Bolanos and Edmeades, 1996; Blum, 2011; Monneveux and Ribaut, 2006; Cabello *et al.*, 2014).

Through recent progress in plant physiology, development of efficient , non-invasive phenotyping tools suitable for drought tolerance breeding has been achieved. Infrared canopy temperature measures plant temperature without destruction of plant organs or tissues in response to drought (Omotobora *et al.*, 2014). It has been used in maize (Keener and Kircher, 1983), in potato (Stark *et al.*, 1991). Photosynthetically active radiation, abscisic acid content, carbon isotope discrimination and stomatal conductance were used in cassava (Adjabeng, 2014) and Normalized Difference Vegetation Index (NDVI) in maize (Spitkó *et al.*, 2016) among others.

In sweetpotato, response to drought has been evaluated by studying changes in the plants under conditions of drought . Such parameters include; chlorophyll content (Mwije *et al.*, 2014) and canopy temperature (Omotobora *et al.*, 2014; Rukundo *et al.*, 2017). Saraswati (2004) and Laurie *et al.* (2015) studied both physiological and morphological traits. However, there is still little information available regarding genetic variability associated with traits in response to drought stress as well as their relationship with yield.

Like potato, little knowledge on the suitability of secondary traits for screening and selection of drought tolerant genotypes is available for sweetpotato compared to cereals. Available studies are based on a small number of genotypes, and little is known about the genetic variation and heritability of these traits (Monneveux *et al.*, 2013) as well as their relation to yield and yield components. The current study aimed to :

1. Assess the effect of drought on physiological and morphological parameters of sweetpotato in controlled experiments with irrigation

2. Determine the genetic variability using various instruments to measure in physiological and morphological traits under irrigation and drought conditions

3. Determine the relationship between instrumental measurements of physiological parameters, morphological and yield traits

4.2 Materials and Method

4.2.1 Location of the experiment

Four field experiments were conducted in locations Nyankpala (2017) and Nyankpala (2018) under drought and irrigated conditions as described in chapter three.

4.2.2 Plant materials

Data for morphological and physiological parameters were collected from field experiments described in chapter three of this study. Weather conditions and soil moisture content are shown in chapter three (Table 3.1). Germplasm used was the BxT mapping population comprised of three hundred and fifteen genotypes and two parents, Beauregard (female parent) and Tanzania (male parent). Beauregard is a leading orange flesh from the United States while Tanzania is a cream fleshed African landrace. Only genotypes with enough vines after field multiplication were selected for the experiments.Two hundred and seventy genotypes were used for the first and second experiments while 260 genotypes were used for the third and fourth experiments.

4.2.3 Experimental design

Experiments 1 and 2 were carried out using a 45 x 6 (45 blocks with 6 genotypes per block) alpha lattice design with two replications per experiment while a 65 x 4 (65 blocks with 4 genotypes per block) alpha lattice design was used for experiments 3 and 4 with two replications for each experiment. Each 16-plant plot had two rows with 8 plants per row. Plants were spaced 0.3 m apart and rows were spaced 1 m apart. Vines measuring 30 cm were planted on ridges, ensuring that at

least 3 nodes were buried. Two hundred and five genotypes common to all environments were used for the combined analysis.

4.2.4 Watering regimes

- **Water-stress treatment**

Watering of both well watered (irrigated) and water stressed (drought) stress treatments commenced immediately after planting and continued till the time of drought imposition at 70 days after transplanting (DAT) to ensure establishment and development of plants prior to drought imposition. Commencement of drought stress at 70 DAT was meant to simulate terminal drought at the root bulking phase of the crop according to Stathers *et al*. (2013). Watering of plants in both treatments was carried out at field capacity till the time of initiation of drought stress 70 Days After Transplanting (DAT). Application of water to the drought treatment was stopped to impose drought (terminal drought) till harvest in only the drought experiments while irrigated experiments received water until harvest. Watering was done a few times when plants in the drought experiments showed signs of wilting (leaf drooping appearance).

- **Soil Moisture Conditions**

The soil moisture content was monitored with the aid of a Procheck tensiometer by Decagon devices. Data on soil moisture (Table 3.1) was collected with aid of a GS3 Decagon moisture sensor and the ECH_2O 10HS connected to a data logger, a component of the Decagon Micro-Climate kit. Each sensor was installed into the soil in each replication per treatment by a gentle push of the probe into the soil and daily rotation of its position within each treatment. Data was collected for both drought and irrigated treatments on volumetric moisture content (VWC).

Appendix 1 depicts soil information for the various experimental environments. Soil texture was mostly sandy loam.

4.2.5 Data collection

Data was collected for morphological parameters and physiological parameters as described in a protocol (unpublished) developed at CIP-Lima by Gemenet *et al.* (2016) for standardized data collection in the Genomic Tools for Sweetpotato Project (GT4SP) drought phenotyping experiments (Appendix 6). Data was collected at two time points: 30 days before drought imposition (DBDI) and 30 days after drought imposition (DADI). Data on parameters were collected with the aid of Field Book App for android (Rife and Poland, 2014) installed on Samsung Tablets model A6-2016.

4.2.5.1 Physiological parameters:

- **Normalized difference vegetation index (NDVI)** was measured using the FieldScout CM 1000 Chlorophyll Meter. Data was collected by focusing the red-light beam from the chlorophyll meter about 80cm above the plant canopy for each of the two rows which comprised a plot. Average reading per row was recorded. The plot mean recorded was the average value of readings from both rows**.**
- **Canopy/ leaf temperature (CT):** The Raytek Raynger MX2 Infrared Thermometer was used for data collection. Measurements were made during the day between 1000 hours to 1400hours when drought stress was well expressed in the plants. The readings were taken from the lower surface of the third fully expanded leaf on two plants from each plot.
- **Chlorophyll content Index (CCI**): The CCM-200plus Chlorophyll Content Meter by Optisciences was used to measure chlorophyll content. Using three leaves from the top

fully developed leaves from each of four selected plants within a plot, the average mean plot chlorophyll content index was measured and recorded.

- **Leaf Area Index (LAI) and Photosynthetically Active Radiation (PAR):** were measured using the AccuPAR LP-80 LAI ceptometer by Decagon devices. One stationary sensor was used to take the above canopy readings to know the amount of incoming solar radiation while the second sensor was used to take the below canopy readings (PAR captured). Canopy measurements in four representative places of each plot were taken. Measurement of the canopies of each of the two rows per plot were done individually and the average reading was computed. The PAR was estimated as the difference between the readings taken above the canopy and the reading taken under the plant canopy (Danquah, 2014).

$$\%\boldsymbol{PAR} = \mathbf{100}\left(\frac{\boldsymbol{PAR\ captured}}{\boldsymbol{Total\ incoming\ radiation}}\right)$$

4.2.5.2 Morphological traits:

Traits measured included: vine diameter, vine length, and number of nodes. To measure the traits, selected vines per plot were tagged with ribbons and used for collection of data at the time points measurement was repeated. Four plants were randomly selected per plot, measurement of diameter for the main vine of each of the plants was done using a Vernier caliper at the base of the plant, 1 cm above soil level. Vine length was measured using a measuring tape spanning from the base of the plant, 1 cm above the ground to the tip., then measurements were recorded. Number of nodes on one main vine per plant was counted and recorded.

4.2.6 Data analysis

Analysis was done using the linear mixed-effect model using 'Eigen' and S4 version 1.1-18-1 (lme4) package of Rstudio version R-3.3.1 (Bates *et al.*, 2014) to estimate BLUP means per trait. Significance testing was done using the lmerTest package (Kuznetsova *et al.*, 2017). For single site analysis, experiments conducted under drought and irrigation (control) conditions, traits were analyzed separately using the alpha lattice design.

$$Y_{ijkl} = \mu + G_i + B_j + R_k + I_l + e_{ijkl}$$

where Y_{ijkl} is the phenotypic response, μ is the mean value, G_i is the genotype, B_j is the effect of block, R_k is the effect of replication, I_l is the $_l^{th}$ effect of interaction between block and replication and e_{ijkl} is the random residual. The random and fixed effect of all the factors were estimated.

For the combined analysis, two hundred and five (205) genotypes common to all 4 environments were selected and analyzed for physiological and morphological traits.

$$Y_{ijk} = \mu + G_i + E_j + +I_k + e_{ijk}$$

where Y_{ijk} is the phenotypic response, μ is the mean value, G_i is the genotype, E_j *is* environment defined by location and treatment, I_k is the k^{th} effect of interaction between genotype and environment *eijk* is the random residual. The random and fixed effect of all the factors were estimated. Broad sense heritability for single trials was estimated as follows (Phuke *et al.*, 2017):

$$H^2 = \sigma^2 g/(\sigma^2 g + \frac{\sigma^2 e}{r})$$

where $\sigma^2 g$ is the genotypic variance and $\sigma^2 e$ is the environmental variance.

Broad-sense heritability (H^2) estimates for combined analysis per trait was calculated from variance components according to International Rice Research Institute (2006):

$$H^2 = \sigma^2 g/(\sigma^2 g + \frac{\sigma^2 \text{ge}}{e} + \frac{\sigma^2 \text{e}}{re})$$

Where e and r are the numbers of environments and replications per environment, respectively.

The genetic coefficient of variation was computed using the formula (Ogunniyan and Olakojo, 2014).

$CV_g = (\sigma^2 g)^{½}/\bar{x}$

Where $\sigma^2 g$ is the genetic variance and $\bar{x}$ is the sample mean.

Reduction due to drought (RDD) in drought versus irrigated treatment was estimated by adopting method of Sallam *et al.* (2018).

RDD-trait $= \left(\frac{XI-XD}{XI}\right) * 100$ OR IDD-trait $= \left(\frac{XI-XD}{XI}\right) * 100$

where RDD is the reduction due to drought in a respective trait, XI and XD refer to the mean performance of each genotype for per trait under irrigated and drought conssditions respectively. If the mean of all genotypes for a trait under drought is larger than the mean under irrigated conditions, increase due to drought (IDD) is estimated.

Pearson's correlation coefficients between the BLUP means per genotype were calculated using the R package Corrplot (Friendly, 2002).

4.3 Results

Table 4.1 presents minimum, maximum, mean values, coefficient of genetic variation, heritability and relative reduction due to drought for canopy temperature, chlorophyll content index, and photosynthetically active radiation per environment. Minimum, maximum, mean values,

coefficient of genetic variation, heritability and relative reduction due to drought and normalized difference vegetation index per environment are presented in Table 4.2.

Table 4.3 represents minimum, maximum, mean values, coefficients of genetic variation, heritability and relative reduction due to drought for vine length, vine diameter and number of nodes per environment.

4.3.1 Trait means under irrigation and drought conditions

The BxT mapping population showed changes in physiological and morphological traits in response to drought in both single site trials (Table 4.1, Table 4.2, Table 4.3), in combined analysis for drought and irrigation (Tables 4.4 and 4.5) and across treatments (Tables 4.6 and Table 4.7) . For single site trials, relative performance per treatment (drought) showed percentage reduction in for Photosynthetically Active Radiation (%PAR), Leaf Area Index (LAI) and chlorophyll content whilst canopy temperature increased after drought imposition. Relative reduction due to drought (RDD) in traits ranged from 7 to 83 % for drought experiment relative to control measured after drought imposition. The highest relative reduction due to drought stress was observed for chlorophyll content in N18 environments while the least reduction was observed for Leaf Area Index (LAI) after drought imposition. Drought resulted in general reduction of vine length, vine diameter and number of nodes (Table 4.3). Relative reduction due to drought (Table 4.3) of vine length was 27.05 % for N17 environment and 18.38 % for N18, 10.87% for vine diameter in environment N17 and 2.53 % in environment N18 while number of nodes reduced by 23.87 % in environment N17 and 5.36 % in environment N18. Mean vine length of plants under irrigation was 64.37 cm under drought and 82.58 cm under irrigation environments. Mean vine diameter of plants under irrigated and drought conditions was 4.17 and 3.89 mm respectively. Mean number of nodes ranged from 12.76 cm under irrigated conditions to 12.13 cm under drought conditions.

For combined analysis within each treatment (Table 4.4), relative reduction under drought conditions was observed for all traits except canopy temperature which increased. The highest reduction was observed in chlorophyll content while the least reduction was in leaf area index. The effect of drought was more severe on chlorophyll content index (CCI) followed by NDVI, Photosynthetically Active Radiation and least in Leaf Area Index. Canopy temperature increased by approximately 24% . Appendix 4.1 - 4.3 represent the best 10, worse 10 genotypes, parents and their means for each physiological trait. The genotype CIP113641.259 had the highest chlorophyll content under irrigation (57.06), while under drought CIP113641.322 had the highest chlorophyll content (31.15) (Appendix 4.1). The least amount of chlorophyll was found in CIP113641.153 (21.31) under irrigation and CIP113641.369 (8.09) under drought. Drought resulted to 53% reduction in chlorophyll content for genotype CIP113641.278. CIP105269.127 had the least canopy temperature (21.45 ^{o}C) under irrigation and CIP113641.308 (27.69 ^{o}C) under drought (Appendix 4.1). The highest canopy temperature was observed in genotype CIP105269.161 (35.55 ^{o}C) and CIP113641.205 (41.33 ^{O}C) under irrigation and drought conditions respectively. Differential responses among genotypes for NDVI, Leaf Area Index and Photosynthetically Active Radiation was also observed. Genotype CIP113641.255 and CIP113641.188 had the highest normalized difference vegetation index of 82.26 and 74.30 under irrigation and drought environments respectively (Appendix 4.2). The largest Leaf Area Index under irrigation was recorded in CIP113641.108 (14.18) and CIP113641.255 (12.15) under drought (Appendix 4.2). In contrast, genotypes CIP113641.408 and CIP113641.205 recorded the least NDVI (63.19 and 44.7) under irrigation and drought conditions , respectively. The least LAI under irrigation was observed in the genotype CIP105269.168 (3.27) which was reduced by drought to 2.66, the least LAI under drought environments. It was also among the least 10 genotypes with high canopy temperature

(38.71 ^{O}C) under drought stress and the least five genotypes with low Photosynthetically Active Radiation (139.81) under irrigation (Appendix 4.3).

For combined analysis within each treatment, among the morphological traits studied (Table 4. 5) vine length had the most reduction (22.38 %) compared to vine diameter (7.97 %) and number of nodes (14.13%). Vine diameter ranged from 7.84 mm in the genotype CIP113641.379 to 2.86 mm in CIP113641.361 as opposed to drought which had a range of 2.42 mm in CIP113641.112 to 1.08 cm in CIP113641.78. Number of nodes varied from 46.67 (CIP113641.358) to 20.32 (CIP113641.299) under irrigation and from 49.41 (CIP113641.234) to 16.94 (CIP105269.198) under drought environments. However, genotype CIP113641.196 showed little reduction in vine length (14.90%) and number of nodes in response to drought (0.94%). The longest vine under irrigation measured 234.52 (CIP113641.387) as opposed to 206.41 (CIP105269.124) under drought stress while the shortest vine length under irrigation was in CIP113641.315 (42.80 cm) and CIP105269.167 (30.04 cm).

Table 4.1 Minimum, maximum values, means($\bar{x}$), coefficient of genetic variation (CVg), broad sense heritability (H^2), reduction due to drought (RDD)% for canopy temperature, chlorophyll content index and Photosynthetically Active Radiation per environment

Trait	Treatment	Environment	min	max	$\bar{x}$	CVg	H^2	RDD(%)
C_temp (^{0}C)	Drought	N17(1)	30.08	37.61	32.84	1.96	19.00	9.68
		N17(2)	18.13	45.17	36.36	2.37	11.00	
	Irrigation	N17(2)	28.64	37.41	33.19	1.43	15.00	8.72
	Drought	N18(1)	24.57	32.58	28.00	1.52	12.00	25.97
		N18(2)	34.67	40.16	37.82	0.51	3.00	
	Irrigation	N18(2)	26.51	39.46	33.76	1.81	8.00	10.74
CCI	Drought	N17(1)	6.90	45.46	22.84	10.93	8.00	9.63
		N17(2)	3.44	50.06	20.64	4.76	1.00	
	Irrigation	N17(2)	1.00	42.44	24.32	2.62	0.00	15.13
	Drought	N18(1)	15.25	46.97	29.37	0.00	0.00	87.33
		N18(2)	4.10	21.13	3.72	47.29	14.00	
	Irrigation	N18(2)	8.52	40.01	23.1	14.62	24.00	83.90
%PAR	Drought	N17(1)	6.20	56.52	31.75	12.24	10.00	3.50
		N17(2)	6.12	55.45	30.64	10.32	8.00	
	Irrigation	N17(2)	11.54	67.67	39.13	4.81	2.00	21.70
	Drought	N18(1)	14.96	67.76	41	8.26	9.00	11.77
		N18(2)	13.98	63.99	36.17	10.82	14.00	
	Irrigation	N18(2)	16.97	66.71	43.84	6.42	8.00	17.49

* C_temp (^{0}C)= canopy temperature, CCI – chlorophyll content index, %PAR- photosynthetically active radiation, Environments- N17- Nyapkala 2017, N18-Nyankpala 2018, (1)- trait measured before drought imposition, (2)- trait measured 30 days after drought imposition

Table 4.2 Minimum, maximum values, means, coefficient of genetic variation(CVg), broad sense heritability (H^2), reduction due to drought (RDD) for Leaf Area Index and normalized difference vegetation index per environment

Trait	Treatment	Environment	min	max	$\bar{x}$	CVg	H^2	RDD(%)
LAI	Drought	N17(1)	1.73	17.37	7.42	16.99	19.00	2.20
		N17(2)	1.19	16.09	7.25	15.13	9.00	
	Irrigation	N17(2)	1.59	19.93	9.27	5.38E-06	1.49E-12	21.77
	Drought	N18(1)	1.56	11.54	5.54	10.84	8.00	10.54
		N18(2)	1.64	10.24	4.95	12.36	9.00	
	Irrigation	N18(2)	2.14	10.55	5.85	8.62	6.00	15.33
NDVI	Drought	N17(1)	32.74	75.13	65.51	2.86	11.00	11.17
		N17(2)	21.5	88.00	58.19	5.11	4.00	
	Irrigation	N17(2)	55.26	85.11	76.61	0.00	0.00	24.04
	Drought	N18(1)	44.65	83.98	77.23	2.10	11.00	15.18
		N18(2)	32.74	75.12	65.51	2.81	11.00	
	Irrigation	N18(2)	58.94	82.41	70.98	3.78	27.00	7.71

* LAI- leaf area index, NDVI- normalized difference vegetation index. Environments- N17- Nyapkala 2017, N18-Nyankpala 2018, (1) - trait measured before drought imposition, (2)- trait measured 30 days after drought imposition

Table 4.3 Minimum, maximum values, means, coefficient of genetic variation, heritability, reduction due to drought per trait and environment

Trait	Environment	Treatment	min	Max	mean	CVg	H^2	RDD(%)
Vine length(cm)	N17	Drought	17.13	168.76	51.13	42.73	64.66	27.05
	N17	irrigation	17.13	218.07	70.09	39.38	62.03	
	N18	Drought	25.5	265.95	77.6	45	63.03	18.38
	N18	Irrigation	38.68	283.67	95.07	39.73	70.25	
Mean Drought					**64.37**	**43.87**	**63.85**	
Mean Irrigation					**82.58**	**39.56**	**66.14**	
Vine diameter (mm)	N17	Drought	2.25	9.00	3.61	17.62	42.33	10.87
	N17	Irrigation	2.37	8.65	4.05	12.21	34.20	
	N18	Drought	1.71	5.88	4.17	4.05	4.60	2.53
	N18	Irrigation	2.03	11.35	4.28	9.92	11.34	
Mean Drought					**3.89**	**10.84**	**23.47**	
Mean Irrigation					**4.17**	**11.07**	**22.77.**	
Number of nodes	N17	Drought	12.01	60.37	21.96	15.05	27.73	23.07
	N17	Irrigation	9.61	47.25	28.54	14.08	38.27	
	N18	Drought	15.53	73.18	30.59	9.21	14.28	5.36
	N18	Irrigation	20.86	52.57	32.32	11.44	34.15	
Mean Drought					**26.28**	**12.13**	**21.00**	
Mean Irrigation					**30.43**	**12.76**	**36.21**	

*Environments- N17- Nyapkala 2017, N18-Nyankpala 2018, 1- trait measured before drought imposition, 2- trait measured 30 days after drought imposition. means per treatment for coefficient of genetic variation (CVg), heritability and trait means are in bold

4.3.2 Broad sense heritability estimates and coefficient of genetic variation

Heritability estimates (H^2) per trait for single trials were lower under conditions of water stress (drought) than conditions without water stress (irrigation) (Table 4.1 and Table 4.2). Heritability values ranged from 1.00 to 14.00 under drought stress and from 0.00 to 27.00 under irrigated stress conditions. For morphological traits (Table 4.3), although mean heritability for all traits was less under drought compared to irrigated conditions, vine length had high heritability under drought (64%) in contrast to vine diameter which had a low heritability (5 %).

Broad sense heritability estimates for combined analysis within treatments followed a similar pattern as the single trials under drought (Table 4.4).Trait heritability estimates were lower under drought conditions with a range from 0 to approximately 21% and from 0 to approximately 40% under irrigated conditions. In contrast, chlorophyll content index showed higher broad sense heritability under drought stress than irrigated conditions. Across treatments (Table 4.6), Leaf Area Index and Photosynthetically Active Radiation had high and moderate heritability respectively. Like the single trial analysis, a high heritability value was observed for vine length under drought conditions for the combined analysis within treatments. Number of nodes recorded slightly moderate heritability while vine diameter had low heritability under drought stress. Across all treatments for morphological traits (Table 4.7), high heritability was recorded for vine length (80%) and number of nodes (50%) while moderate heritability was observed for vine diameter (30%). Lower coefficient of genetic variation (CVg) estimates were observed under drought relative to irrigated conditions in the combined analysis within treatments for all physiological traits studied (Table 4.4). Across treatments (Table 4.6), Leaf Area Index had the largest CVg followed by photosynthetic active radiation (%PAR).

For combined analysis within treatment, vine length and number of nodes recorded higher coefficient of variation under drought in contrast to vine diameter which had lower coefficient of variation under drought conditions (Table 4.5). Across treatments (Table 4.7), coefficient of genetic variation was highest for vine length and lowest for vine diameter.

4.3.3 Variance components and patterns of G X E interaction

Table 4.3 represents variance components for physiological traits under irrigated and drought collected 30 days after drought imposition. For combined analysis of physiological traits within treatment (Table 4.4), genetic variation for Photosynthetically Active Radiation was significant at $p<0.05$ under irrigation but non- significant variation was observed for other traits under both conditions of drought and irrigation. Genetic variation was, however, lower under drought conditions relative to irrigated conditions. GxE interaction was significant for only canopy temperature ($p<0.001$) under conditions of drought and irrigation. Chlorophyll content had highly significant G x E interaction under drought ($p<0.001$) and ($p<0.05$) under irrigated conditions. All other traits had non-significant GxE interaction under both drought and irrigated conditions. For combined analysis within treatments (Table 4.5), genetic variation observed for all morphological traits studied was highly significant ($p<0.001$) under conditions of irrigation and drought, however, genetic variation was higher under irrigated conditions. Genotype by environment interaction was also significant under both conditions of drought and irrigation but lower values were observed under drought conditions. Although environmental variance was higher under drought conditions, variation was significant for both conditions of drought and irrigation. G: G x E: E ratio for vine length, vine diameter and number of nodes under irrigation was 1: 0.30: 0.33, 1:3.33: 0.08 and 1: 1.64: 5.15 respectively. Genetic variance ratios for traits

under drought was 1: 0.43: 0.49 for vine length, 1: 5: 4.67 for vine diameter and 1: 1.63: 5.14 for number of nodes.

Across environments (Table 4.5) , genetic variation was significant ($p<0.001$) for leaf area index and chlorophyll content index ($p<0.01$) but not significant for other traits. Genotype by environment interaction was not significant .Variation for morphological traits in the combined analysis across treatments (Table 4.6) was largely due to the genotypic variance. Significant genotypic variation and environmental variation ($p< 0.001$) was observed for all traits. However, G x E interaction was not significant for all the morphological traits studied. The G : G x E : E ratio for vine length, vine diameter and number of nodes was 1: 0.29 : 0.41 , 1: 3.43: 1.03 and 1: 1.42: 2.38 respectively.

Table 4.4 Means and variances for combined analysis within treatments for physiological traits measured at 30 days after drought initiation

Random term	NDVI		% PAR		LAI		Canopy.temp		CC	
	irrigation	drought	irrigation	drought	irrigation	drought	irrigation	drought	irrigation	drought
Mean	73.75	62.44	179.68	152.03	7.65	7.03	28.20	37.04	35.46	18.60
G	0.00NS	0.00NS	164.01*	3.11E-12NS	0.67NS	1.58E-13NS	0.33NS	0.20NS	6.13 NS	1.53NS
G X E	10.64NS	16.34NS	512.93NS	216.09NS	2.05NS	1.14NS	4.54***	1.06***	5.42*	11.50***
H^2	0.00	0.00	39.01	2.88E-12	39.56	2.76E-11	12.62	27.27	69.35	20.99
CVg	0	0	7.13	1.16E-06	10.72	5.65E-06	2.03	1.20	6.98	6.64
RDD%	15.33		15.39		8.03		23.87		47.55	

NDVI-Normalized difference vegetative index, %PAR- percent photosynthetic active radiation, LAI- Leaf Area Index , Canopy.temp- canopy temperature, CC- chlorophyll content, G- genetic variance, G x E- Genotype by environment interaction, H^2- broad sense heritability, CVg- coefficient of genetic variation
*, **,***significant at the 0.05, 0.01, 0.001 probability level, NS-not significant

Table 4.5 Variance components for morphological traits within treatments combined environments

	Vine length		Vine diameter		Number_nodes	
Random term	irrigation	drought	irrigation	drought	irrigation	Drought
Mean	82.10	63.73	4.23	3.89	30.55	26.23
G	704.14***	534.24***	0.12***	0.03***	6.58***	5.98***
G X E	217.36***	228.30 ***	0.40***	0.15***	11.60***	9.78***
H^2	75.77	68.45	36.63	20.65	43.67	22.77
CVg	32.32	36.27	8.16	4.44	8.40	9.32
RDD%	22.38		7.97		14.13	

*G- genetic variance, G x E- Genotype by environment interacion, , H^2- broad sense heritability, CVg- coefficient of genetic variation
*, **,***significant at the 0.05, 0.01, 0.001 probability level, NS-not significant

Table 4.6 Mean and variances for combined analysis across treatments for physiological traits after drought imposition for combined environments

Random term	NDVI	%PAR	LAI	Canopy.temp	CC
G	0.41 NS	38.62 NS	1.58***	8.10E-13NS	1.58**
G X E	5.61 NS	467.85 NS	1.97NS	3.10 NS	20.08 NS
Mean (μ)	68.09	165.86	7.34	32.62	27.03
CVg	0.94	3.75	17.13	2.76E-06	4.65
H^2	3.02	11.19	7.35	2.63e-11	22.66

*G- genetic variance, G x E- Genotype by environment interacion, H^2- broadsense heritability, CVg- coefficient of genetic variation
*, **,***significant at the 0.05, 0.01, 0.001 probability level, NS-not significant

Table 4.7 Mean and variances for combined analysis across treatments for morphological traits after drought imposition

Random term	Vine length	Vine diameter	Number of nodes
G	677.24***	8.66E-04***	7.68***
G x E	199.17NS	2.97E-03NS	10.91NS
Mean (μ)	72.91	4.21	28.38
CVg	35.69	0.70	9.76
H^2	80.52	30.43	50.16

G- genetic variance, G x E- Genotype by environment interacion, E- environmental variance, H^2- broad sense heritability, CVg- coefficient of genetic variation

*,***,NS- significant at 0.05, 0.001 , not significant

4.3.4 Correlation between morphological, physiological parameters and yield, yield related traits

Relationship between percent change (relative to control) for physiological parameters and percent reduction for root yield (RY), foliage yield (FY), biomass (biom) and harvest index (hi) are represented in Table 4.7. Leaf Area Index (LAI) and Photosynthetically Active Radiation (%PAR) had low but highly significant and positive correlation (r=0.19 at P= 0.01), (r=0.17 and P= 0.05) with root yield, foliage yield (r=0.20, P=0.01) (r=0.14, P=0.05) and biomass (r=0.24, P= 0.001) (r=0.19, P= 0.01). While Photosynthetically Active Radiation (%PAR) had a positive non-significant relationship with hi, correlation between Leaf Area Index and harvest index was negative and non-significant. Chlorophyll content index was positive and non-significantly correlated with storage root yield, foliage yield, biomass and harvest index. Normalized difference vegetation index (NDVI) had positive, non-significant correlations with storage root yield, biomass and harvest index, but was negatively correlated with foliage yield.

Between physiological parameters, significant and positive correlation was observed between leaf area index and Photosynthetically Active Radiation (r=0.65) and between chlorophyll content and leaf area index (r=0.16). Significant and negative correlation was observed between chlorophyll content index and canopy temperature (r=-0.61). Chlorophyll content index was non-significant and positively correlated with storage root yield , foliage yield and biomass but negatively correlated with harvest index.

Correlation between vine length had a significant and positive correlation with foliage yield (0.14) and biomass (r=0.16) all at (P=0.05). However, vine length had a positive but non-significant correlation with storage root yield and harvest index. The relationship between number of nodes, storage root yield and biomass were negative and non-significant, but a negative and significant correlation was observed between number of nodes and harvest index

(r= -0.16 at P=0.01). Vine length was highly correlated (P=0.001) with number of nodes. Storage root yield was significantly and highly correlated with foliage yield (r=0.30 at P= 0.001) and biomass (r=0.73 at P=0.001) as well as harvest index (r= 0.40 at P=0.01). Foliage yield was significantly and positively correlated with biomass (r=0.87 at P=0.001) but negatively correlated with harvest index (r=-0.16 at P=0.05).

Table 4. 8 Pearson correlation coefficients for physiological parameters, yield and yield related traits for 205 sweetpotato genotypes

	cc	LAI	CT	%PAR	NDVI	RY	FY	biom	hi
cc	1								
LAI	0.16*	1							
CT	-0.61***	-0.04 NS	1						
%PAR	0.06NS	0.65***	0.05 NS	1					
NDVI	0.08 NS	0.03 NS	-0.02 NS	0.07 NS	1				
RY	0.04 NS	0.19**	-0.07 NS	0.17*	0.1	1			
FY	0.04 NS	0.20**	-0.03 NS	0.14*	0.08 NS	0.30***	1		
biom	0.05 NS	0.24***	-0.06 NS	0.19**	0.09 NS	0.73***	0.87***	1	
hi	-0.02 NS	-0.03 NS	-0.05 NS	0.01 NS	0.09 NS	0.40***	-0.16*	0.08 NS	1

*,**,***= significant at P<0.05, P<0.01 and P<0.001 respectively, NS = not significant (P>0.05), CC = chlorophyll content, LAI = leaf area index, CT (^{o}C)= canopy temperature , %PAR = Percentage of captured photosynthetically active radiation, NDVI= normalized difference vegetation index, RY (t/ha) = root yield, FY (t/ha) = folige yield, biom (t/ha) = above ground biomas yield, HI = harvest index.

Table 4. 9 Correlation between morphological traits and storage root yield, foliage yield, biomass and harvest index

	vine_diam	num_nodes	vine_len	RY	FY	biom	hi
vine_diam	1						
num_nodes	0.06NS	1.00					
vine_len	-0.05 NS	0.53***	1.00				
RY	0.06 NS	-0.07 NS	0.10 NS	1.00			
FY	0.07 NS	0.03 NS	0.14*	0.30***	1.00		
Biom	0.09 NS	-0.02 NS	0.16*	0.73***	0.87***	1.00	
Hi	0.03 NS	-0.16*	-0.12 NS	0.40**	-0.16*	0.08 NS	1.00

*, **,*** = significant at P=0.05, P< 0.01 and P=0.001 respectively, NS = not significant , vine_diam= vine diameter (cm) , vine_len = vine length (cm), num_nodes = number of nodes, RY = root yield (t/ha), FY = foliage yield (t/ha), biom = biomass), hi = harvest index

4.4 Discussion

4.4.1 Genetic variation per traits under drought

The identification of genetic variability for yield and yield related traits and physiological traits under water stress (drought) conditions and full irrigation (non-stress) conditions is of great interest for breeders because selected genotypes with favorable traits can be used as parents in future crosses (del Pozo *et al.*, 2016). Generally, genetic variation for canopy temperature, NDVI, chlorophyll content index and Leaf Area Index were not significant under irrigated and drought conditions except for Photosynthetically Active Radiation in irrigated environments. This is possibly due to spatial variability associated with using hand held devices when measuring physiological traits in a large mapping population. Deery *et al*. (2016) reported challenges associated with the use of hand-held canopy temperature measuring devices which is laborious and challenging due to interference by environmental fluctuations. The resulting low heritability estimates hampers genotype selection for large scale experiments. In contrast, the use of airborne thermography using a radiometrically-calibrated thermal camera is a preferable method for measuring canopy temperature. This method is accurate, a very short image acquisition time, subject to less environmental interference and cost saving. Field phenotyping of yield and stress related traits is a challenge. Managing trials, handling spatial variability and defining important environmental conditions in target environment and data management are important considerations of phenotyping. Progress in technological development such as remote- sensing are hopeful ways to improve field-based phenotyping (Araus *et al*., 2018).

The observed non-significant genetic variation may also be attributed to the age of the plants at the time of data collection. Data was collected on sweetpotato plants 100 days after planting (30 days after drought imposition). Similar observation was reported by Laurie *et al.* (2015)

for which no significant effect was found for control and severe drought stressed sweetpotato varieties at 120 days after planting for leaf area index. This is possibly related to the age of the plants as they were close to at the end of their growth phase. Cassana *et al*. (2010) reported a reduction in chlorophyll fluorescence ratio due to leaf aging in sweetpotato plants grown in-vitro. Omotobora *et al*. (2014) observed non-significant differences among genotypes when canopy temperature was measured under drought stress for sweetpotato plants at 120 days after planting.

4.4.2 Effect of stress on genotypes based on morpho-physiological traits in sweetpotato

Drought stress elicited variable responses on the physiological and morphological traits of sweetpotato studied. Observed reduction in Leaf Area Index under drought stress in the present study is consistent with previous studies by Laurie *et al*. (2009), Gajanayake *et al.* (2016). Gajanayake *et al.* (2014) observed a lower biomass and yield in cultivars Beaurgard and Evangeline owing to decrease in leaf area, photosynthetic activities and chlorophyll concentrations under moisture stress conditions . Osmotic potential in leaf tissues of sweetpotato also decreased depending on reduced soil water content, genotypes and their interaction (Yooyongwech *et al.*, 2014). As a response to decreasing soil moisture, plant water status is maintained by lowering the rate of transpiration in order to slow leaf expansion. Reduction of leaf area in sweetpotato under conditions of low moisture is an adaptive mechanism for moisture conservation (Gajanayake *et al.*, 2016).

Findings from the present study also revealed that drought caused a reduction in chlorophyll content of sweetpotato. A marked reduction in chlorophyll content indicates enhanced tolerance to drought (Ramya *et al.*, 2016). Yooyongwech *et al*. (2013) also observed a decrease in photosynthetic rate in sweetpotato under drought condition. Both chlorophyll a and b are sensitive to soil moisture stress (Farooq *et al*., 2009). Laurie (2014) suggested that sweetpotato

plants respond to drought stress through antioxidant systems and chlorophyll degradation. This can be used as a screening tool for drought tolerance.

Observed increase in canopy temperature of sweetpotato genotypes in response to drought stress compared to irrigated conditions agrees with Bazzaz *et al*. (2015) who reported that increase in canopy temperature in wheat genotypes under drought was attributed to a rise in respiration and reduction in transpiration rate due to stomata closure. Low temperature is associated with a high rate of photosynthesis due to increased respiration (Jones, 1983). This is suggesting that genotypes with low canopy temperature have high chlorophyll content due to increased photosynthetic activity as stomata close at a reduced rate. This supports the negative relationship observed between reduction in canopy temperature and chlorophyll content reported in this study. Canopy temperature also had a negative correlation with root yield. Similar finding was reported in cassava by Adjabeng (2014) in cassava.

Reduction in Photosynthetically Active Radiation following imposition of drought stress was observed in this study. In an experiment on orange flesh sweetpotato, Laurie *et al.* (2009) reported that decrease in the photosynthetic rates in sweetpotato varieties indicated that photosynthesis was possibly influenced by metabolic impairment. This suggests that drought effect on leaf area and leaf photosynthetic ability also influences storage root yield.

Drought also affected morphological traits in sweetpotato by causing reduction in vine length, number of nodes and vine diameter. Vine length had the most reduction due to drought, followed by number of nodes. Vine diameter had the least rate of reduction due to drought. This agrees with findings by Laurie (2014) who reported severe decrease in sweetpotato stem length (10-fold relative to the control) in response to drought. Solis (2012) also stated that drought stress resulted to decreased stem length and internode diameter. Gajanayake *et al.* (2016) reported reductions in the node number with increasing water deficit. Growth and

developmental parameters such as vine length, nodes per vine, and whole-plant leaf area declined linearly with increasing moisture deficit. Reduction of leaf expansion and vine length due to drought is suggestive that the growth of the shoot will be affected as it is the source of biomass to be partitioned to the storage root (Motsa *et al*., 2015). Since drought results to reduction in leaf expansion and vine length (Lebot, 2009; Saraswati *et al.*, 2004) it can be inferred that the above ground biomass (shoot) will be affected as it is the only source of biomass to partitioned for storage root development.

4.4.3 Relationship between morpho-physiological traits and yield, yield related traits

Positive and strong association between Leaf Area Index and root yield, foliage yield and biomass as well as between Photosynthetically Active Radiation and foliage yield, root yield and biomass were observed in the present study. Like our research findings, Laurie *et al.* (2015) reported a strong correlation between yield and Leaf Area Index in three trials accentuating the relationship between canopy cover and storage yield in sweetpotato. Leaf Area Index is a measure of canopy cover based on estimations of leaf area per unit ground surface area. It can be considered as a signal of efficient photosynthesis, available soil moisture and other processes that enhance plant adaptation to their environments. It portrays plant canopy structure, roles and developments and relates with vital processes associated with physiology; photosynthesis, respiration and transpiration (Breda, 2003). Lethwaite and Triggs (2012) reported yield reductions in sweetpotato cultivars with low canopy cover under conditions of moisture stress. Ludlow and Muchow (1990) attributed the association between leaf area and yield to photosynthesis. Reduction in leaf area under drought is a survival mechanism but could be detrimental to plant productivity if leaf area index is below a value of 3. The relationship between leaf area index, photosynthesis and canopy cover possibly explains the observed highly significant and positive correlation in the study between Photosynthetically Active

Radiation and storage root yield, biomass as well as between Leaf Area Index and storage root yield and biomass.

In contrast to findings by Laurie *et al.* (2015), non-significant low correlation was observed between root length and root yield. However, positive correlation between percent reductions in vine length, foliage yield and biomass as well as number of nodes and harvest index suggests that these traits contribute to shoot development. In turn, strong correlations between root yield, foliage yield, biomass and harvest index indicate possible contributions of vine length and number of nodes to root yield. Andrade *et al.* (2017) opined that vine length and other morphological traits contribute to vine survival and have high heritability. Vine length and diameter are pertinent for vine survival.

Suitable secondary traits for selection should have adequate genetic variability, be easy and not costly to measure, non-destructive, fast and accurate, be suitable to use during part or the entire growth phase of the plant and be informative about the performance of the crop in relation to environment (Tuberosa, 2011). Although there was no significant genetic variability among physiological traits, positive correlation between leaf area index, Photosynthetically Active Radiation and yield was recorded. However, these traits had low heritability under drought stress due to shortcomings with the devices for measurement. Selection of drought tolerant genotypes using these traits will not be efficient. In comparison, morphological traits studied had significant genetic variability, despite the age of the plants, were easy to measure, did not require the use of expensive phenotyping equipment, they also had high to moderate levels of heritability under drought stress. In addition , percent reduction in vine length had positive correlation with foliage yield and biomass, while number of nodes was strongly correlated with vine length. The use of vine length and possibly number of nodes can be explored as secondary traits for selection of drought tolerant sweetpotato genotypes. Proper

timing for the use of physiological traits in relation to the phenology of the crop and drought stress can also be explored to identify suitable traits for selection.

4.5 Conclusion

Drought affected all traits resulting in reduction in traits but an increase in plant canopy temperature in drought stressed environment. Among the physiological traits studied, chlorophyll content index had the greatest reduction (47.55 %) in response to drought stress while vine length had the most reduction among the morphological traits (22.38 %).

Genetic variability of genotypes per physiological trait under each treatment was highly influenced was not significant due to interference by spatial variation associated with the use of hand-held devices for phenotyping large scaled trials. Furthermore, the age of plants at the time of data collection possibly contributed to the inability to capture genetic variation among genotypes. Despite the age of plants, genotypes showed significant genetic variation per morphological trait in response to drought stress .

Leaf Area Index and Photosynthetically Active Radiation were strongly associated with root yield however, their heritability values under drought were low. In contrast, morphological traits were strongly correlated to foliage yield and harvest index which in turn had high correlation with root yield. Vine length had high heritability values and moderate heritability for vine diameter and number of nodes under drought. This infers that they can be exploited for the selection of drought tolerant sweetpotato.

CHAPTER FIVE

5.0 Quantitative trait loci linked to yield traits related to drought tolerance in a sweetpotato bi-parental mapping population

5.1 Introduction

Climate change including drought affects sweetpotato making it necessary to breed for sweetpotato varieties which are resilient and drought tolerant in order to contribute towards global food security (Motsa *et al.*, 2015). Major constraints to breeding sweetpotato include its highly heterozygous nature, outcrossing polyploidy with many small chromosomes (2n = 6x = 90), poor or no flowering, self -incompatibility and cross incompatibility with successful crosses producing less than two seeds. The factors including the quantitatively inherited nature of its economic traits such as yield, quality resistance traits (Cervantes-Flores *et al*., 2008; 2011) slow the pace of breeding (Baafi *et al.*, 2015).

Quantitative traits of biological and economic importance such as agricultural traits (yield, quality, resistance to diseases and abiotic stress) are intricate in nature. They are influenced by numerous genetic loci (quantitative trait loci), environmental factors and a combination of both genetic and environmental factors (genotype by environment interactions). Quantitative trait loci (QTL) mapping is an unbiased phenotype-dependent scheme that identifies and synchronizes statistics-based relationships between genotypes of genetic markers and phenotypic values for quantitative traits. It is used in localizing QTLs which influence a trait(s) of interest to regions on the chromosome containing marker loci that are significantly linked to the quantitative trait. This explains the genetic architecture of traits and their variations (Miles, 2008).

Genomic research in sweetpotato lags behind compared to essential crops such as cereals due to its previously perceived “poor person” or “orphan” crop status. Consequently, it has received little attention towards improvement from the public (universities, the CGIAR, and NGOs) and

private sectors (such as seed companies and agro-based industries). It's extremely complex genetics and trait segregation patterns was also a draw back. However, with changing trends more attention and technological development for polyploids including sweetpotato improvement is ongoing (Mwanga *et al.*, 2017).

Several traits have been mapped in sweetpotato using QTLs. Cervantes-Flores *et al.* (2011) mapped 13 QTLs for root dry matter, 12 QTLs for starch content and 8 QTLs for beta-carotene and root-knot nematode resistance using 240 genotypes from the BxT mapping population which explained variations ranging from 15–24% dry-matter content, 17–30% starch content and 17–35% beta-carotene content respectively. Yu *et al.* (2014) identified 6 QTLs for starch content using a mapping population comprising 202 individuals from a cross between Xushu 18 and Xu 781, accounting for 9.1 - 38.8% of the variation in starch content and Li *et al.* (2014) identified 9 QTLs for storage root yield using the same mapping population, explaining 17.7–59.3% of the variation in storage root yield.

Analysis of QTLs associated with economic traits such as yield in relation to conditions such as drought could be beneficial in sweetpotato. Identification of favorable alleles and development of cross combinations with needed traits such as yield will improve breeding activities through the transfer of elite alleles from one gene pool to another for improvement with respect to the needed trait through QTL analysis Cervantes-Flores *et al.* (2011), Liu, (2017).

The mapping population used in this study has previously been studied by Cervantes-Flores (2006) and QTLs mapped for root knot nematode, dry matter , starch and beta-carotene using amplified fragment length polymorphic markers (AFLP). Following evaluation of genotypes for yield and yield related traits under irrigated and drought conditions in parts of Ghana

(Chapter three of this study), the current study aims at identifying quantitative trait loci related to drought for storage root yield, foliage yield, biomass and harvest index.

5.2 Materials and Methods

5.2.1 Plant Materials

The sweetpotato BxT biparental mapping population comprising of 315 progeny derived from the cross 'Tanzania' (female) x 'Beauregard' (male) was used for this study. 'Tanzania' is a sweetpotato landrace from sub-Saharan Africa characterized by a cream-fleshed, high dry matter (ca.30%), resistant to root-knot nematodes (Cervantes-Flores *et al.*, 2002) and tolerant to Sweet Potato Feathery Mottle Virus (SPFMV) and Sweetpotato Chlorotic Stunt Virus which combine in a complex resulting to Sweetpotato Virus Disease (SPVD) (Mwanga *et al.*, 2002). 'Beauregard', the most widely grown sweetpotato in the U.S is orange flesh has a low dry matter (ca. 18%). The mapping population was provided by the International Potato Center (CIP) Peru for studies in Ghana at the International Potato Center, Ghana-Kumasi.

Lau *et al.* (2018) reported higher expression of co-regulated clusters of genes involved in photosynthesis and the pentose-phosphate pathway in Beauregard may contribute to its chlorophyll content being more resilient to drought than Tanzania, suggesting Beauregard may be more tolerant to drought than Tanzania.

5.2.2 Phenotyping Sites and Experimental Design

Field evaluation of genotypes (as reported in chapter three) under drought, irrigated and rainfed conditions in parts of Ghana; Nyankpala, Wenchi, Fumesua comprised three irrigated experiments (two experiments in Nyankpala, one in Wenchi) and three drought experiments (two experiments in Nyankpala, one in Wenchi) in 2017 and 2018. Two rainfed experiments (one in Nyankpala and the other in Fumesua) in 2016. Nyankpala is located in the northern

parts of Ghana, Wenchi is situated in the Brong-ahafo region (transition ecozone) of Ghana and Fumesua (forest ecozone), in the Ashanti region of Ghana.

5.2.3 Phenotypic data collection and analysis

Phenotypic data collected based on yield and yield related traits were used in computation of the derived traits : root yield (t/ha) , foliage yield (t/ha), biomass (t/ha) and harvest index using the Statistical tools for genetic improvement R package (St4gi), an interface tool for Highly interactive Data Analysis Platform (HIDAP). HIDAP supports clonal crop breeders at the International Potato Center (CIP) with regards to data collection, data quality and data analysis in clonal crop breeding.

5.2.4 Genotypic data acquisition and genetic linkage map construction

Genotypic data for 315 genotypes and two parents from the BxT mapping population was genotyped in collaboration with the North Carolina State University (NCSU), using a GBS (genotype-by-sequencing) method optimized for hexaploid sweetpotato called GBSpoly (Olukolu *et al*. Under preparation). GBS tags were aligned against the relative diploid *Ipomea trifida* genome (Wu *et al*., 2018) using Bowtie2 (Langmead *et al*., 2012) and allele read counts per locus using the Tassel-GBS pipeline (Glaubitz *et al*., 2014) modified for polyploids was obtained. The read counts were utilized for dosage calling in the software SuperMASSA (Serang *et al*., 2012) aided by VCFpoly script (Pereira *et al.*, 2019). Based on the dosage calls, genetic linkage map was developed using MAPpoly software for linkage mapping in polyploids (Mollinari *et al*., 2019). The integrated map had 15 linkage groups of 16,107 GBS-generated and phased SNPs with a total length of 1,303.1 cM and an average distance between markers of about 0.08 cM (unpublished). The QTL genotype conditional probabilities based on the map and the predicted genotypic means were used in QTL mapping using the QTLpoly software for QTL mapping in polyploids (Pereira *et al.*, 2019).

A random-effect multiple interval mapping model (REMIM) where score-based tests (Qu *et al*., 2013) were performed every 1 cM following a stepwise method was used. First, the forward search added one QTL at a time into a multiple QTL model using a less conservative threshold (p-value < 0.01). Then, the backward elimination tested each QTL again conditional to all the others in the model using a more conservative threshold (p-value < 0.001). Under the more conservative threshold, forward and backward procedures are repeated until no more QTLs are added or dropped from the model. A region of 20 cM on either side of QTLs already in the model when searching for a new QTL was avoided. Total phenotypic variances were estimated using restricted maximum likelihood (REML) as implemented in the R package Sommer (Covarrubias-Pazaran, 2016). QTL heritabilities were computed as the ratio of QTL and total phenotypic variances.

The GBS method optimized for hexaploid sweetpotato, the genetic linkage methods and QTL mapping methods applied were developed as part of the genomic tools for sweetpotato improvement (GT4SP) project and have been used in-house awaiting publication. Variant calling was based on *I. trifida* reference genome, one of the two diploid relatives of hexaploid sweetpotato, developed also as part of GT4SP. Methods and linkage maps will be available in sweetpotatobase (https://sweetpotatobase.org/).

5.3 Results

QTL analysis was done to study the genetic basis of drought tolerance observed in the BxT mapping population under irrigated , drought stress and rainfed conditions. A summary of single environments, traits, marker, linkage group, position of QTL peak, P-value, heritability and percentage variation explained (PVE) for QTL analysis is shown in Table 5.1 and combined environments in Table 5.2. A total of 32 QTLs were detected for yield and yield related traits (Table 5.1). A total of eleven QTLs were detected under irrigated conditions, eleven QTLs under drought and seven QTLs under rainfed conditions using data from single

site trials. Three QTLs were detected under drought conditions for combined analysis. These QTLs were found on all linkage groups except linkage groups 5 and 8. Eight QTLs were associated with storage root yield (RY) (Figure 5.1), ten QTLs were related to foliage yield (Figure 5.2), nine were linked to biomass (Figure 5.3) and five for harvest index (Figure 5.4).

Observed phenotypic variations for storage root yield (RY) under irrigated conditions explained by the QTLs ranged from 27.01 % to 34.89%. QTLs explained 16.43% of variations under drought stress in environment N18. QTLs were not detected under drought stress for environments N17 and W17. Under rainfed conditions, QTLs explained 17% of the observed phenotypic variation in environment N16. No QTLs were detected for environment F16.

Table 5.1 Quantitative trait loci related to yield and yield related traits in the sweetpotato biparental mapping population in single sites

ENV	Trait	Marker	S/N	LG	Position	P-value	H^2	PVE
N17_irr	RY	Tl_S10_25840155	1	10	138.31	0.00	0.15	33.29
		Tf_S15_3594488	2	15	57.4	0.00	0.18	
N18_Irr	RY	Tf_S2_25419900	1	2	177.15	0.00	0.14	27.01
		Tf_S14_5928189	2	14	58.11	0.00	0.13	
N18_Drgt	RY	Tf_Tl_S14_2342931_S14_2604132	1	14	19.49	0.00	0.16	16.43
W17_Irr	RY	Tf_Tl_S7_3162800_S7_3326725	1	7	45.48	0.00	0.13	34.89
		Tf_Tl_S12_19525059_S12_23050052	2	12	110.17	0.00	0.22	
N16_r	RY	Tf_S4_1845053	1	4	29.03	0.00	0.17	17.00
N17_Drgt	FY	Tf_Tl_S6_15375151_S6_14556603	1	6	39.34	0.00	0.19	30.75
		Tf_Tl_S10_3063888_S10_3709055	2	10	36.09	0.00	0.12	
N18_Drgt	FY	Tf_Tl_S3_13440195_S3_16138822	1	3	179.21	0.00	0.16	52.94
		Tf_Tl_S9_3875536_S9_4679570	2	9	71.09	0.00	0.14	
		Tl_S10_25737488	3	10	136.22	0.00	0.13	
		Tf_S15_2779657	4	15	46.19	0.00	0.10	
W17_Drgt	FY	Tf_Tl_S13_17125139_S13_24888039	1	13	96.11	0.00	0.17	17.43
N16_r	FY	Tf_Tl_S3_15932831_S3_18791200	1	3	151.36	0.00	0.16	16.00
F16_r	FY	Tf_Tl_S7_1045750_S7_1192649	1	7	15.29	0.00	0.16	16.00
N18_Irr	BIOM	Tf_S2_25419900	1	2	177.15	0.00	0.14	25.86
		Tf_Tl_S14_5451933_S14_6504293	2	14	56.08	0.00	0.12	
N18_Drgt	BIOM	Tl_S10_25334934 117.65	1	10	131.44	0.00	0.14	14.29
W17_Irr	BIOM	Tf_Tl_S12_18291707_S12_21655796	1	12	101.1	0.00	0.17	17.28
W17_Drgt	BIOM	Tf_Tl_S13_17125139_S13_24888039	1	13	96.11	0.00	0.18	18.06
N16_r	BIOM	Tf_Tl_S3_15932831_S3_18791200	1	3	151.36	0.00	0.16	16.00
F16_r	BIOM	Tf_Tl_S7_1045750_S7_1192649	1	7	15.29	0.00	0.16	16.00
W17_Irr	HI	Tf_Tl_S1_26515694_S1_31836857	1	1	190.08	0.00	0.10	28.49
		Tl_S7_2915625	2	7	36.18	0.00	0.19	
W17_Drgt	HI	Tf_Tl_S6_21562896_S6_21634432	1	6	100.1	0.00	0.15	29.08
		Tl_S10_25737488	2	10	136.22	0.00	0.15	
F16_r	HI_	Tl_S15_23143000	1	1	182.15	0.00	0.13	13.00

*Environments-N17 (Nyankpala 2017), Drgt-Drought, Irr- Irrigation, r-rainfall, RY- storage root yield, FY- foliage root yield, BIOM- biomass, HI- harvest index, H2-heritability, PVE- percent variation explained, LG- linkage group, position - Position of QTL peak in centiMorgans

Table 5. 2 Quantitative trait loci related to yield and yield related traits in the sweetpotato biparental mapping population for combined environments

ENV	Trait	Marker	S/N	LG	Position	P-value	H^2	PVE
Drought	FY	Tf_Tl_S11_6399413_S11_8520032	1	11	127.39	0.00	0.28	27.93
Drought	Biom	Tf_Tl_S6_20081764_S6_20063749	1	6	182.35	0.00	0.16	36.65
		Tf_Tl_S11_6399413_S11_8520032	2	11	91.02	0.00	0.21	

Drgt-Drought, RY- root yield, FY - foliage root yield, BIOM- biomass, HI- harvest index, H^2-heritability, PVE-percent variation explained, LG- linkage group, position -Position of QTL peak in centiMorgans

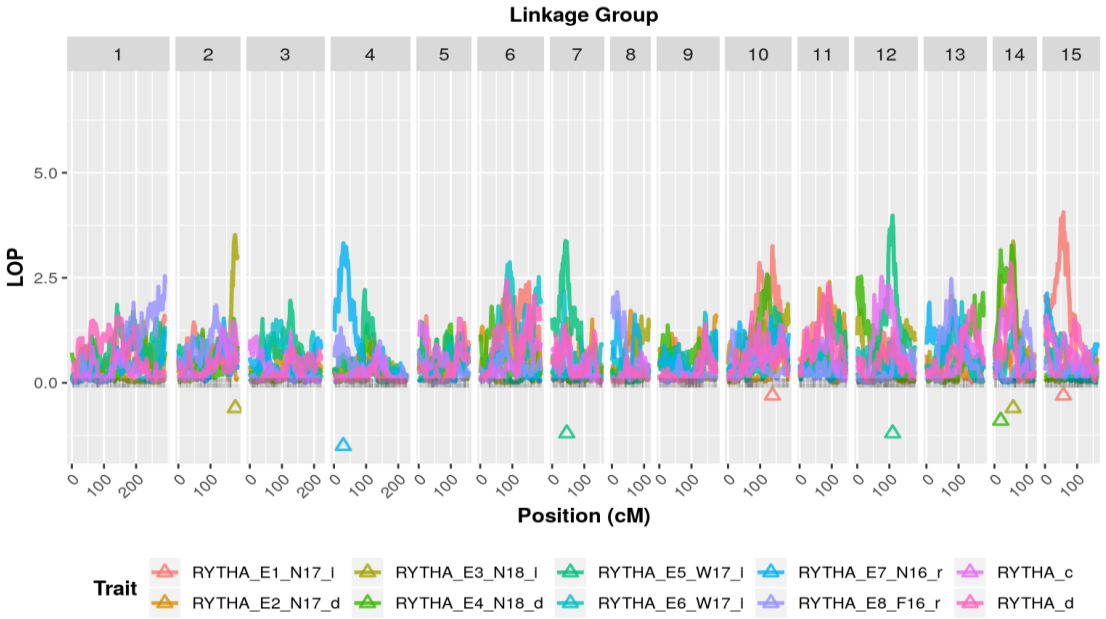


Figure 5.1 QTL plots of the B x T mapping population for root yield (RY) t/ha under single and combined environments for drought and irrigation.
Environments: E1- N17 irrigation, E2- N17_Drought, E3- N18
Irrigation, E4-N18 Drought ,E5 -WI7 Irrigation, E6- W17 Drought, E7- N16 rainfall, E8- F16 rainfall, c- combined irrigated environments, d- combined drought environments

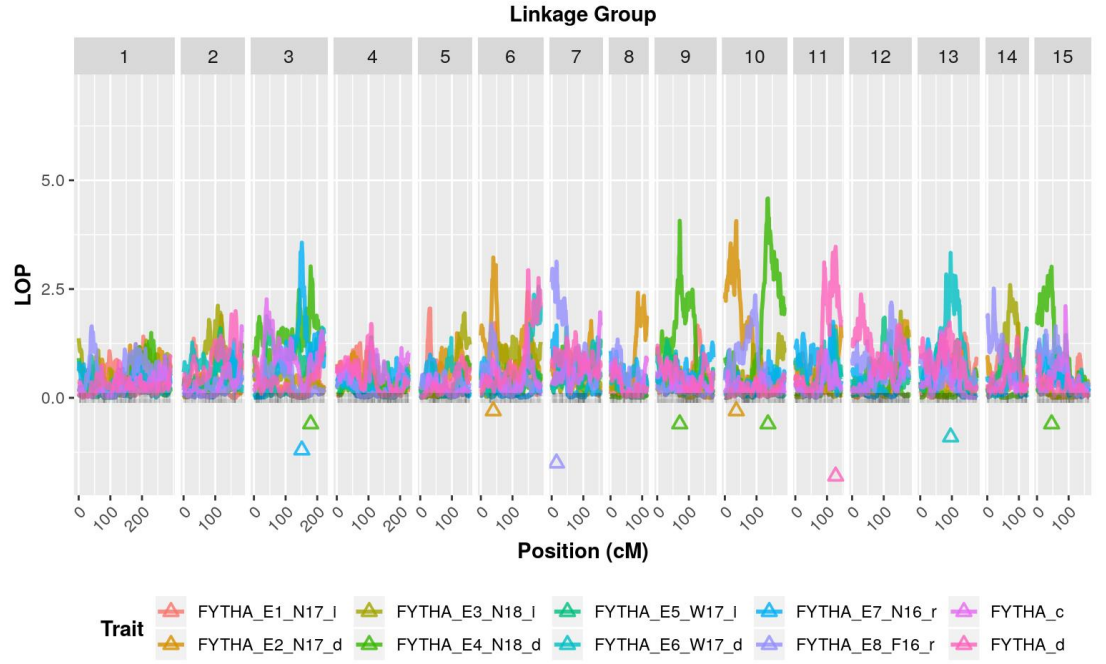


Figure 5.2 QTL plots of the B x T mapping population for foliage yield (FY) t/ha under single and combined environments for drought and irrigation.
Environments: E1- N17 irrigation, E2- N17_DrougsE3- N18, Irrigation, E4-N18 Drought ,E5 -WI7 Irrigation, E6- W17 Drought, E7- N16 rainfall, E8- F16 rainfall, c- combined irrigated environments, d- combined drought environments

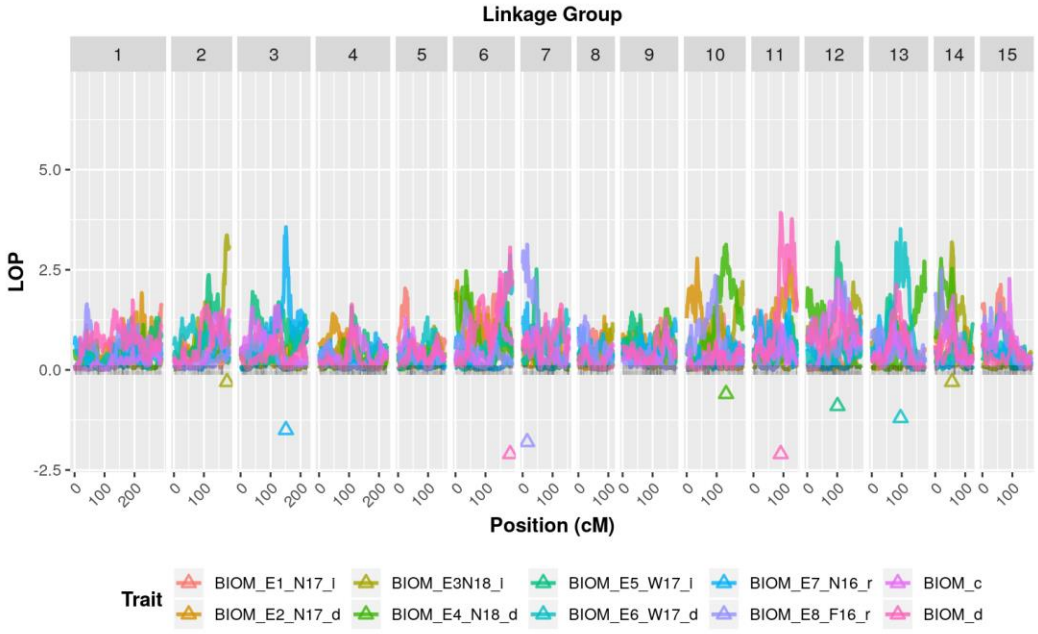


Figure 5.3 QTL plots of the B x T mapping population for biomass (BIOM) t/ha under single and combined environments for drought and irrigation.
Environments: E1- N17 irrigation, E2- N17_Drought, E3- N18 Irrigation, E4-N18 Drought ,E5 -WI7 Irrigation, E6- W17 Drought, E7- N16 rainfall, E8- F16 rainfall, c- combined irrigated environments, d- combined drought environments

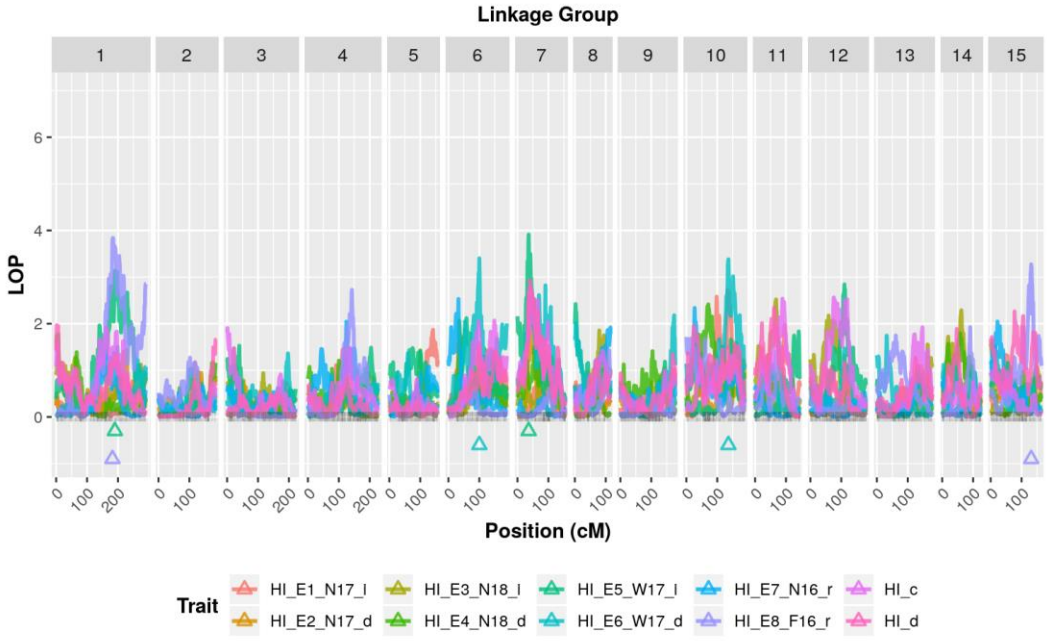


Figure 5.4 QTL plots of the B x T mapping population for harvest index (HI) under single and combined environments for drought and irrigation.
Environments: E1- N17 irrigation, E2- N17_Drought, E3- N18 Irrigation, E4-N18 Drought ,E5 -WI7 Irrigation, E6- W17 Drought, E7- N16 rainfall,E8- F16 rainfall, c- combined irrigated environments, d- combined drought environments

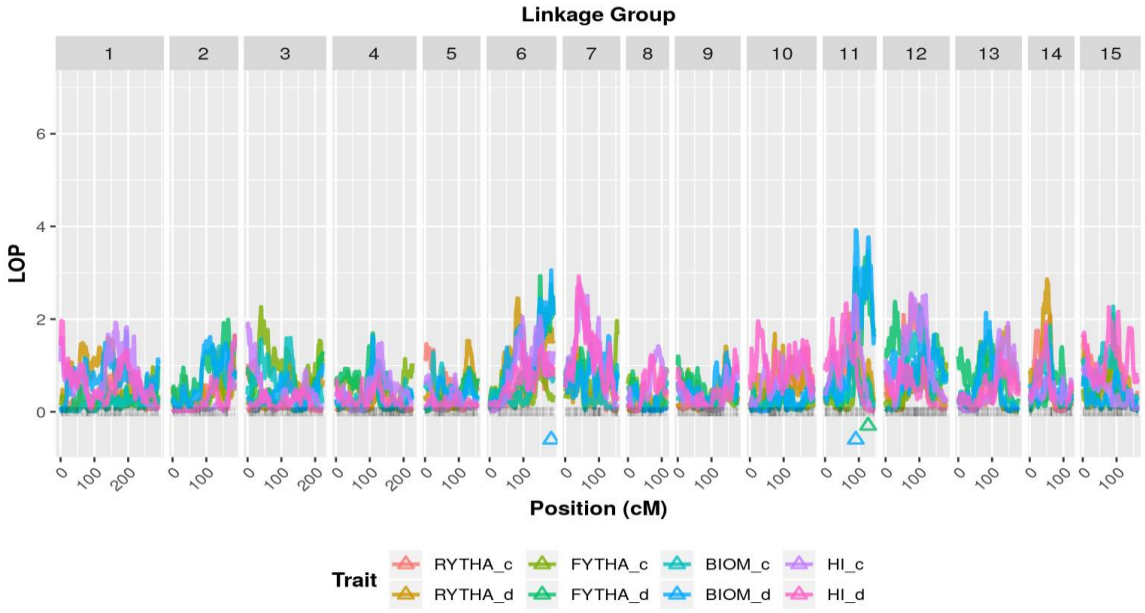


Figure 5.5 QTL plots of the B x T mapping population for root yield (RY), foliage yield (FY), biomass (BIOM) and harvest index (HI) for combined environments for drought and irrigation.
Environments: c- combined irrigated environments, d- combined drought environments

QTLs associated with foliage yield under drought conditions explained variations ranging from 17.43 to 52.94%. QTLs associated with foliage yield under irrigated conditions were not detected. Total variation attributed to QTLs under rainfed conditions was 16%. Phenotypic variations for biomass was between 17.28 to 25.86% under irrigation, 14.29 to 18.06% under drought and 16% under rainfed conditions.

QTLs explained 28.49% of observed phenotypic variation for harvest index under irrigated environments, 29.08% under drought in environment W17. Under rainfed conditions, QTLs described 29% of the phenotypic variation recorded. Under both treatments, some QTLs were found on linkage group 10, localized in the 131.44 to 138.31 cM interval with one QTL for foliage yield under drought located in the 10 cM to 40 cM interval. QTLs found in the 131.44 to 138.31 cM interval included QTLs for foliage yield explaining 12% variation in environment N17D, foliage yield in environment N18D accounting for 13 %variation, biomass in environment N18D

explaining 14% and attributing to 15% phenotypic variation in harvest index for environment W17D, all under drought stress. It also accounted 15 % phenotypic variation for storage root yield in environment N18 under irrigation.

Besides QTLs found on linkage group 10, sixteen significant QTLs were identified on linkage group 14, 7, 15,1, 6 and 12. QTLs found on linkage group 14 included QTLs for root yield under irrigated conditions, root yield under drought and biomass under irrigation. Each explained 13% , 16% and 12% variation respectively. QTLs situated on linkage group 7 on positions 36.18 cM and 45.48 cM were associated with storage root yield under irrigated conditions, harvest index under drought stress and explained 12% and 19% variation respectively. QTL found on linkage group 7, position 15.29 cM was associated with phenotypic variation for foliage yield and biomass under rainfed conditions. Two QTLs associated with phenotypic variation in root yield under irrigated conditions and foliage yield under drought stress were identified on linkage group 15 on positions 57.40 and 46. 19 cM and explained 18 and 10% phenotypic variations observed in root yield and foliage yield respectively. QTLs on linkage group 1 were identified for harvest index under irrigation and rainfed conditions. They were located on position 190.08 and 182.15 cM respectively. Genomic regions (positions 39.34 and 100.1 cM) on linkage group 6 were associated with variations in foliage yield and harvest index under drought stress, accounting for 19% and 15% variations in foliage yield and harvest index respectively. On linkage group 12, QTLs for root yield and biomass under irrigated conditions were detected on positions 110.17 cM and 101.1 cM. Each QTL explained 22% and 17% phenotypic variations for root yield and biomass respectively.

Certain QTLs were found on the same linkage group and position and associated with more than one trait. These include 10 QTLs on linkage groups 2, 10, 13, 3 and 7. The QTL found on linkage group 2, position 177.15 cM explained 14% phenotypic variation in root yield and 14% variation

of biomass under irrigated conditions. The QTL on linkage group 10, position 136.22 cM accounted for 13% variation in foliage yield and 15% variation in harvest index under drought. Under drought stress, a QTL detected on linkage group 13, position 96.11 cM was associated with 17% phenotypic variation for foliage yield and 18% variation in biomass. Under rainfall conditions, a QTL located at position 151.36 cM linkage group 3 explained 16% variation in foliage yield and 16% in biomass while a QTL located on linkage group 7 (position 15.29 cM) was associated with root yield under rainfed conditions and harvest index under irrigation. The QTL explained 16% observed phenotypic variation in foliage yield and 19% variation in harvest index.

Four QTLs found on linkage group 9, 11 and 6 were detected in only drought stressed environments. The QTL located on linkage group 9 between support intervals of 50 and 60 cM

explained 14% of the observed phenotypic variation in foliage yield in the environment N18D. Under combined environments within treatments (Figure 5.5), QTLs found on linkage group 11 within support intervals between 50 and 150 cM (Appendix 5.5) explained 28% phenotypic variation in foliage yield and 21% variation in biomass. QTL on linkage group 6 located within an interval of 80 to 180 cM (Appendix 5.5) accounted for 16% phenotypic variation in biomass. Four QTLs were detected at different loci on linkage group 7 were associated with variation for root yield, foliage yield , biomass and harvest index under non- stressed conditions (irrigation and rainfed environments). In contrast, three QTLs located on linkage group 10 were linked to variations in foliage yield, biomass and harvest index under drought conditions.

5.4 Discussion

To understand the genetic basis of sweetpotato tolerance to drought, quantitative trait loci for genes related to root yield, foliage yield, biomass and harvest index under drought stressed and irrigated

and rained conditions were mapped. Twenty-nine possible QTLs were identified with 8 QTLs related to root yield. Although not under irrigated conditions, Li, *et al.* (2014) detected QTLs associated with root yield in sweetpotato that explained 10.2–59.3% of the variation in storage root yield. This explained a wider variation than obtained in the present study, however, observed percent variation explained was within the reported range by Li *et al.* (2014).

The co-localization of QTLs on linkage groups 2, 10, 13, 3 and 7 for root yield, foliage yield, biomass and harvest index suggest the possible presence of gene(s) with pleiotropic effects. In potato, Tessema (2017) reported the co-localization of QTLs for shoot, root and tuber yield on linkage group 5 and suggested that the gene(s) had pleiotropic effects. Thus, linkage groups identified in this study can be considered to contain important QTLs which can be exploited in breeding programmes for sweetpotato improvement.

The occurrence of four QTLs on linkage group 9 in a single environment and on linkage groups 11 and 6 for combined environments under only drought stressed environments is indicative of drought specific QTLs . Khan *et al.* (2015) reported the occurrence of 24 drought specific QTLs (found in only one treatment) in potato .The absence of QTLs in the combined environment for irrigated environment is a limitation for comparison with combined environments under drought conditions. However, in comparison between the detected QTLs in both single and combined environments, the absence of these QTLs under irrigated environments suggests the possibility of potential drought specificity which may be vital for certain environments and can be exploited for marker-assisted breeding.

The association of QTLs located on linkage groups 7 and 10 with yield and yield related traits under specified environments is suggestive that these QTLs may be environment specific. Furthermore, QTLs on linkage group 7 and 10 may influence growth and development of

sweetpotato (roots and shoots) in response to non- stressed environments (irrigated and rainfed conditions) and drought stressed environments respectively. There exists a strong correlation between root yield, foliage yield, biomass and harvest index, suggesting that these traits are related to root yield and can be used for indirect selection for root yield considering their heritability and ease of measurement.

Physiological traits and morphological traits studied showed positive correlation with some of the yield related traits. Leaf Area Index and photosynthetically active radiation showed association with root yield , foliage yield and biomass (Table 4.7). Vine length had a positive correlation with foliage yield and biomass (Table 4.8) as well as a high heritability under drought stress (Table 4.4). Further study will be needed to understand if the morphological traits studied contribute to root yield through foliage yield or biomass. QTL mapping can also be done to identify genomic regions associated with phenotypic traits in this mapping population.

5.5 Conclusion

Detected QTLs associated with traits in drought environments provide insightful information regarding traits suitable for genetic improvement of sweetpotato yield in drought environments. They are also revealing of genetic variation within the studied mapping population which can be explored for improvement of sweetpotato yield under drought stressed conditions. Further studies will be required to confirm stability of these QTLs.

CHAPTER SIX

6. GENERAL CONCLUSIONS AND RECOMMENDATIONS

6.1. General conclusions

Drought reduced root yield, foliage yield, biomass and harvest index. There exists genetic variability for these traits indicating that progress can be made for selection of drought tolerant genotypes through direct selection. Geometric mean productivity (GMP), mean productivity (MP), drought tolerant index (DTI) were identified as suitable traits for selection of drought tolerant genotypes. There was no difference in the use of either estimated genotype BLUP means and drought tolerant indices. Genotypes were grouped based on their means into four groups : drought tolerant and high yielding, drought tolerant and low yielding, drought susceptible and high yielding, drought susceptible and low yielding adapting the method of Gerloff (1977).

For physiological traits ,in the combined analysis for control and drought treatments, genetic variability was not detected due to spatial variability associated with the use of handheld devices for measurements. However, drought resulted in reduced amount of chlorophyll , photosynthetically active radiation, Leaf Area Index and increased canopy temperature in sweetpotato plants. These responses in turn affected the growth and development of the plants. Drought stressed plants had decreased vine length, vine diameter and a smaller number of nodes relative to the control. Genetic variability within genotypes for morphological traits and positive correlation with yield related traits suggests their potential as secondary traits for selection of drought tolerant sweetpotato.

Quantitative trait loci (QTLs) related to root yield, foliage yield, biomass and harvest index were identified under irrigated, drought and rainfed conditions for single sites and across all drought environments. Thirty-two QTLs were detected in association with these traits. QTLs on linkage

group 10 associated with root yield, foliage yield, biomass and harvest index were detected mainly under drought conditions. Conversely, QTLs on linkage group 7 were detected under irrigated and rainfed conditions, suggesting that they are possibly environmental specific and useful for selection of drought tolerant sweetpotato genotypes.

6.2 Recommendations

- The study identified some drought tolerant genotypes from the BxT mapping population. These genotypes should be included in preliminary yield trials for further evaluation in Ghana.
- Physiological traits such as stomatal conductance, carbon isotope ratio, abscisic acid, proline quantification and root system architecture (RSA) be exploited to identify traits with for utilization as secondary traits.
- Quantitative Trait Loci Identified for root yield, foliage yield, biomass and harvest index should be validated and deployed in breeding for drought tolerant genotypes.
- Gene(s) influencing foliage yield and harvest index under drought condition located on linkage group 10 position 136.22 cM should be identified using the *Ipomea trifida* or Ipomea triloba genome as these are the *Ipomea* species with available genome sequence.
- Vine length is an easy to measure trait and was highly heritable under drought conditions. Quantitative Trait Loci for vine length should be mapped to enable its utilization for Marker Assisted Selection in breeding sweetpotato for drought tolerance.
- Physiological traits for screening of sweetpotato genotypes for drought tolerance should be done at different phenological phases to identify the best time for collection of data in order to successfully determine genetic variability within genotypes and under varying treatments. Breeders can adopt the use of high through-put phenotyping methods such as

the use of unmanned or manned aerial vehicles for physiological traits can be explored for large scale phenotyping.

- Other parameters such as stomatal conductance, Carbon Isotope Discrimination, abscisic acid, proline quantification and root system architecture (RSA) not used in this study can be explored for drought phenotyping to identify parameters which are highly heritable and suitable as secondary traits for screening and identification of drought tolerant genotypes.

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APPENDICIES

Appendix 1 Soil information for environments studied

LOCATION	Treatment	pH 1:2.5 H2O	%ORGANIC CARBON	%TOTAL NITROGEN	%ORGANIC MATTER	Exchangeable cations cmol/kg				
						Ca	Mg	K	Na	TEXTURE
W17	Drought(50cm)	6.22	0.62	0.05	1.06	0.94	0.27	0.33	0.19	LOAMY SAND
	Drought(20cm)	5.99	0.55	0.05	0.95	0.99	0.4	0.24	0.15	LOAMY SAND
	Irrigation(50cm)	5.55	0.4	0.03	0.69	1.34	0.54	0.26	0.12	LOAMY SAND
	Irrigation(20cm)	5.21	0.6	0.05	1.03	1.34	0.6	0.36	0.24	LOAMY SAND
N17	Drought(50cm)	5.70	0.67	0.06	1.15	1.94	0.46	0.05	0.03	LOAMY SAND
	Drought(20cm)	5.66	0.4	0.03	0.69	2.01	0.87	0.14	0.06	LOAMY SAND
	Irrigation(50cm)	5.60	0.58	0.05	1.00	2.4	0.87	0.09	0.06	LOAMY SAND
	Irrigation(20cm)	5.35	0.48	0.04	0.83	1.87	1.34	0.05	0.03	LOAMY SAND
N18	Drought(50cm)	5.46	0.33	0.03	0.56	1.22	0.86	0.08	0.03	LOAMY SAND
	Drought(20cm)	5.48	0.59	0.06	1.01	1.28	1.07	0.11	0.04	LOAMY SAND
	Irrigation(50cm)	5.73	0.72	0.08	1.23	1.6	0.85	0.07	0.03	LOAMY SAND
	Irrigation(20cm)	5.66	0.49	0.05	0.84	1.38	1.01	0.09	0.03	LOAMY SAND
	Drought(50cm)									
N16	Rainfed(50cm)	5.22	0.51	0.05		1.36	0.2	0.56		
	Rainfed(20cm)	5.49	0.47	0.05		1.56	0.44	0.57		

Appendix 2 Soil information for environments studied

LOCATION	TREATMENT	T.E.B	EX. ACIDITY (cmol/kg)	ECEC (cmol/kg)	BASE SAT. (cmol/kg)	AVi.BRAYS ppmP	MECHANICAL ANALYSIS % SAND	% CLAY	% SILT
W17	Drought (50cm)	1.73	0.25	1.98	86.87	15.11	84.00	2.00	14.00
	Drought (20cm)	1.78	0.37	2.15	82.96	26.14	82.80	2.00	15.20
	Irrigation (50cm)	2.25	0.5	2.75	80.58	13.55	80.00	3.00	17.00
	Iirrigation (20cm)	2.41	0.65	3.06	78.78	23.60	86.00	3.00	11.00
N17	Drought (50cm)	2.54	0.45	2.99	84.96	3.33	53.00	4.00	43.00
	Drought (20cm)	2.86	0.45	3.31	86.22	1.95	50.00	2.00	48.00
	Irrigation (50cm)	3.62	0.48	4.09	86.72	5.54	47.00	3.00	50.00
	Irrigation (20cm)	2.95	0.58	3.52	83.28	3.79	49.00	2.00	49.00
N18	Drought (50cm)	2.19	0.63	2.82	77.94	5.42	54.00	42.00	4.00
	Drought (20cm)	2.50	0.58	3.07	81.30	5.62	51.00	40.00	9.00
	Irrigation (50cm)	2.56	0.53	3.08	82.97	5.62	50.00	45.00	5.00
	Irrigation (20cm)	2.52	0.60	3.12	81.36	6.50	53.00	43.00	4.00
N16	Rainfed (50cm)						47.88	4.08	48.04
	Rainfed (20cm)						47.88	4.08	48.04

Appendix 3.1 Genotype, mean yield under drought (RY_d), irrigation(RY_c), drought tolerant indices (DTI, MP, GMP, HAR, DSI, PR, SUS, YSI) for drought tolerant and high yielding genotypes

Genotype	RY_d	RY_c	DTI	MP	GMP	HAR	DSI	PR	SUS	YSI	Group
CIP113641.308	4.29	11.29	4.29	7.79	6.96	-0.74	1.07	62.02	7.00	0.38	DTHY
CIP105269.186	4.33	11.25	4.33	7.79	6.98	-0.71	1.06	61.52	6.92	0.38	DTHY
CIP113641.183	4.36	10.65	4.36	7.51	6.82	-0.80	1.01	59.06	6.29	0.41	DTHY
CIP113641.42	2.10	10.13	2.10	6.11	4.61	-0.57	1.36	79.29	8.03	0.21	DTHY
CIP113641.355	3.57	9.07	3.57	6.32	5.69	-0.79	1.04	60.66	5.50	0.39	DTHY
CIP105269.135	6.70	8.93	6.70	7.81	7.73	-0.87	0.43	24.98	2.23	0.75	DTHY
CIP113641.69	3.14	8.82	3.14	5.98	5.26	-0.82	1.11	64.41	5.68	0.36	DTHY
CIP113641.14	3.60	8.71	3.60	6.16	5.60	-0.64	1.01	58.66	5.11	0.41	DTHY
CIP105269.102	2.55	8.60	2.55	5.57	4.68	-0.67	1.21	70.40	6.06	0.30	DTHY
CIP113641.108	3.81	8.52	3.81	6.16	5.69	-0.60	0.95	55.33	4.71	0.45	DTHY
CIP113641.307	3.56	8.42	3.56	5.99	5.48	-0.76	0.99	57.67	4.86	0.42	DTHY
CIP105269.125	3.28	8.32	3.28	5.80	5.23	-0.68	1.04	60.55	5.04	0.39	DTHY
CIP113641.251	2.87	8.27	2.87	5.57	4.87	-0.69	1.12	65.36	5.41	0.35	DTHY
CIP113641.282	7.64	8.26	7.64	7.95	7.95	-0.91	0.13	7.56	0.63	0.92	DTHY
CIP113641.247	5.39	8.20	5.39	6.80	6.65	-0.87	0.59	34.22	2.81	0.66	DTHY
CIP113641.278	3.59	8.20	3.59	5.89	5.42	-0.80	0.97	56.24	4.61	0.44	DTHY
CIP105269.197	2.58	8.19	2.58	5.39	4.60	-0.69	1.18	68.47	5.61	0.32	DTHY
CIP113641.127	3.44	8.16	3.44	5.80	5.30	-0.87	0.99	57.87	4.72	0.42	DTHY
CIP105269.115	2.55	8.05	2.55	5.30	4.53	-0.95	1.17	68.32	5.50	0.32	DTHY
CIP113641.38	2.36	7.93	2.36	5.14	4.32	-0.68	1.21	70.27	5.57	0.30	DTHY
CIP113641.255	3.52	7.87	3.52	5.70	5.27	-0.95	0.95	55.24	4.35	0.45	DTHY
CIP113641.3	3.22	7.71	3.22	5.47	4.98	-0.78	1.00	58.28	4.50	0.42	DTHY
CIP113641.181	2.38	7.58	2.38	4.98	4.25	-0.75	1.18	68.54	5.19	0.31	DTHY
CIP113641.244	3.98	7.44	3.98	5.71	5.44	-0.81	0.80	46.52	3.46	0.53	DTHY
CIP113641.303	2.96	7.36	2.96	5.16	4.67	-0.78	1.03	59.79	4.40	0.40	DTHY
CIP105269.147	2.38	7.33	2.38	4.86	4.18	-0.82	1.16	67.55	4.95	0.32	DTHY
CIP105269.119	4.48	7.30	4.48	5.89	5.72	-0.89	0.66	38.68	2.82	0.61	DTHY
CIP113641.351	3.19	7.10	3.19	5.15	4.76	-0.72	0.95	55.02	3.91	0.45	DTHY
CIP113641.321	2.61	7.01	2.61	4.81	4.27	-0.71	1.08	62.81	4.40	0.37	DTHY
CIP113641.33	2.02	6.74	2.02	4.38	3.69	-0.83	1.20	70.02	4.72	0.30	DTHY
CIP113641.324	2.01	6.70	2.01	4.36	3.67	-0.77	1.20	69.94	4.69	0.30	DTHY
CIP105269.118	2.49	6.56	2.49	4.53	4.04	-0.74	1.07	62.03	4.07	0.38	DTHY
CIP113641.113	3.81	6.55	3.81	5.18	4.99	-0.82	0.72	41.87	2.74	0.58	DTHY
CIP113641.234	2.22	6.51	2.22	4.37	3.80	-0.73	1.13	65.89	4.29	0.34	DTHY
CIP113641.401	3.26	6.49	3.26	4.87	4.60	-0.79	0.85	49.75	3.23	0.50	DTHY
CIP113641.375	2.34	6.44	2.34	4.39	3.88	-0.86	1.09	63.67	4.10	0.36	DTHY
CIP105269.83	2.81	6.39	2.81	4.60	4.24	-0.63	0.96	55.94	3.57	0.44	DTHY
CIP105269.45	2.89	6.35	2.89	4.62	4.28	-0.79	0.94	54.49	3.46	0.46	DTHY

Appendix 3.2 Genotype, mean yield under drought (RY_d), irrigation(RY_c), drought tolerant indices (DTI, MP, GMP, HAR, DSI, PR, SUS, YSI for drought tolerant and high yielding genotypes

Genotype	RY_d	RY_c	DTI	MP	GMP	HAR	DSI	PR	SUS	YSI	Group
CIP113641.205	2.04	6.33	2.04	4.19	3.60	-0.69	1.16	67.73	4.29	0.32	DTHY
CIP113641.225	4.09	6.32	4.09	5.20	5.08	-0.92	0.61	35.31	2.23	0.65	DTHY
CIP113641.393	2.76	6.26	2.76	4.51	4.15	-0.78	0.96	55.96	3.50	0.44	DTHY
CIP113641.313	2.04	6.22	2.04	4.13	3.56	-0.92	1.16	67.24	4.18	0.33	DTHY
CIP113641.312	3.36	6.21	3.36	4.79	4.57	-0.86	0.79	45.90	2.85	0.54	DTHY
CIP113641.61	2.74	6.04	2.74	4.39	4.07	-0.86	0.94	54.71	3.31	0.45	DTHY
CIP113641.366	3.31	6.04	3.31	4.68	4.47	-0.77	0.77	45.09	2.72	0.55	DTHY
CIP113641.242	3.18	5.99	3.18	4.59	4.37	-0.91	0.81	46.91	2.81	0.53	DTHY
CIP113641.280	2.92	5.93	2.92	4.42	4.16	-0.92	0.87	50.70	3.00	0.49	DTHY
CIP113641.200	2.44	5.75	2.44	4.09	3.75	-0.87	0.99	57.57	3.31	0.42	DTHY
CIP113641.163	4.10	5.65	4.10	4.87	4.81	-0.93	0.47	27.46	1.55	0.73	DTHY
CIP113641.392	2.92	5.53	2.92	4.22	4.02	-0.84	0.81	47.15	2.61	0.53	DTHY
CIP113641.371	3.31	5.50	3.31	4.41	4.27	-0.81	0.69	39.86	2.19	0.60	DTHY
CIP113641.137	2.24	5.50	2.24	3.87	3.51	-0.83	1.02	59.34	3.26	0.41	DTHY
CIP113641.315	2.19	5.50	2.19	3.85	3.47	-0.69	1.03	60.10	3.31	0.40	DTHY
CIP113641.51	2.67	5.45	2.67	4.06	3.81	-0.91	0.88	51.06	2.78	0.49	DTHY
CIP113641.39	3.40	5.28	3.40	4.34	4.24	-0.85	0.61	35.53	1.88	0.64	DTHY
CIP105269.24	2.47	5.28	2.47	3.87	3.61	-0.76	0.91	53.25	2.81	0.47	DTHY
CIP113641.322	2.24	5.16	2.24	3.70	3.40	-0.90	0.97	56.64	2.92	0.43	DTHY
CIP113641.299	2.72	5.08	2.72	3.90	3.72	-0.94	0.80	46.54	2.37	0.53	DTHY
CIP113641.198	3.11	5.00	3.11	4.06	3.94	-0.77	0.65	37.93	1.90	0.62	DTHY
CIP105269.141	2.45	4.94	2.45	3.70	3.48	-0.80	0.87	50.47	2.50	0.50	DTHY
CIP105269.52	4.80	4.89	4.80	4.84	4.84	-0.99	0.03	1.76	0.09	0.98	DTHY
CIP113641.337	3.01	4.86	3.01	3.93	3.82	-1.05	0.65	38.04	1.85	0.62	DTHY
CIP113641.262	3.17	4.83	3.17	4.00	3.91	-0.86	0.59	34.42	1.66	0.66	DTHY
CIP105269.166	2.19	4.76	2.19	3.47	3.23	-0.86	0.93	54.11	2.58	0.46	DTHY

Appendix 3.3 Genotype, mean yield under drought (RY_d), irrigation(RY_c), drought tolerant indices (DTI, MP, GMP, HAR, DSI, PR, SUS, YSI for drought tolerant and low yielding genotypes

Genotype	RY_d	RY_c	DTI	MP	GMP	HAR	DSI	PR	SUS	YSI	Group
CIP440132	2.16	4.50	2.16	3.33	3.12	-0.81	0.89	51.96	2.34	0.48	DTLY
CIP113641.180	2.07	4.46	2.07	3.27	3.04	-0.91	0.92	53.63	2.39	0.46	DTLY
CIP105269.160	3.23	4.41	3.23	3.82	3.77	-0.84	0.46	26.75	1.18	0.73	DTLY
CIP113641.18	2.50	4.37	2.50	3.44	3.31	-0.91	0.73	42.66	1.86	0.57	DTLY
CIP113641.188	2.45	4.32	2.45	3.39	3.25	-0.88	0.75	43.36	1.88	0.57	DTLY
CIP113641.169	2.09	4.13	2.09	3.11	2.94	-0.90	0.85	49.30	2.04	0.51	DTLY
CIP113641.232	2.02	4.06	2.02	3.04	2.87	-0.83	0.86	50.18	2.04	0.50	DTLY
CIP105269.132	2.70	4.06	2.70	3.38	3.31	-0.88	0.57	33.45	1.36	0.67	DTLY
CIP113641.184	1.69	4.02	1.69	2.85	2.60	-0.96	1.00	58.11	2.34	0.42	DTLY
CIP113641.414	3.75	3.95	3.75	3.85	3.85	-0.97	0.09	5.13	0.20	0.95	DTLY
CIP113641.431	2.12	3.86	2.12	2.99	2.86	-0.78	0.78	45.20	1.75	0.55	DTLY
CIP113641.9	2.13	3.69	2.13	2.91	2.81	-0.92	0.72	42.16	1.56	0.58	DTLY
CIP113641.291	2.55	3.21	2.55	2.88	2.86	-0.80	0.35	20.49	0.66	0.80	DTLY
CIP113641.316	2.54	3.01	2.54	2.78	2.77	-1.00	0.27	15.54	0.47	0.84	DTLY
CIP105269.42	2.72	2.82	2.72	2.77	2.77	-0.90	0.06	3.61	0.10	0.96	DTLY
CIP113641.99	2.67	2.81	2.67	2.74	2.74	-0.93	0.09	4.95	0.14	0.95	DTLY
CIP113641.380	2.57	2.78	2.57	2.68	2.67	-1.23	0.13	7.66	0.21	0.92	DTLY
CIP105269.161	2.15	2.51	2.15	2.33	2.32	-1.00	0.25	14.47	0.36	0.86	DTLY
CIP113641.7	2.00	2.45	2.00	2.23	2.22	-0.94	0.31	18.15	0.44	0.82	DTLY
CIP105269.177	2.70	2.26	2.70	2.48	2.47	-1.03	-0.33	-19.48	-0.44	1.19	DTLY
CIP113641.283	2.35	2.10	2.35	2.23	2.22	-0.97	-0.20	-11.77	-0.25	1.12	DTLY

Appendix 3.4 Genotype, mean yield under drought (RY_d), irrigation(RY_c), drought tolerant indices (DTI, MP, GMP, HAR, DSI, PR, SUS, YSI for drought susceptible and high yielding genotypes

Genotype	RY_d	RY_c	DTI	MP	GMP	HAR	DSI	PR	SUS	YSI	Group
CIP105269.108	1.51	4.80	1.51	3.15	2.69	-0.63	1.18	68.57	3.29	0.31	DSHY
CIP105269.128	1.43	5.46	1.43	3.44	2.79	-0.92	1.27	73.84	4.03	0.26	DSHY
CIP105269.133	1.20	5.70	1.20	3.45	2.62	-0.81	1.36	78.88	4.50	0.21	DSHY
CIP105269.184	1.62	6.31	1.62	3.96	3.19	-0.68	1.28	74.38	4.69	0.26	DSHY
CIP105269.194	1.83	5.06	1.83	3.45	3.05	-0.91	1.10	63.80	3.23	0.36	DSHY
CIP105269.23	1.69	5.96	1.69	3.82	3.17	-0.71	1.23	71.71	4.27	0.28	DSHY
CIP105269.39	1.35	6.27	1.35	3.81	2.91	-0.78	1.35	78.43	4.91	0.22	DSHY
CIP105269.7	1.57	7.07	1.57	4.32	3.34	-0.62	1.34	77.75	5.50	0.22	DSHY
CIP113641.15	1.92	6.75	1.92	4.33	3.60	-0.64	1.23	71.52	4.82	0.28	DSHY
CIP113641.153	1.18	5.38	1.18	3.28	2.52	-0.80	1.34	78.04	4.19	0.22	DSHY
CIP113641.156	0.99	7.25	0.99	4.12	2.68	-0.66	1.48	86.38	6.26	0.14	DSHY
CIP113641.160	1.41	7.26	1.41	4.34	3.20	-0.64	1.38	80.56	5.85	0.19	DSHY
CIP113641.165	1.16	5.48	1.16	3.32	2.52	-0.76	1.36	78.87	4.32	0.21	DSHY
CIP113641.182	1.90	5.84	1.90	3.87	3.33	-0.82	1.16	67.51	3.94	0.32	DSHY
CIP113641.20	1.62	6.57	1.62	4.10	3.26	-0.65	1.29	75.32	4.95	0.25	DSHY
CIP113641.206	1.43	4.85	1.43	3.14	2.63	-0.91	1.21	70.58	3.42	0.29	DSHY
CIP113641.211	0.76	5.28	0.76	3.02	2.01	-0.61	1.47	85.53	4.51	0.14	DSHY
CIP113641.259	1.72	6.10	1.72	3.91	3.24	-0.85	1.23	71.83	4.38	0.28	DSHY
CIP113641.264	1.35	5.94	1.35	3.64	2.83	-0.70	1.33	77.22	4.58	0.23	DSHY
CIP113641.279	1.73	7.13	1.73	4.43	3.51	-0.66	1.30	75.71	5.40	0.24	DSHY
CIP113641.286	1.43	6.28	1.43	3.85	3.00	-0.70	1.33	77.21	4.85	0.23	DSHY
CIP113641.289	1.44	6.20	1.44	3.82	2.99	-0.71	1.32	76.79	4.76	0.23	DSHY
CIP113641.293	1.56	4.77	1.56	3.17	2.73	-0.78	1.16	67.31	3.21	0.33	DSHY
CIP113641.298	1.73	5.18	1.73	3.45	2.99	-0.76	1.15	66.64	3.45	0.33	DSHY
CIP113641.33	0.78	5.34	0.78	3.06	2.04	-0.72	1.47	85.36	4.56	0.15	DSHY
CIP113641.358	1.03	4.78	1.03	2.91	2.22	-0.50	1.35	78.39	3.75	0.22	DSHY
CIP113641.387	1.96	6.21	1.96	4.08	3.49	-0.84	1.18	68.46	4.25	0.32	DSHY
CIP113641.429	1.49	5.30	1.49	3.39	2.81	-0.59	1.24	71.97	3.81	0.28	DSHY
CIP113641.57	1.94	5.10	1.94	3.52	3.15	-0.84	1.07	61.98	3.16	0.38	DSHY
CIP113641.58	1.37	4.96	1.37	3.17	2.61	-0.67	1.24	72.39	3.59	0.28	DSHY

Appendix 3.5 Genotype, mean yield under drought (RY_d), irrigation(RY_c), drought tolerant indices (DTI, MP, GMP, HAR, DSI, PR, SUS, YSI for drought susceptible and low yielding genotypes

Genotype	RY_d	RY_c	DTI	MP	GMP	HAR	DSI	PR	SUS	YSI	Group
CIP105269.100	1.54	4.45	1.54	3.00	2.62	-0.81	1.12	65.35	2.91	0.35	DSLY
CIP105269.101	1.76	3.88	1.76	2.82	2.61	-0.95	0.94	54.71	2.12	0.45	DSLY
CIP105269.114	1.33	2.48	1.33	1.91	1.82	-0.83	0.79	46.17	1.14	0.54	DSLY
CIP105269.119	0.79	0.97	0.79	0.88	0.87	-1.13	0.33	19.05	0.19	0.81	DSLY
CIP105269.120	1.22	3.41	1.22	2.31	2.04	-0.86	1.10	64.17	2.19	0.36	DSLY
CIP105269.124	0.74	3.86	0.74	2.30	1.69	-0.66	1.39	80.94	3.13	0.19	DSLY
CIP105269.135	1.36	3.99	1.36	2.67	2.33	-1.20	1.13	65.97	2.63	0.34	DSLY
CIP105269.144	0.47	2.25	0.47	1.36	1.03	-0.92	1.36	78.97	1.77	0.21	DSLY
CIP105269.156	0.88	2.52	0.88	1.70	1.49	-0.91	1.12	65.07	1.64	0.35	DSLY
CIP105269.157	1.13	2.50	1.13	1.81	1.68	-0.78	0.94	54.73	1.37	0.45	DSLY
CIP105269.160	1.76	2.25	1.76	2.01	1.99	-1.00	0.38	21.89	0.49	0.78	DSLY
CIP105269.163	0.66	1.07	0.66	0.87	0.84	-0.84	0.66	38.24	0.41	0.62	DSLY
CIP105269.167	0.56	1.81	0.56	1.19	1.00	-1.07	1.19	69.36	1.26	0.31	DSLY
CIP105269.176	1.54	2.67	1.54	2.10	2.03	-0.93	0.73	42.46	1.13	0.58	DSLY
CIP105269.177	1.24	2.64	1.24	1.94	1.81	-0.97	0.91	53.24	1.41	0.47	DSLY
CIP105269.198	1.27	3.03	1.27	2.15	1.96	-1.13	1.00	58.17	1.76	0.42	DSLY
CIP105269.22	1.97	3.00	1.97	2.49	2.43	-0.70	0.59	34.28	1.03	0.66	DSLY
CIP105269.42	1.25	1.83	1.25	1.54	1.51	-0.84	0.54	31.39	0.57	0.69	DSLY
CIP105269.50	0.69	2.91	0.69	1.80	1.42	-0.59	1.31	76.27	2.22	0.24	DSLY
CIP105269.51	1.05	3.65	1.05	2.35	1.96	-0.96	1.22	71.19	2.60	0.29	DSLY
CIP105269.52	1.88	2.70	1.88	2.29	2.26	-1.01	0.52	30.19	0.81	0.70	DSLY
CIP105269.54	1.89	3.15	1.89	2.52	2.44	-0.95	0.69	39.91	1.26	0.60	DSLY
CIP105269.59	1.80	2.60	1.80	2.20	2.16	-1.05	0.53	30.60	0.79	0.69	DSLY
CIP105269.6	1.06	3.51	1.06	2.29	1.93	-0.85	1.20	69.64	2.44	0.30	DSLY
CIP105269.68	0.70	1.15	0.70	0.92	0.89	-0.89	0.67	39.15	0.45	0.61	DSLY
CIP105269.75	1.28	2.22	1.28	1.75	1.68	-1.10	0.73	42.38	0.94	0.58	DSLY
CIP105269.82	0.94	1.62	0.94	1.28	1.23	-0.81	0.72	41.86	0.68	0.58	DSLY
CIP105269.85	0.81	2.80	0.81	1.80	1.50	-0.51	1.22	71.21	1.99	0.29	DSLY
CIP105269.9	1.80	2.01	1.80	1.90	1.90	-1.05	0.18	10.60	0.21	0.89	DSLY
CIP105269.90	1.06	1.55	1.06	1.31	1.28	-1.21	0.54	31.21	0.48	0.69	DSLY
CIP105269.99	0.74	1.76	0.74	1.25	1.14	-0.77	0.99	57.84	1.02	0.42	DSLY
CIP113641.101	1.02	4.21	1.02	2.62	2.08	-0.81	1.30	75.72	3.19	0.24	DSLY
CIP113641.112	0.85	4.65	0.85	2.75	1.99	-0.58	1.41	81.79	3.81	0.18	DSLY
CIP113641.12	1.40	4.67	1.40	3.04	2.56	-0.85	1.20	69.91	3.26	0.30	DSLY
CIP113641.140	0.72	3.46	0.72	2.09	1.58	-0.56	1.36	79.28	2.75	0.21	DSLY
CIP113641.152	0.93	2.15	0.93	1.54	1.41	-0.82	0.97	56.68	1.22	0.43	DSLY
CIP113641.169	1.43	2.38	1.43	1.91	1.85	-1.00	0.69	40.00	0.95	0.60	DSLY
CIP113641.174	0.91	2.40	0.91	1.66	1.48	-0.91	1.07	62.25	1.50	0.38	DSLY
CIP113641.180	1.69	4.02	1.69	2.85	2.60	-0.96	1.00	58.11	2.34	0.42	DSLY

Appendix 3.6 Genotype, mean yield under drought (RY_d), irrigation(RY_c), drought tolerant indices (DTI, MP, GMP, HAR, DSI, PR, SUS, YSI for drought susceptible and low yielding genotypes

Genotype	RY_d	RY_c	DTI	MP	GMP	HAR	DSI	PR	SUS	YSI	Group
CIP113641.19	1.67	4.56	1.67	3.11	2.76	-0.89	1.09	63.40	2.89	0.37	DSLY
CIP113641.196	1.39	4.40	1.39	2.89	2.47	-0.77	1.18	68.42	3.01	0.32	DSLY
CIP113641.198	1.74	4.05	1.74	2.89	2.65	-0.93	0.98	57.14	2.31	0.43	DSLY
CIP113641.205	1.77	4.56	1.77	3.16	2.84	-0.78	1.05	61.22	2.79	0.39	DSLY
CIP113641.225	1.31	2.17	1.31	1.74	1.68	-0.75	0.68	39.53	0.86	0.60	DSLY
CIP113641.227	0.29	1.28	0.29	0.78	0.61	-0.42	1.33	77.54	0.99	0.22	DSLY
CIP113641.228	0.85	3.57	0.85	2.21	1.74	-0.78	1.31	76.13	2.72	0.24	DSLY
CIP113641.232	1.85	4.08	1.85	2.97	2.75	-0.97	0.94	54.60	2.23	0.45	DSLY
CIP113641.235	1.61	2.28	1.61	1.94	1.92	-1.11	0.50	29.27	0.67	0.71	DSLY
CIP113641.237	0.95	2.61	0.95	1.78	1.57	-0.94	1.09	63.64	1.66	0.36	DSLY
CIP113641.250	0.31	1.62	0.31	0.97	0.71	-0.84	1.39	80.86	1.31	0.19	DSLY
CIP113641.266	1.30	3.32	1.30	2.31	2.08	-0.58	1.04	60.81	2.02	0.39	DSLY
CIP113641.27	1.27	4.41	1.27	2.84	2.37	-0.67	1.22	71.14	3.14	0.29	DSLY
CIP113641.283	0.76	0.98	0.76	0.87	0.86	-1.27	0.38	22.27	0.22	0.78	DSLY
CIP113641.284	0.78	2.23	0.78	1.50	1.32	-0.86	1.12	65.17	1.45	0.35	DSLY
CIP113641.299	1.71	1.90	1.71	1.80	1.80	-0.98	0.17	10.12	0.19	0.90	DSLY
CIP113641.30	1.95	3.77	1.95	2.86	2.71	-1.06	0.83	48.16	1.81	0.52	DSLY
CIP113641.301	0.51	3.14	0.51	1.83	1.27	-0.56	1.44	83.65	2.63	0.16	DSLY
CIP113641.313	1.54	4.41	1.54	2.97	2.61	-0.93	1.12	65.02	2.87	0.35	DSLY
CIP113641.316	0.82	0.80	0.82	0.81	0.81	-1.15	-0.04	-2.31	-0.02	1.02	DSLY
CIP113641.324	1.28	4.20	1.28	2.74	2.32	-0.74	1.19	69.49	2.92	0.31	DSLY
CIP113641.328	0.86	2.58	0.86	1.72	1.49	-0.64	1.14	66.59	1.72	0.33	DSLY
CIP113641.337	0.68	1.50	0.68	1.09	1.01	-0.98	0.95	55.05	0.83	0.45	DSLY
CIP113641.349	0.67	3.67	0.67	2.17	1.57	-0.68	1.40	81.71	3.00	0.18	DSLY
CIP113641.353	0.88	2.24	0.88	1.56	1.40	-0.85	1.04	60.66	1.36	0.39	DSLY
CIP113641.359	1.30	4.05	1.30	2.68	2.30	-0.70	1.17	67.89	2.75	0.32	DSLY
CIP113641.360	1.06	4.65	1.06	2.86	2.22	-0.90	1.33	77.17	3.59	0.23	DSLY
CIP113641.361	1.00	4.66	1.00	2.83	2.16	-0.70	1.35	78.55	3.66	0.21	DSLY
CIP113641.367	1.48	4.47	1.48	2.97	2.57	-0.85	1.15	66.84	2.99	0.33	DSLY
CIP113641.369	0.96	5.74	0.96	3.35	2.34	-0.99	1.43	83.29	4.78	0.17	DSLY
CIP113641.37	0.37	1.34	0.37	0.85	0.70	-0.66	1.24	72.32	0.97	0.28	DSLY
CIP113641.370	1.66	4.61	1.66	3.13	2.76	-0.74	1.10	64.06	2.95	0.36	DSLY
CIP113641.376	0.48	2.83	0.48	1.66	1.17	-0.95	1.43	82.95	2.35	0.17	DSLY
CIP113641.379	1.59	2.26	1.59	1.93	1.90	-1.14	0.51	29.54	0.67	0.70	DSLY
CIP113641.384	1.92	4.35	1.92	3.13	2.89	-0.75	0.96	55.93	2.43	0.44	DSLY
CIP113641.385	1.89	4.56	1.89	3.22	2.94	-0.67	1.00	58.44	2.66	0.42	DSLY
CIP113641.390	1.41	3.71	1.41	2.56	2.29	-0.85	1.06	61.91	2.30	0.38	DSLY
CIP113641.403	1.06	3.34	1.06	2.20	1.88	-0.83	1.17	68.28	2.28	0.32	DSLY
CIP113641.408	0.62	1.87	0.62	1.24	1.07	-0.74	1.15	67.00	1.25	0.33	DSLY

Appendix 3.7 Genotype, mean yield under drought (RY_d), irrigation(RY_c), drought tolerant indices (DTI, MP, GMP, HAR, DSI, PR, SUS, YSI for drought susceptible and low yielding genotypes

Genotype	RY_d	RY_c	DTI	MP	GMP	HAR	DSI	PR	SUS	YSI	Group
CIP113641.411	1.55	4.16	1.55	2.85	2.54	-0.93	1.08	62.83	2.61	0.37	DSLY
CIP113641.413	0.97	2.41	0.97	1.69	1.53	-0.72	1.02	59.56	1.44	0.40	DSLY
CIP113641.45	0.61	2.38	0.61	1.50	1.20	-0.78	1.28	74.56	1.78	0.25	DSLY
CIP113641.59	1.53	1.59	1.53	1.56	1.56	-0.85	0.06	3.78	0.06	0.96	DSLY
CIP113641.62	0.84	2.79	0.84	1.82	1.54	-0.58	1.20	69.76	1.95	0.30	DSLY
CIP113641.63	0.74	1.01	0.74	0.87	0.86	-1.13	0.46	26.83	0.27	0.73	DSLY
CIP113641.68	1.49	4.55	1.49	3.02	2.60	-0.70	1.16	67.34	3.06	0.33	DSLY
CIP113641.70	0.28	1.27	0.28	0.78	0.59	-0.87	1.34	78.23	1.00	0.22	DSLY
CIP113641.78	1.44	3.14	1.44	2.29	2.13	-0.73	0.93	54.05	1.70	0.46	DSLY
CIP113641.80	1.41	4.63	1.41	3.02	2.56	-0.69	1.19	69.53	3.22	0.30	DSLY
CIP113641.86	0.16	1.73	0.16	0.94	0.52	-0.38	1.56	90.90	1.57	0.09	DSLY
CIP113641.93	0.33	1.58	0.33	0.96	0.72	-1.02	1.36	79.24	1.25	0.21	DSLY
CIP440166	0.14	0.62	0.14	0.38	0.29	-0.33	1.34	78.11	0.49	0.22	DSLY

Appendix 4.1 Mean of top 20 and bottom 20 performing genotypes for chlorophyll content (CC) and canopy temperature(CT) under irrigated (Irr) and drought conditions (Drgt)

Genotype	CC_Irr	Genotype	CC_Drgt	Genotype	CT_Irr	Genotype	CT_Drgt
CIP113641.259	57.06	CIP113641.322	31.15	CIP105269.127	21.45	CIP113641.308	27.69
CIP105269.161	55.11	CIP105269.115	29.83	CIP113641.431	21.69	CIP105269.120	34.06
CIP113641.86	51.99	CIP105269.50	29.06	CIP113641.108	22.29	CIP105269.100	34.70
CIP113641.237	51.59	CIP113641.301	28.47	CIP113641.278	22.48	CIP113641.351	34.86
CIP113641.232	48.98	CIP105269.157	28.07	CIP113641.411	22.76	CIP113641.431	35.02
CIP113641.313	48.43	CIP113641.152	28.02	CIP105269.125	23.25	CIP113641.319	35.12
CIP105269.52	48.34	CIP113641.321	26.26	CIP113641.174	23.36	CIP113641.37	35.16
CIP113641.312	47.44	CIP105269.160	26.25	CIP105269.167	23.49	CIP113641.301	35.49
CIP113641.113	46.39	CIP105269.104	26.18	CIP105269.59	23.51	CIP113641.235	35.54
CIP105269.118	46.01	CIP113641.1	25.98	CIP113641.156	23.62	CIP105269.144	35.58
CIP113641.369	24.99	CIP113641.360	12.22	CIP113641.38	33.17	CIP105269.168	38.71
CIP113641.278	24.95	CIP105269.163	11.86	CIP113641.206	33.36	CIP113641.45	38.75
CIP113641.367	24.63	CIP113641.278	11.71	CIP113641.232	33.47	CIP113641.322	38.90
CIP113641.291	24.23	CIP113641.174	11.64	CIP113641.359	33.56	CIP113641.20	38.97
CIP105269.125	23.88	CIP113641.153	11.35	CIP113641.152	33.58	CIP113641.163	39.01
CIP105269.163	23.54	CIP105269.198	11.14	CIP113641.237	34.59	CIP113641.19	39.04
CIP113641.431	22.94	CIP113641.93	10.86	CIP113641.313	34.87	CIP105269.22	39.29
CIP105269.127	22.43	CIP105269.135	10.78	CIP113641.259	35.01	CIP105269.85	39.53
CIP113641.59	22.17	CIP105269.100	9.50	CIP113641.62	35.05	CIP105269.90	41.26
CIP113641.153	21.31	CIP113641.369	8.09	CIP105269.161	35.55	CIP113641.205	41.33
CIP440132 (P)	37.12	CIP440132 (P)	16.66	CIP440132 (P)	28.73	CIP440132 (P)	37.48
CIP440166 (P)	29.54	CIP440166 (P)	20.53	CIP440166 (P)	29.45	CIP440166 (P)	37.56
min	21.31		8.09		21.45		27.69
max	57.06		31.15		35.55		41.33
mean	36.50		19.35		28.56		36.96
SE	0.606		0.384		0.256		0.138

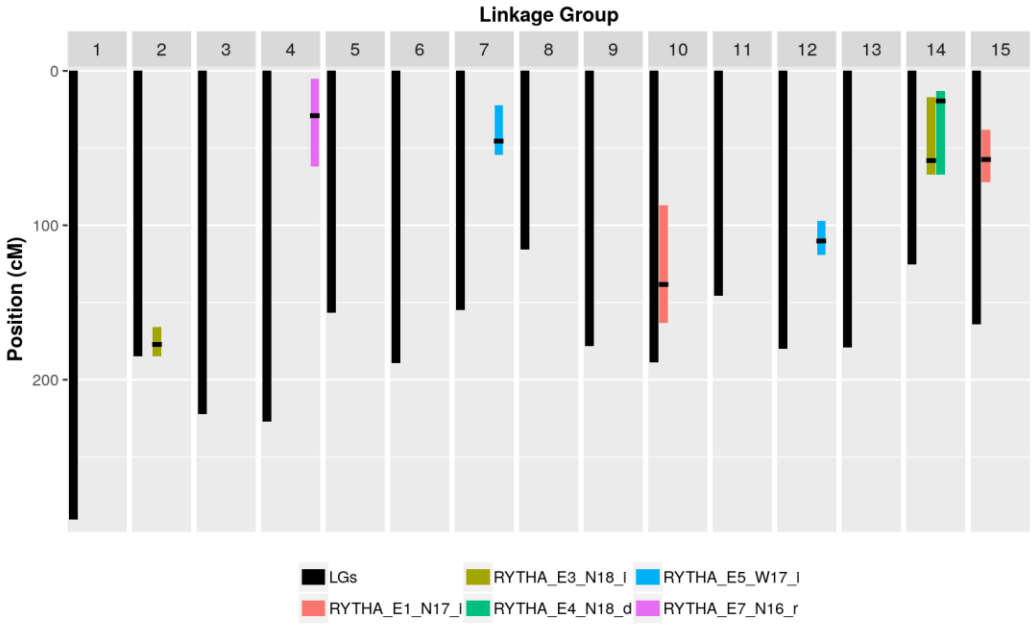
Appendix 4.2 Mean of top 20 and bottom 20 performing genotypes for NDVI and LAI under irrigated (Irr) and drought (Drgt) conditions

Genotype	NDVI_Irr	Genotype	NDVI_Drgt	Genotype	LAI_Irr	Genotype	LAI_Drgt
CIP113641.255	82.26	CIP113641.188	74.3	CIP113641.108	14.18	CIP113641.255	12.15
CIP113641.62	81.22	CIP105269.39	71.7	CIP113641.255	12.97	CIP113641.108	11.94
CIP105269.108	79.17	CIP105269.119	71.7	CIP105269.121	12.69	CIP113641.235	11.22
CIP105269.115	78.49	CIP113641.235	71.6	CIP113641.93	12.12	CIP105269.54	11.18
CIP113641.328	78.37	CIP113641.86	71.6	CIP105269.54	11.88	CIP105269.121	10.97
CIP113641.140	78.33	CIP113641.301	71.5	CIP105269.177	11.76	CIP113641.355	10.95
CIP113641.86	78.21	CIP105269.141	71.2	CIP113641.235	11.72	CIP105269.177	10.78
CIP113641.384	78.16	CIP113641.108	70.2	CIP113641.355	10.93	CIP113641.172	10.53
CIP113641.370	78.09	CIP113641.385	70.1	CIP113641.172	10.91	CIP105269.100	10.43
CIP113641.358	77.9	CIP113641.57	70.1	CIP105269.102	10.89	CIP113641.408	10.23
CIP113641.200	68.42	CIP105269.128	52.6	CIP105269.99	5.15	CIP105269.75	4.44
CIP113641.184	68.28	CIP113641.156	52.5	CIP113641.37	5	CIP105269.43	4.43
CIP113641.315	68.19	CIP113641.27	52.5	CIP113641.1	4.87	CIP105269.38	4.33
CIP113641.137	67.88	CIP113641.328	52.5	CIP105269.124	4.83	CIP113641.358	4.24
CIP105269.160	67.5	CIP113641.20	52.2	CIP113641.266	4.69	CIP105269.82	4.09
CIP105269.45	67.25	CIP105269.156	51.8	CIP113641.188	4.37	CIP113641.200	4.08
CIP113641.58	67.02	CIP105269.120	50	CIP105269.9	4.23	CIP113641.237	3.94
CIP105269.43	66.97	CIP113641.19	49.6	CIP113641.237	4.17	CIP113641.37	3.81
CIP113641.316	65	CIP113641.413	48.6	CIP113641.328	3.65	CIP105269.9	3.68
CIP113641.408	63.19	CIP113641.205	44.7	CIP105269.168	3.27	CIP105269.168	2.66
CIP440132 (P)	69.45	CIP440132 (P)	61.7	CIP440132 (P)	9.29	CIP440132 (P)	8.06
CIP440166 (P)	75.88	CIP440166 (P)	65.1	CIP440166 (P)	5.04	CIP440166 (P)	4.96
min	63.19		44.68		3.27		2.66
max	82.26		74.31		14.18		12.15
mean	72.97		61.27		8.12		7.41
SE	0.279		0.468		0.175		0.161

Appendix 4.3 Mean of top 20 and bottom 20 performing genotypes for Photosynthetically Active Radiation (PAR) under irrigated and drought conditions

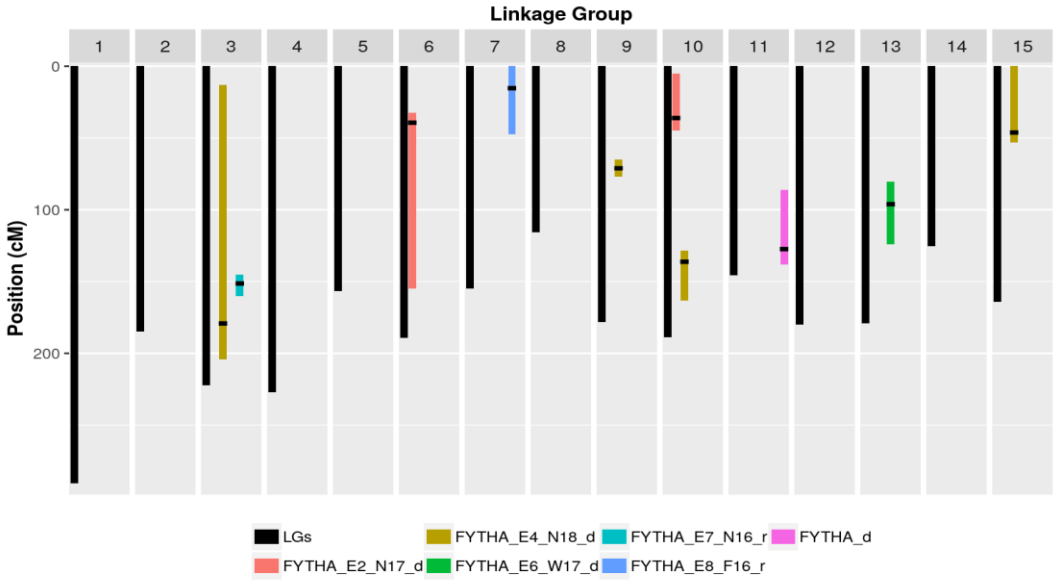
Genotype	%PAR_Irr	Genotype	%PAR_Drgt
CIP113641.255	302.51	CIP113641.39	209.14
CIP113641.112	297.05	CIP105269.119	208.17
CIP105269.121	267.67	CIP105269.23	198.54
CIP113641.93	266.13	CIP105269.68	186.69
CIP113641.172	247.98	CIP113641.284	185.45
CIP113641.108	241.35	CIP113641.27	181.03
CIP113641.315	233.66	CIP113641.355	178.78
CIP113641.234	229.60	CIP113641.78	178.53
CIP113641.182	227.46	CIP113641.12	178.52
CIP105269.177	222.23	CIP105269.121	177.70
CIP105269.99	148.06	CIP113641.379	131.93
CIP113641.237	147.23	CIP105269.52	130.54
CIP105269.83	145.93	CIP113641.152	130.24
CIP113641.328	143.36	CIP105269.104	127.04
CIP105269.9	142.91	CIP105269.161	126.95
CIP113641.266	141.67	CIP105269.22	126.16
CIP105269.168	139.81	CIP105269.45	123.19
CIP105269.82	134.29	CIP113641.413	121.50
CIP113641.188	130.27	CIP105269.167	104.24
CIP105269.135	119.66	CIP105269.99	79.21
CIP440132 (P)	194.99	CIP440132 (P)	151.81
CIP440166 (P)	151.80	CIP440166 (P)	156.56
min	119.66		79.21
max	302.51		209.14
mean	194.35		154.18
SE	2.722		1.631

Appendix 5.1 Support intervals for QTLs detected on linkage groups for storage root yield (RY) per environment



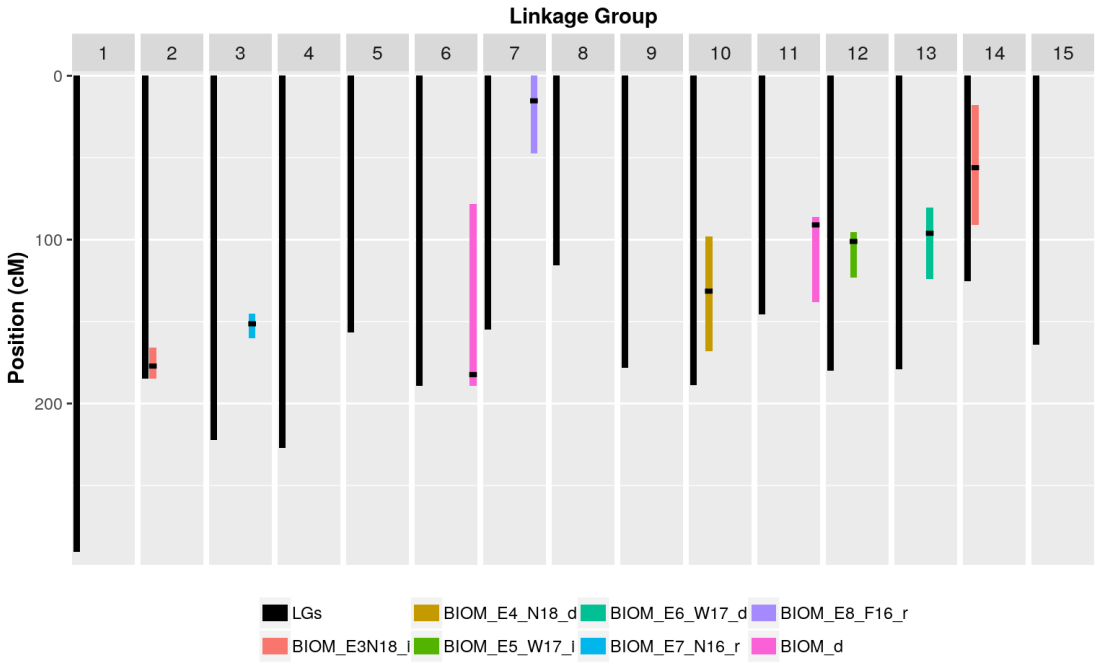
*Environments: E1- Nyankpala 2017 irrigation, E3- Nyankpala 2018 irrigation, E4- Nyankpala 2018 drought, E5- Wenchi 2017 irrigation, E7- Nyankpala 2016 rainfall

Appendix 5.2 Support intervals for QTLs detected on linkage groups for foliage yield (FY) per environment



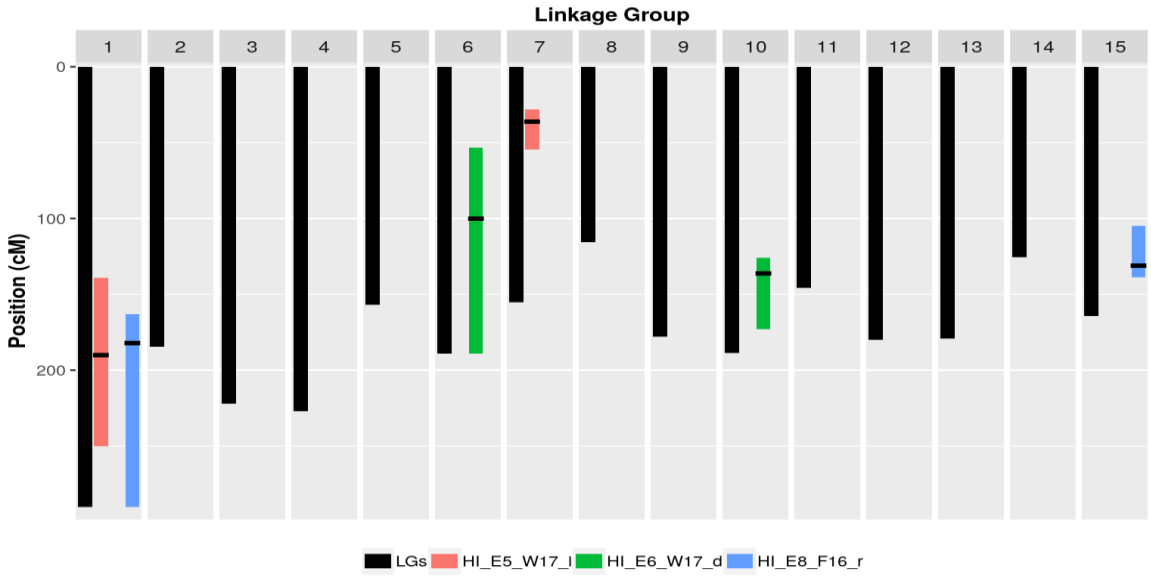
*Environments: E2- Nyankpala 2017 drought, E4- Nyankpala 2018 drought, E6- Wenchi 2017 drought, E7- Nyankpala 2016 rainfall, E8-Fumesua 2016 rainfall, FY_d- foliage yield for combined drought environments

Appendix 5.3 Support intervals for QTLs detected on linkage groups for biomass (BIOM) per environment



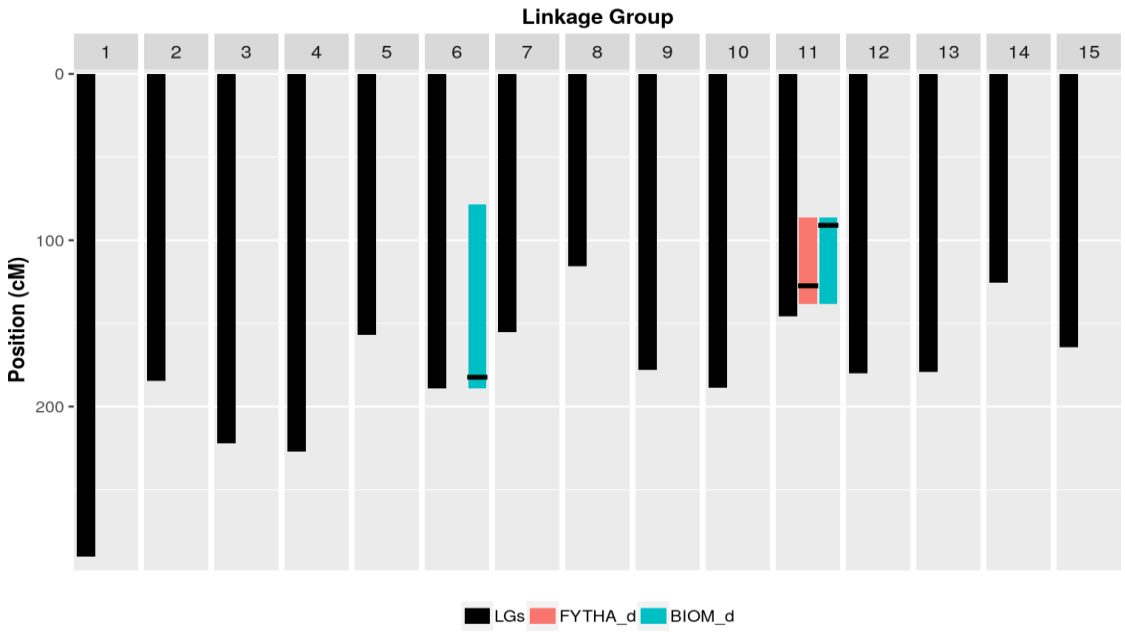
*Environments: E3- Nyankpala 2018 irrigation, E4- Nyankpala 2018 drought, E5- Wenchi 2017 irrigation, E6- Wenchi 2017 drought, E7- Nyankpala 2016 rainfall, E8-Fumesua 2016 rainfall, BIOM_d- biomass for combined drought environments

Appendix 5.4 Support intervals for QTLs detected on linkage groups for harvest index (harvest index) per environment



*Environments: E5- Wenchi 2017 irrigation, E6- Wenchi 2017- drought, E8- Fumesua 2016 rainfall

Appendix 5.5 Support intervals for QTLs detected on linkage groups for foliage yield (FY) and biomass (BIOM) under combined drought environments



Environments: c- combined irrigated environments, d- combined drought environments

Appendix-6

Protocol for Drought Tolerance Field Evaluation of Sweetpotato (developed for the GT4SP drought phenotyping experiments)

Gemenet DC, Gruneberg Wolfgang, Awais Khan*

(A) Experimental design:

The experimental design chosen will depend on the objectives of a given experiment. For quantitative genetic experiments under field conditions, we shall mainly apply α-lattice designs unless otherwise dictated by the experiment.

- **α-lattice design**

This is a resolvable incomplete block design which is just an improved form of the randomized complete block design (RCBD) that most people are familiar with. The improvement involves partitioning the variation within one complete block (normally referred to as replication) into incomplete blocks (normally just referred to as blocks). The partitioning is meant to further increase the precision of an experiment by reducing experimental error through comparing of genotypes under more uniform conditions. This design is desirable for quantitative genetic studies which have to deal with many genotypes because many genotypes imply larger replications (complete blocks) and as the replications get larger, conditions within the replications get more heterogeneous.

- **Basics of the α-lattice design in a simple language**

The design depends on the number of genotypes and the number of rows desired for each genotype in one replication. For the purpose of this protocol, we shall use n = number of genotypes = 300. If we have 300 genotypes for an RCBD, it means each genotype will appear in one replication one

time thereby making 300 genotypes in one replication and we will compare the 300 genotypes together. The same goes for the lattice design. All the genotypes will appear in each replication one time; however each genotype will be compared with its close neighbors before being compared with those in the whole replication. To be able to do this, we need to have 2 numbers which when multiplied, result in 300 (our genotype number). We can have several options of this e.g. 20 x 15, 30 x 10, 50 x 6 etc. If a α-lattice design is indicated as a 20 x 15 α-lattice design it means that there will be 20 incomplete blocks in one replication and each of these incomplete blocks will be having 15 plots (i.e. 15 genotypes being compared). That is, the first number indicates the number of blocks in a replication and the second number indicates the number of plots in each block, where plot = genotypes. Since the objective is to be able to compare genotypes with relatively uniform conditions, the fewer the number of plots in an incomplete block, the better the design. For example, if we have a between-row spacing of 1m, and two rows per genotype, in this case 50 x 6 α-lattice design will have more precision than a 20 x 15 because in the first design, we are comparing genotypes which are 12 m apart while in the second design we compare genotypes which are 30 m apart. So for the purposes of this protocol we will take the 50 x 6 α-lattice design.

- **Setting of an α-lattice**

Assume our example above: 300 genotypes to be planted with spacing of 1m between rows and 0.3 m between plants in a row and each genotype having two-rows of eight plants each i.e. two-row plots. Consider also that we have selected the 50 x 6 α-lattice design implying that in each replication we will have 50 incomplete blocks each consisting of 6 plots (where a plot = the number of rows per genotype). This therefore implies we will have 12 rows in each incomplete block (i.e. 2 rows per genotype (plot) x 6 plots per incomplete block). Because we have 12 rows for each incomplete block, it means that we can only set the experiment to have the number of rows which

is a multiple of 12 on each strip of land (since the term 'Block' is used as part of the design here, we will not use it to refer to the number of pieces of land included in each replication. We shall refer to these as strips (Fig. 1). In this case therefore we can have P incomplete blocks per each strip and T strips per replication as long as P x T = our total number of incomplete blocks per replication which is 50. This means that we could have P = 2 and T = 25, or P = 5 and T = 10 or vice versa. The guiding principle of choosing how many Ps or Ts is that we intend to make the complete block, i.e. the replication as compact as possible i.e. as square as possible. This will therefore depend on the spacing. Assuming our example (Fig 1), the spacing between rows is 1 m implying that each incomplete block = 12 m and each strip = 2.4 m i.e. 0.3 m between plants and 8 plants per row, if we choose P = 2 and T = 25 it means that we will have 24 m (12 rows per incomplete block x 2 incomplete blocks per strip) x 85 m (25 strips per replication x 2.4 m per strip + 25 m which forms the 1 m spacing from one strip to the next). This is hardly a square! What happens if we lay it the other way round i.e. P = 25, T = 2, then we have 300 m (= 12 m per incomplete block x 25 incomplete blocks) x 5.8 m (2.4 m per strip x 2 strips per replication + 1 m between the strips). This is also hardly a square! So the 2 and 25 for either P or T is not our design of choice. Let us then look at the P = 5 and T = 10 scenario. This will give us 60 m (12 m per incomplete block x 5 incomplete blocks per strip) x 34 m (2.4 m per strip x 10 strips per replication + 10 m between the strips). What happens if we change the setting the other way round? In this case P = 10 and T = 5 and we have 120 m x 17 m.

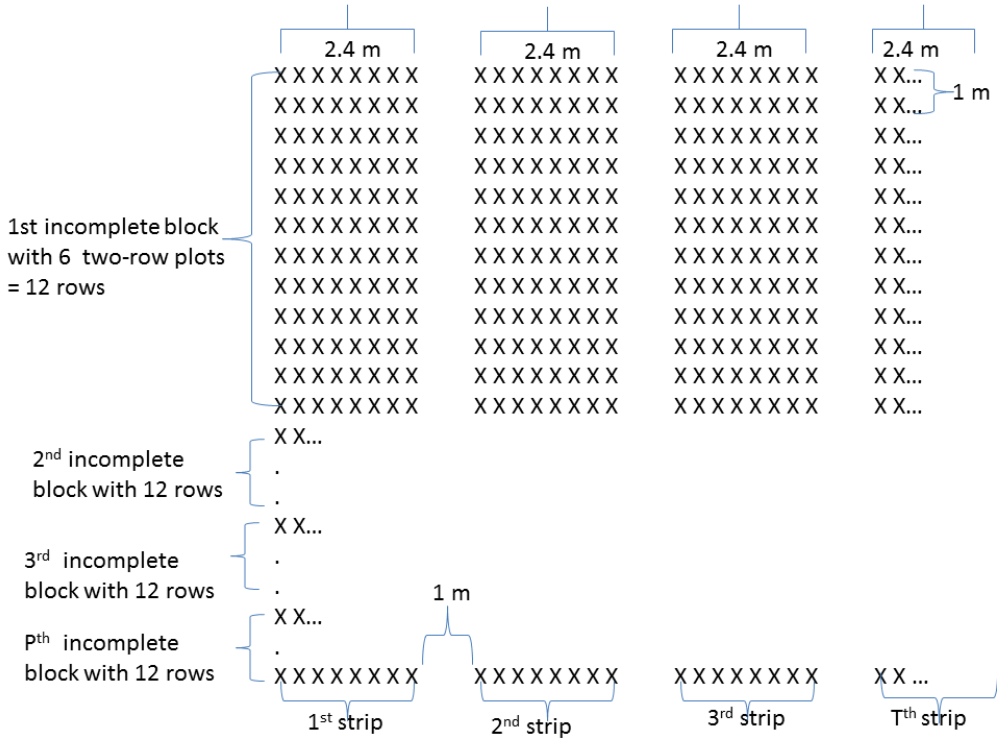


Fig. 1: A representation of an α-lattice resolvable design involving 6 two-row plots in each incomplete block showing the 1st through the Pth incomplete block and the 1st through the Tth strip.

In a scenario with 300 genotypes planted in two-row plots with inter-row spacing of 1 m and intra-row spacing of 0.3 m with 8 plants per row, we would use a 50 x 6 α-lattice design, with each replication having 60 rows (this number only refers to the actual experiment excluding the border rows) i.e. 5 incomplete blocks of 12 rows each, and 10 strips of 2.4 m each with 1m spacing between the strips requiring about 60 m x 34 m per replication.

(B) Soil analysis

Soil sampling will be done based on the field plan before the experiment is started. Each intended replication will have two composite samples i.e. each replication will be divided into two halves and 5 soil samples will be randomly taken from each half in a zigzag way in a way that takes a representation of the sampled area. The 5 soil samples will then be mixed to form one composite

sample for each half of a replication. The soil will be collected using a clean and rust-free auger to a depth of 20 cm and 50 cm. About 500g of the composite samples will be clearly labeled and submitted to the laboratory for analysis. For example if we have two drought treatments (stressed and irrigated) each with three replications, we will need 12 composite samples from the 20 cm depth (2 composite samples for each replication for 3 replications and 2 drought treatments) and 12 composite samples from the 50cm depth, making a total of 24 samples.

(C) Weather station

A weather station will be installed in the field to measure rainfall, temperature together with relative humidity, and solar radiation. Photosynthetically active radiation PAR could then be calculated as half of the total solar radiation. Data should be recorded every 20 minutes per day and uploaded regularly. For this purpose, a microclimate kit from Decagon devices will be used**.**

(D) Drought treatments

Given the size of quantitative genetic populations, we shall only have two treatments water stressed (terminal stress) and non-stressed (control) treatment).

A drip irrigation system which avoids water flow between treatments and gives better control and precision on irrigation should be installed in the field where the experiments will be carried out.

- ***Control experiment (irrigated experiment)***

This treatment needs to be raised with optimum water conditions for sweet potato. Frequency, duration and intensity of irrigation will depend on the soil types, weather conditions and crop stage. We will maintain soil at field capacity for the control experiment. There is therefore need to determine the actual field capacity of the soil where the experiment is held. As a rule of thumb,

field capacity for most soils has been estimated to be around -1/3 = -0.33 bars or -0.033 Mpa and the soil water potential for the control should therefore not decline below -0.033 Mpa. These levels of water will be maintained by monitoring using soil water potential sensors.

- ***Water-stress treatment (drought experiment)***

For this treatment, the experiment will be irrigated like the control experiment until the time of initiation of drought stress (45 days after transplanting (DAT)). Then irrigation will be suspended until just above permanent wilting point (≥ -1.2 MPa) then maintained until harvest. The levels of stress will be maintained by monitoring with soil water potential sensors.

- **Soil water potential monitoring**

A water potential sensor (with a 75 m extension cable) measuring both soil water potential and soil temperature will be installed in each replication for each treatment. This sensor will be moved around the replication to take representative measurements for the replication in order to make irrigation decisions.

- ***Equipment:***

MPS-6 calibrated water potential sensors from Decagon devices (*A Procheck tensiometer was used in my study)*

EM50 data logger

(E) Data collection

All physiological traits listed below will be measured at least once before initiation of the stress to be able to monitor progress of the stress.

- **Physiological parameters**

All physiological traits will be measured at least two times (preferably 3 times) in the whole growth cycle.

For my study: Physiological parameters were measured at two time points. The first measurement was done 40 days after planting, which was 30 days prior to imposition of water stress. The second measurement was done 30 days after drought imposition, which was 100 days after planting.

These traits will include:

- ***Normalized difference vegetation index (NDVI)***

Instrument: Green seeker (*A FieldScout CM 1000 NDVI Chlorophyll Meter was used in my study*)

Hold the green seeker 80 cm above the canopy. With the trigger still engaged, move the seeker along the canopy of the first row, release the trigger after moving along the row and record the average reading for the row. Do this again for the remaining rows of the plot and record the average readings. The plot mean will be the average of the single row readings per plot. In cases where the canopy is filling the space in-between the rows, the green seeker will be moved along the four rows at once before releasing the trigger and the average per plot will be recorded as the plot reading.

- ***Canopy temperature (CT)***

Equipment: Infra-red thermometer (*The Raytek Raynger MX2 Infrared Thermometer was used in my study*)

Measurements should be made when drought stress is well developed since the correlation between canopy temperature and plant water status becomes stronger with decreasing plant water status. The measurements should be done between 10.00-2.00. Place the thermometer 50 cm above the canopy and focus on leaves only while avoiding other materials including soil. For the 4-row plot, we will take measurements on top of each of the rows and get the average of the rows as the plot mean. In cases where the canopy is covering the space between the rows, then we will measure the top of the rows and in-between the rows to get the plot mean based on these readings.

- ***Chlorophyll content (CC)***

Equipment: SPAD 502 plus chlorophyll meters (*The CCM-200plus Chlorophyll Content Meter by Optisciences was used in my study*)

In a 4-row plot of4 plants each, we will measure 3 leaves per plant and 4 representative plants per plot. Clamp the effective open chamber of the spectrophotometer on a selected leaf and make the reading. The mean plot chlorophyll content will be the mean of all readings (i.e. 3 leaves x 4 plants = 12) taken from a given plot. Preferably use the top fully developed leaves.

- ***Leaf Area Index (LAI) and Photosynthetically Active Radiation (PAR)***

Equipment: AccuPAR LP-80

One stationary sensor will be used to take the above canopy readings while the second sensor will be used to take the canopy readings. Use the sensor to take canopy measurements in four representative places of the plot. We will measure the canopies of each of the rows individually and get the average. In cases where the canopies are filling the space between the rows, we will get the plot average including measurement in-between the rows also**.**

- **Agromorphological traits**

Agronomic traits will be measured according to the breeding trial protocol by Gruneberg and colleagues (2009). Please check that the acronyms are in line with those used in SASHA project so we use standardized protocols.

- **Pre-harvest data**

1. NOPS= number of plant planted per plot where plot is equal to genotype
2. NOPE = Number of plants established per plot (to be determined 3 weeks after planting)
3. VIR1 = The first score of virus attack to be done 5 weeks after planting scored on a scale of 1-9 as described below

Scale	Description
1	No virus symptoms
2	Unclear virus symptoms
3	Clear virus symptoms on <5% of plants per plot
4	Clear virus symptoms on between 6% and 15% of plants per plot
5	Clear virus symptoms on between 16% and 33% of plants per plot
6	Clear virus symptoms on between 34% and 66% of plants per plot
7	Clear virus symptoms on between 67% and 99% of plants per plot
8	Clear virus symptoms in all plants per plot (not stunted)
9	Severe virus symptoms in all plants per plot (stunted)

4. VIR2 = the second score of virus attack done 1 month before harvesting and scored as described in VIR1 above
5. ALT1 = the first scores for Altanaria symptoms to be carried out 5 weeks after planting on a scale of 1-9 as described below

Scale	Description
1	No symptoms
2	Unclear symptoms
3	Clear symptoms on <5% of plants per plot
4	Clear symptoms on between 6% and 15% of plants per plot
5	Clear symptoms on between 16% and 33% of plants per plot
6	Clear symptoms on between 34% and 66% of plants per plot
7	Clear symptoms on between 67% and 99% of plants per plot
8	Clear symptoms in all plants per plot (not fully defoliated)
9	Severe symptoms in all plants per plot (fully defoliated)

6. ALT2 = the second scores for Altanaria symptoms to be carried out 1 month before harvest as described for ALT1 above.

- **At harvest data**

8. NOPH = Number of plants harvested.

9. NOPR = Number of plants with storage roots.

10. NOCR = Number of commercial storage roots per net plot

11. NONC = Number of non-commercial storage roots per net plot

12. CRW = Weight of commercial storage roots per net plot in kg

13. NCRW= Weight of non- commercial storage roots per net plot in kg

14. VW = Weight of vines per net plot in kg