IDENTIFICATION OF MORPHO-PHYSIOLOGICAL TRAITS FOR DROUGHT TOLERANCE AND THEIR ASSOCIATED GENOMIC REGIONS IN ANDEAN GENE POOL OF COMMON BEAN (*Phaseolus vulgaris* L.)

BY

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I56/35458/2019

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE IN PLANT PHYSIOLOGY AND BIOCHEMISTRY

FACULTY OF SCIENCE AND TECHNOLOGY

DEPARTMENT OF BIOLOGY

UNIVERSITY OF NAIROBI

2023

DECLARATION

I hereby declare that this is my own original work and that it has not been presented for the award of a degree in any other University or Institute of higher learning for examination or award of a degree. Where other people's work has been used, this has been properly acknowledged and referenced in according with the University of Nairobi's requirements

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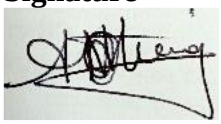
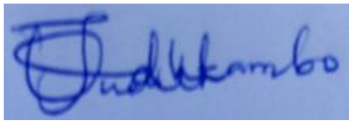
Dr. Nicholas A. Otieno Department of Biology University of Nairobi P.O. Box 30197 NAIROBI KENYA Email: nicholas.amimo@uonbi.ac.ke	 ……	**23/05/2023** ……
Dr. Judith A. Odhiambo Department of Biology University of Nairobi P.O. Box 30197 NAIROBI KENYA Email: judyaswani@uonbi.ac.ke	 ……	**23/05/2023** ……
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DEDICATION

I dedicate this thesis to: my beloved son Chilobe and my mother Elizabeth Moonga

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to my supervisors Dr. N. A. Otieno, Dr J. A. Odhiambo and Dr. K. Kamfwa for their patience, kindness, timely guidance, support, encouragement and criticism during the entire study period.

I am indebted to the Kirkhouse Trust for awarding me the scholarship to pursue a Masters degree in plant physiology and biochemistry through the project that it is funding at the University of Zambia which paid for my course work at the University of Nairobi and research in Zambia. Special gratitude goes to Dr. K. Kamfwa, the project principal investigator at the University of Zambia for administering funds, providing the study materials, and making constructive suggestions for the research and ensuring that I completed my study program.

I thank members of Dr. Kamfwa's Laboratory for their support through assistance with field, greenhouse and laboratory experiments. It is also my pleasure to express my gratitude to my family and friends for their support and understanding during the entire period of my study.

Above all, I thank Almighty God for bringing me this far in my academic pursuit, His free gift of life and enabling me carry out the study for he wills it all.

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ACRONYMS

ADP – Andean Diversity Panel

CIAT - The International Center for Tropical Agriculture

CID – Carbon Isotope Discrimination

DS – Drought stress(ed)

EL – Electrolyte Leakage

GART – Golden valley Agricultural Research Trust

GWAS – Genome Wide Association Study

HI – Harvest Index

HSW – Hundred Seed Weight

NS – Non stress(ed)

PHI – Pod Harvest Index

QTL – Quantitative Trait Loci (Locus)

PN – Number per plant (Pod load)

SDW – Shoot Dry weight

UNZA – University of Zambia

ABSTRACT

Drought is a major abiotic common bean (*Phaseolus vulgaris* L.) production constraint worldwide. There is limited knowledge on the role and relative importance of agronomic and morpho-physiological traits as well as the genetic architecture of drought tolerance of Andean common bean. The objectives of this study were to (*i*) determine the role and relative importance of agronomic and morpho-physiological traits in drought tolerance of the Andean gene pool of common beans, and (*ii*) identify common bean genomic regions associated with these traits. In this study, field and pot experiments were conducted at the University of Zambia (UNZA) (28˚20' E, 15˚ 25' S) research farm, Golden Valley Research Trust (GART) (28" 10' E, 14" 50' S), and Zambia Agricultural Research Institute - Kabwe (28° 50' E S, 14° 39' S). In the first objective, 20 Andean genotypes were evaluated with three field trials and one pot experiment. Significant correlations of seed yield with Harvest index (HI), pod harvest index (PHI), and carbon isotope discrimination (CID) under drought stress (DS) were observed, which suggested the important role of photo-assimilate partitioning efficiency (measured by PHI and HI) and water use efficiency (measured by CID) in the observed drought tolerance. The genotypes Kibala, OAC Inferno and Kijivu showed high seed yield and low CID under DS, and were categorized as water savers and recommended for use in environments prone to severe intermittent or terminal drought. Nine genotypes (Pink Panther, Kardinal, H9659-27-10, Mrondo, PI638816, G17913, PR0737, Krimson, and H9659-27-1) showed high seed yield and high CID under DS, and were categorized as water spenders and recommended for use in environments prone to intermittent drought. Drought tolerant genotypes including Krimson, OAC Inferno and SEQ11 showed significantly lower electrolyte leakage than the drought tolerant checks under DS indicating that these genotypes had lower cell membrane damage under DS, which could be one of the physiological mechanism that could explain their observed drought tolerance. In the second objective, 155 $F_{4:5}$ recombinant inbred lines (RILs) derived from a cross of Kijivu, which was identified as drought tolerant in this study and Bukoba, a drought susceptible Andean genotype were used to map the quantitative trait loci (QTL) for drought tolerance. These RILs were evaluated for drought tolerance in four field experiments conducted in three locations. In addition, a pot trial was conducted to assess the photosynthetic response of the 155 RILs under drought stress. The RIL population was genotyped with 12,000 Single Nucleotide Polymorphism (SNP) markers and composite interval mapping was conducted. QTL "hotspots" for drought tolerance were identified on chromosomes *Pv06* (21.0 Mbp -25.0 Mbp), *Pv07* (2.0 Mbp - 9.0 Mbp) and *Pv10* (37.0 Mbp - 41.0 Mbp). These three genomic regions showed extensive co-localization of seed yield, geometric mean, seed weight, partitioning indices, photosynthetic traits, pod load, and drought susceptibility index. These QTL overlapped with previously identified genomic regions for drought tolerance, suggesting that genes underlying these QTL have stable expression from drought tolerance in diverse environments and genetic backgrounds. Some of the identified QTL are novel. In this study, it is evident that there is complex genetic architecture of drought tolerance in the Andean gene pool of common bean involving several loci with additive gene action. The three "QTL hotspots" could be targeted for use in marker-assisted selection to enhance selection efficiency for drought tolerance in the Andean gene pool of common bean.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Common bean (*Phaseolus vulgaris* L.) is one of the most abundantly grown legume crop all over the world occupying a big area and having a prominent position in international food grain trade with annual productivity exceeding 23 million tonnes (Broughton et al., 2003). It is a source of income, food security, and nutrition for many households in African and Latin American countries (Akibode & Maredia, 2012). It plays a major role in food nutritional quality because of its high protein, dietary fiber, and carbohydrate content. In addition, it provides essential dietary minerals including iron (Fe) and zinc (Zn), and is consumed by various age groups (Wortmann et al., 1998).

In Africa, common bean is mainly produced in east and southern Africa. However, not all countries in this region are self-sufficient in production. For example, Zambia is a net importer of common beans mainly from Tanzania due to low productivity per unit area (Katungi et al., 2009; Sunga, 2017). Zambia's common bean on-farm national average yield is as low as 0.56 tonnes per hectare (Mulenga et al., 2021). The annual production is about 55,000 tonnes with an individual consumption rate of 10 kg per annum (Sichilima et al., 2016; Mulenga et al., 2021).

Despite its economic importance, common bean yields remain low due to biotic and abiotic stresses. Drought is the most important abiotic stress of common beans responsible for significant yield losses of up to 100% depending on drought duration and intensity, and cultivar susceptibility to drought. About 60% areas of extensive common bean production are prone to drought (Urrea et al 2009). In Africa, eastern Kenya, northern Tanzania, and Mpumalanga (in South Africa) are bean-producing areas that experience frequent and severe drought (Farrow and Muthoni-Andriatsitohaina, 2020). In Southern Africa, there was a significant drop in common bean yield between 1998 and 2018 (Farrow and Muthoni-Andriatsitohaina, 2020). This drop was partly attributed to deficit of moisture in the soil caused by drought. Development and use of drought tolerant varieties could be the most cost-effective mitigation strategy for drought, particularly in Africa where there is lack of irrigation infrastructure. Genetic variation for drought tolerance exists within *P. vulgaris* and closely related species including tepary bean (*P. acutifolious*) have been used to support the genetic enhancement of drought tolerance. Within common bean, drought

tolerance is found in both the Andean (large-seeded) and Middle American (small-seeded) gene pool, however, the Middle American gene pool (particularly the races Durango and Mesoamerican) possess more drought tolerance than the Andean gene pool (Beebe et al., 2013).

Genotypes belonging to race Durango from the Middle American gene pool have the highest levels of tolerance to drought and have been used as sources of tolerance to improve other market classes especially those in the Middle American gene pool. Mesoamerican (small-seeded) genotypes with drought tolerance have been identified and used by different breeding programs to produce progenies with superior drought tolerance. However, breeding efforts to transfer tolerance from races Durango or Mesoamerican to large-seeded Andean genotypes have been hampered by poor agronomic traits of progenies from inter-gene pool crosses (Beebe et al., 2013).

Several agronomic, morphological, and physiological traits related to drought tolerance have been recognized by different researchers (Beebe et al., 2013; Rezene et al., 2014; Polania et al 2016; Dramadri et al 2021). Common bean genetic enhancement for drought tolerance requires characterization of these traits and how they relate to seed yield under drought conditions. Assimilate partitioning indices such as harvest index (HI) and pod harvest index (PHI) have been identified as important target physiological traits in breeding for drought tolerance. Common bean genotypes that are efficient in remobilizing assimilates to the seed during drought stress tend to be high yielding. Because of PHI's high heritability and strong correlation with seed yield, it has been recommended for use to indirectly select for drought tolerance (Mukeshimana et al., 2014). Photosynthesis is highly sensitive to drought stress. Under drought stress, photosynthetic activity and subsequently production of photo-assimilates is significantly diminished resulting in reduced biomass accumulation, pod filling, grain filling, and ultimately seed yield (Rezene et al., 2014; Kamanga et al., 2018; Sedlar et al., 2020). Variation in photosynthetic activity under drought stress has been observed in the Andean gene pool of common beans (Dramadri et al., 2021).

Drought tolerance is a genetically complex trait involving several genes that affect various agronomic, morphological, and physiological characteristics. The genetic and physiological mechanisms leading to optimal drought stress tolerance differ based on environmental factors, such as when drought stress is imposed (e.g., early-season, intermittent, or terminal drought), and whether other stresses are simultaneously imposed (e.g. disease, pest, heat, soil nutrient deficiencies). This environmental and genetic complexity results in lower heritability for drought

stress tolerance. Quantitative trait loci (QTL) analyses and genome-wide association studies (GWAS) have been used to identify genomic regions for traits associated with drought tolerance. These drought-related traits include seed yield and its components, partitioning indices, and plant total biomass, among others. QTL for these traits have been reported on all eleven common bean chromosomes using mapping populations of RILs evaluated under DS and NS conditions field trials (Schneider et al., 1997; Acosta-Díaz et al., 2004; Beebe et al., 2006; Mukeshimana et al., 2014; Berny Mier y Teran et al., 2019; Sedlar et al., 2020; Berny Mier y Teran et al., 2020).

As expected, the percentage of variation explained by these QTL have generally been low for seed yield but high for traits such as harvest index, pod harvest index, and plant biomass measured under moisture stress. Co-localizations have been reported for some of the identified QTL for traits associated with drought tolerance. For example, co-localized seed yield QTL under drought stress have been identified on chromosomes *Pv01* (Trapp et al., 2016; Berny Mier y Teran et al., 2020), *Pv05* (Trapp et al., 2016; Berny Mier y Teran et al., 2020), *Pv06* (Berny Mier y Teran et al., 2019; Dramadri et al., 2021); *Pv07* (Berny Mier y Teran et al., 2020), *Pv09* (Mukeshimana et al., 2014; Berny Mier y Teran et al., 2020), and *Pv10* (Hoyos-Villegas et al., 2016; Berny Mier y Teran et al., 2020). Identification of QTL in different environments and populations suggest that these QTL are stable and could potentially be used in marker-assisted selection for drought tolerance.

1.2 PROBLEM STATEMENT

In Africa, about 73% of common bean is grown in areas prone to drought resulting in significant yield losses (Buruchara 2007). Common bean in Africa is mostly grown by poor resource farmers who depend on natural climatic conditions for their crop production (CIAT, 2008). These farmers are heavily constrained with financial resources and cannot make investments in irrigation facilities (CIAT, 2008). As a result, huge yield losses resulting from drought are incurred. Depending on drought severity and variety, yield losses can reach 100% (Urrea et al., 2009). The low yields in common beans may lead to malnutrition and food insecurity to low income households because this crop is a cheap source of protein and calories especially in developing countries.

In Zambia, the northern parts of the country are significant common bean producing areas. The high concentration of production in these areas is favored by conducive climatic conditions for

bean production (Wortmann et al., 1998; Hamazakaza et al., 2014). The areas receive higher amounts of rainfall (an average of 1000mm per annum) and have a longer growing season (120-180 days) compared to other parts of the country making farmers able to grow the crop twice in a single rain season (Hamazakaza et al., 2014). The first crop is used for seed production while the second crop is for food production. The duration of the rainy season as well as the rainfall pattern has drastically changed (Phiri et al., 2013) and this has been attributed to climate change making the second crop subjected to terminal drought due to reduction in the period of the season (Hamazakaza et al., 2014). It has been predicted that by 2050 the reduction in common beans production in Zambia (the main common bean growing area) will be mainly attributed to the reduction in the amount of rainfall and duration of the season (Hummel et al., 2018). This will be exacerbated by farmers' failure to invest in irrigation facilities due to lack of resources (CIAT, 2008). As a result, huge yield losses resulting from droughts will be incurred.

Most previous studies on common bean drought tolerance have focused on the Middle American gene pool as compared to the Andean gene pool genotypes that are mostly grown in Africa. Middle American common bean genotypes are known to produce high yields under drought stress compared to the large-seeded Andean beans (Beebe et al., 2013). There is limited knowledge of drought tolerance within the Andean gene pool.

Although there is genetic variation for drought tolerance that exists within the common bean, only limited progress has been made in developing genotypes tolerant to drought. This is partly because most of the RIL populations that have been used in the drought tolerance QTL studies were derived from Middle American parents or the inter gene pools making the Andean drought tolerance genetic architecture not well understood (Asfaw and Blair 2011; Mukeshimana et al., 2014; Diaz et al. 2020). This has provided more insights on the drought tolerance architecture enabling more progress in breeding for drought tolerance in the small-seeded Middle American genepool than in the large-seeded Andean types, which are more popular in East and Southern Africa. Identification of Andean genotypes tolerant to drought and understanding the genetic basis of that tolerance is important for supporting genetic improvement of Andean beans for tolerance to drought. QTL studies using Andean germplasm are needed to identify important drought tolerance QTL in the Andean gene pool. Additionally, such studies are important for determining the stability of drought QTL identified from the Middle American gene pool in the Andean genetic background.

1.3 JUSTIFICATION

Drought is a major abiotic production limitation of common beans and is responsible for significant yield losses. It is envisaged that climate change will increase the severity and frequency of drought in many bean-producing areas in Africa. The crop is mostly grown by small-scale farmers that are resource-poor and cannot afford the cost of irrigation to mitigate the drought effect on the crop. Therefore, there is a need to develop a cost-effective strategy to mitigate the negative effects of drought. It is also important to develop drought tolerant common bean genotypes adapted to the Zambian environment. Knowledge on the common bean drought tolerance genetics is therefore an invaluable resource in a breeding program.

Identification of morpho-agronomic and physiological traits with a strong correlation to drought could provide more insights into drought tolerance mechanisms in common bean and support the development of effective identification and selection strategies for drought tolerance. Genetic enhancement for drought tolerance requires the characterization of several agronomic, morphological and physiological traits associated with drought tolerance and how they relate to seed yield under drought stress. Common bean genotypes that are efficient in remobilizing assimilates to the seed during drought stress have been found to result in high yields. Therefore, traits that can be used to indirectly select for the high seed yield in common beans under drought stress and non-stress conditions need to be identified.

Despite physiology related studies that have revealed the role of some traits in plant drought tolerance, the mechanisms behind these traits especially in common beans are not yet well-defined and their relative importance is still not understood. Therefore, the study of physiological and other traits and mechanisms used to tolerate drought in common bean need to be explored as this would contribute to the criteria to be used in selection of genotypes tolerant to drought as well as help to enhance the understanding on the genetic architecture for drought tolerance.

The purpose of this research was to characterize the morpho-agronomic and physiological response of Andean genotypes to drought stress as well as identify the genomic regions associated with drought tolerance.

1.4 OBJECTIVES

1.4.1 General objective

To characterize agronomic and morpho-physiological traits associated with drought tolerance in Andean gene pool of common bean (*Phaseolus vulgaris* L.) and identify their associated genomic regions.

1.4.2 Specific objectives

1. To evaluate morphological, agronomic, and physiological traits associated with drought tolerance in selected Andean genotypes of common bean
2. To identify Quantitative Trait Loci (QTL) for morphological, agronomic, and physiological traits associated with drought tolerance in an Andean population of Recombinant Inbred Lines (RILs) of common bean

1.4.3 Research hypothesis of the study

Agronomic, morphological, and physiological traits are important in selecting Andean genotypes of common bean that are tolerant to drought and their associated genomic regions.

CHAPTER TWO

LITERATURE REVIEW

2.1 CLIMATE CHANGE AND DROUGHT EFFECTS ON PLANTS

Over time, there have been alterations in the average external or weather conditions in most parts of the world and this is referred to as climate change (Werndl, 2016). Greenhouse gases released into the atmosphere cause global warming resulting into climate change. It is likely to have a negative impact on hydrology, biodiversity, and agriculture (Aydinalp and Cresser, 2008). There has been an increase in temperatures and a reduction and/or changes in rainfall patterns in most parts of the world. The environmental event or phenomenon characterized by deficiency of rainfall for a duration significant enough to lead to insufficient moisture in the soil and plant damage is referred to as drought. (Carrao et al., 2016). Drought is categorized into intermittent drought and terminal drought. Intermittent drought occurs discontinuously during the life of the plant or crop causing intervals of drought within the crop's growing season. On the other hand, terminal drought is a prolonged drought that continues till the end of the plant life cycle. It is more lethal to plant development than intermittent drought as it mainly affects the later plant developmental stages especially the reproductive stage in many crops. According to Carrao et al. (2016), tropical regions are more vulnerable to drought as the people are neither prepared nor can deal with this challenge due to its occurrence at a faster rate and lack of resources to deal with it.

2.2 EFFECTS OF DROUGHT ON COMMON BEAN PRODUCTION

Drought adversely affects the physiological and biochemical processes of the common bean plant leading to low productivity. Most of the common bean worldwide is produced under rain-fed conditions accounting for over 60% of common bean growing areas (Rao et al., 2013). In Africa, a small percentage area is optimally suitable for growing the *Phaseolus* beans as most areas are either marginally suitable or not suitable at all to grow these beans (Parker et al., 2022).

Grain yield and its components including plant stand, pod load, seeds per pod, and seed weight are severely reduced due to drought (Razene et al., 2014). Morphological traits such as shoot biomass,

leaf size, number of leaves, and plant height also are reduced under drought stress conditions (Lanna et al., 2016; Kusvuran and Dasgan, 2016). Drought at the beginning of the common bean life cycle is very detrimental as it affects the plant stand leading to a reduction in yield components per unit area, however, if rectified in the later stages, it might not affect seed weight. When imposed at the reproduction stage, drought stress interferes with the production, translocation, and partitioning of assimilates resulting in poor pod formation, seed set, and seed filling. This has been found to have a direct effect on pod load and seed weight (Asfaw et al., 2012: Lanna et al., 2016). Root attributes such as rooting depth, length, thickness, and volume are important for drought adaptation as they maximize water uptake (Asfaw and Blair, 2011). On the other hand, shoot attributes optimize the use of the water absorbed by roots for grain production. Reduction in leaf area is an important shoot morphological attribute for drought adaptation. Thus, reduction in leaf area or senescence normally results in decreased seed yield (Mukeshimana et al., 2014; Dramadri et al., 2021).

Depending on the growth stage at which plants are exposed to drought and the level of tolerance plants may have, phenological traits such as days from planting to flowering and physiological maturity tend to reduce due to drought stress. If drought is imposed at the seedling stage, there is a reduction in the number of days from planting to the time plants reach 50% flowering and physiological maturity in the stressed trial compared to the non-stressed, but if it is imposed at a later stage i.e. at flowering or mid pod fill, only the latter varies (Ghanbari et al., 2013; Dramadri et al., 2019). Mukeshimana et al., (2014) subjected an inter-gene pool common bean RIL population to drought and observed that drought-stressed plants had shorter number of days from planting to seed fill and physiological maturity compared to the non-stressed ones.

Reduction in soil moisture content due to drought causes low water uptake and reduction in mineral uptake such as Iron (Fe), Zinc (Zn), Phosphorus (P), and Nitrogen (N) (Ghanbari et al., 2013). Smith et al. (2019), observed a reduction in N content where the protein content was directly reduced in the drought-stressed common beans. In addition, growing common beans under drought stress condition increases levels of phytic acid, an anti-nutritional factor that limit micronutrient uptake in the human diet (Hummel et al., 2018). This makes micronutrients such as Zn and Fe to be less bioavailable in the common bean grain.

Common bean portrays variations in accumulation of biomass and photosynthate partitioning when exposed to drought stress. These are reflected in the canopy biomass, pod partitioning index, PHI, and HI (Beebe et al., 2013). It has also been observed that photosynthesis reduction is mainly due to the low availability of primary raw materials of carbon dioxide (CO_2) and water in smaller quantities. Therefore, when the leaf's relative water content reduces, photosynthesis becomes suppressed. Under mild drought conditions, stomatal closure by restricting CO_2 entry into the leaves causes reduction in photosynthesis rate. As stress levels become severe, leaf water potential becomes more negative and other factors such as RuBisCO enzyme inhibition, photo-oxidation, and photorespiration come into play, reducing carbon assimilation (Kamanga et al., 2018). In addition, as drought stress becomes severe, the reaction centre of photosystem II is down-regulated as water molecules required to provide the electrons are in limited supply. This down-regulation of the photosystem II leads to a further decrease in the assimilation of carbon dioxide and also affects the electron transport chain leading to the reduced production of photosynthates (Jain, 2018). However, the down-regulation of photosystem II helps to protect the plant from excess incident light (Pinheiro and Chaves, 2010).

Drought stress also affects the content of photosynthetic pigment, chlorophyll, which is the major absorbent of light energy in the presence of light, though the effect of drought on chlorophyll content is species specific. In species like onion (*Allium cepa*), chlorophyll content increases while in young peaches (*Prunus persica*) and most other plants it reduces. The level of chlorophyll content variation due to drought stress depends on the drought stress severity (Kamanga, et al., 2018). Rezene et al. (2013) and Darkwa et al. (2016), observed a reduction in chlorophyll content in the stressed treatment in common beans compared to the non-stressed.

There is also a change in the allocation of photo-assimilates. Under mild drought stress, the major sink is the roots as the plant tries to take up more water. In this situation, the energy demand increases hence increasing the rate of respiration in the roots (Zlatev and Lidon, 2012). As the stress gets severe, the sink changes and the photo-assimilates get accumulated in other parts of the plant. In grain crops like common beans, it can lead to flower and pod abortion if the crop is stressed with drought at the reproductive stage and the reproductive organs are not the sink which reduces the economical yield capacity. Severe prolonged drought stress can lead to plant death resulting in no economical yield. For instance, drought stress has more impact at the reproductive

stage than other growth stages (Rao., 2014). However, the percentage reduction varies based on the severity of drought stress imposed on common beans (Rezene et al., 2013; Darkwa et al., 2016).

2.3 MECHANISMS OF DROUGHT TOLERANCE IN COMMON BEAN

Drought resistance is defined as the various means plants use to survive periods of environmental insufficient water supply. In agriculture, crops are said to be drought resisrant if they can survive, reproduce or have the least reduction of the economical yield when grown under insufficient water supply compared to adequate water supply (Aslam et al., 2015). Drought resistance in common beans results from a synergy of mechanisms involving both the root and the shoot system. Based on the mode of adaptation, drought resistance mechanisms include escape, avoidance, and tolerance (Beebe et al., 2013).

Plants that use drought escape mechanisms complete their life cycle within the period of water supply before the onset of drought. They are mostly annuals and their seed remain dormant during the dry season (Jain, 2018). This drought resistance mechanism is important for terminal drought. In common beans, days to flowering and days to physiological maturity are important as they may predict the type of drought a crop may be able to withstand. Darkwa et., al (2016) reported two common bean genotypes with shorter days to physiological maturity and high yield under drought stress conditions. The genotypes portrayed developmental plasticity mechanisms under drought stress conditions as they had relatively longer days to physiological maturity when they were grown under optimal conditions.

According to Beebe et al. (2013), drought avoidance is where the plant maintains high water potential by reducing the impact of the drought stress. This drought resistance mechanism is achieved through deep rooting system and reduction of water loss through the shoot which helps maintain high tissue water potential. The mode of drought avoidance by plants can be classified as water spenders, water savers, or water collectors. Water spenders have a deep root system and aggressively absorb water. They do not face negative water potential during the dry season due to access to water. For instance, Asfaw and Blair, (2011) observed a large volume of root system in the drought resistant common bean genotype BAT 477. Water savers have small leaf area, few leaves, and hairy covering on leaf surfaces (Jain 2018). Polania et al. (2016a) classified two common bean genotypes SER 16 and SCR 16 as a water spender and a water saver respectively.

On the other hand, it has been reported that traits such as leaf rolling, transpiration rate, and other root and shoot attributes like leaf pubescence and waxiness are important for drought avoidance (Aslam et al., 2015).

Drought tolerance is the plant's ability to carry out normal physiological and biochemical functions even at low water potential (very negative water potential) (Jain, 2018). When plants are exposed to drought conditions, many physiological and biochemical processes are increased or decreased (Baroowa and Gigoi., 2013). Processes such as osmotic adjustment, antioxidant production, plant growth regulators, aquaporins, stress responsive proteins, transcription factors, and signaling pathways have been reported to actively participate in drought tolerance in several plant species (Aslam et al., 2015). For instance, drought has been reported to increase the levels of endogenous abscisic acid and reduction of stomatal conductance in common beans (Traub et al., 2017). Proline content may not play a role in drought tolerance under moderate drought stress while other compatible solutes do (Rosales et al., 2012; Traub et al., 2017).

Several studies have been carried out on mechanisms plants use to adapt to drought stress. These studies have been done mostly by measuring several traits related to plant physiology, morphology, phenology, and biochemistry. However, there are unclear demarcations among drought escape, avoidance, and tolerance mechanisms when it comes to measuring drought related traits. Therefore, a combination of traits related to drought escape, avoidance, and tolerance mechanisms are normally measured for assessing drought tolerance/resistance. (Beebe et al., 2013 and Darkwa et al., 2016).

According to Kamanga et al. (2018), the physiological traits associated with drought tolerance include; maintenance of photosynthesis rate, low rate of transpiration, low electrolyte leakage, maintenance of plant tissue mineral concentration, WUE, and maintenance of relative water content. Polania et al., (2016a) evaluated physiological traits related to drought stress tolerance mechanisms in common beans by assessing the crops'; effective use of water (EUW), canopy biomass, dry matter partitioning indices, CID, and leaf stomatal conductance. The researchers further used data on physiological traits such as CID and stomatal conductance to determine whether the common bean genotypes were water spenders or water savers. They found that water spenders had a positive correlation of yield with CID but not the water savers and plants with higher stomatal conductance had higher EUW as they tapped more water in the soil and were likely

to be water spenders. In addition, the genotypes that resisted/tolerated drought by early maturity were able to compensate for the effect of yield penalty by increasing dry matter partitioned to grain.

Other drought related physiological traits that have been studied in common beans and other crops include; water potential, osmotic adjustment through the accumulation of compatible solutes, plant growth regulators' levels, accumulation of reactive oxygen species (ROS), and antioxidant defence (Franca et al., 2000; Farooq et al., 2009; Sedlar et al., 2020). Franca et al., (2000) evaluated the ability of four common bean genotypes to maintain cell membrane integrity, growth rate, and transpiration rate at low water potential and observed the need for incorporating several traits when evaluating drought tolerance of plants. They also highlighted that despite these parameters being useful, the relationships between them and drought tolerance (mainly determined by seed yield) in common beans could be complex. Solute accumulation of sugars, sorbitol, inositols, amino acids, proline, quaternary ammonium compounds, ureides, inorganic (K^+, Ca^{2+}, Mg^{2+}) and organic (PO_4^{3-}, No^{3-}, Cl^-) ions have been reported to have a contribution to osmotic adjustment in common beans including other grain legumes under drought stress (Amede and Schubert, 2003). However, there is a variation in their contribution to osmotic adjustment in legumes, for example, water soluble sugars concentration increases in chickpeas (Cicer arietinum) and reduces in common beans and faba beans (*Vicia faba*) (Amede and Schubert, 2003).

Photosynthesis, a complex and diverse biochemical process is the driving metabolic process in plants. Modern gadgets such as the SPAD Chlorophyll meter or MultispeQ (Photosynq) devices have been used to collect data on photosynthesis related traits; relative chlorophyll content, leaf temperature differential (LTD), photosystem I and II activity, linear electron flow (LEF), photosystem II quantum yield (Phi2), the proportion of incoming light lost through non-regulated processes (PhiNO) and proportion of incoming light that goes towards non-photochemical quenching (PhiNPQ) (Traub et al., 2018; Dramadri et al., 2021). These gadgets have been used to measure the aforelisted data in a very short period (approximately less than 30 seconds) indicating the status of photosynthesis in a plant leaf. Such comprehensive measurements have helped to describe the complexity and diversity of photosynthesis across plant populations which cannot be explained by phenotypic traits alone (Kuhlgert et al., 2016). Genotypes that are more sensitive to drought stress are likely to have more photosynthetic variation under drought stress and non-

stressed conditions. Significant differences in LEF, SPAD, Phi2, PhiNO, and PhiNPQ also have been observed in common beans grown under DS and NS showing that it behaves differently depending on the water treatments (Sedlar et al., 2020; Dramadri et al., 2021). Depending on the drought resistance mechanism (avoidance or tolerance) at play, LTD can help to determine whether the genotype is a water saver or a water spender. It is directly related to stomatal conductance, CID, transpiration rate, and plant rooting depth (in case of water spenders). Water savers transpire less compared to water spenders and are likely to have lower LTD compared to water spenders (Polania et al., 2016a, b).

2.4 IMPORTANCE OF MORPHO-AGRONOMIC AND PHYSIOLOGICAL TRAITS IN DROUGHT TOLERANCE

The response of different crop species or a specific crop genotype to drought determines their level of drought tolerance (Beebe et al., 2013). Morphological, agronomic, and physiological traits provide the basis for differences in the plant's performance under drought stress. Even though many phenotypic traits in common beans are used to evaluate drought tolerance, most focus has been on yield components, plant attributes, and photosynthate partitioning to grain on drought stressed beans. These traits are reliable in identifying common bean drought tolerance because they have a high correlation with seed yield (Rezene et al., 2014; Mukeshimana et al., 2014; Darkwa et al., 2016; Polania et al., 2016a; 2016b; Dramadri et al., 2021). These traits increase the selection efficiency for drought tolerance other than just assessing the yield component. Therefore, a combination of these traits is useful in assessing common bean adaptation to drought as most of them are highly heritable and genetically variable, and the methods for evaluating them are relatively reliable and cost effective (Beebe et al., 2013). Plants under drought stress have variation in morpho-agronomic traits such as days from planting to flowering and physiological maturity, seed weight, and pod load. Usually, a decrease in the performance of such traits normally contributes to a significant reduction in the geometric mean of yield (Polania et al 2016a).

Morphological features are an important first step of categorising plants under drought stress. However, they have certain limitations due to their plasticity, a tendency of a plant species to physically change appearance in response to the environment. Thus, plants develop morphological features that reduce the adverse effects of drought stress through adaptation (Rezene et al., 2012).

The early visual expressions of plants in response to drought include leaf rolling, senescence, and abscission (Gonçalves et al., 2019).

2.5 GENETIC SOURCES OF DROUGHT TOLERANCE IN COMMON BEAN

Common beans (*P. vulgaris*) possess sources of genetic tolerance to drought within its primary gene pool. However, *P. acutifolius* possess higher levels of tolerance than *P. vulgaris*. Successful introgression of drought tolerance has been performed from *P. acutifolius* genotype, G40001 to *P. vulgaris* and resulted in the development of interspecific genotypes with higher levels of drought tolerance (Mejia-Jimenez et al., 1993). However, there have been challenges in moving tolerance genes from *P. acutifolius* to *P. vulgaris* as interspecific crosses require embryo rescue, therefore hampering the efforts of routine transfer of tolerance genes (Beebe et al., 2013).

Within the *P. vulgaris* species, the middle American gene pool tends to possess higher levels of tolerance than the Andean. There are differences in drought tolerance among races within the middle American gene pool. The middle American races, Durango and Mesoamerican, tend to possess higher levels of tolerance than the other races within this gene pool. Crossing of the races, Durango and Mesoamerica, within the Middle American gene pool has been successful and genotypes from the two races have been used as a genetic source of drought tolerance within the gene pool (Beebe et al., 2013). However, breeding efforts to transfer tolerance from races Durango or Mesoamerican to large-seeded Andean genotypes, have been hampered largely by poor agronomic traits of progenies from inter-gene pool crosses due to less compatibility among parental genotypes (Beebe et al., 2013).

Despite the low levels of tolerance within the Andean gene pool, there are a few genotypes that have demonstrated higher levels of tolerance. These are mostly from the Neuva Granada and Peru races of the Andean gene pool (Pérez-Vega et al., 2011). For example, Kijivu (ADP 33) and Portillo have been reported to be drought tolerant (Mndolwa et al 2018; Dramadri et al., 2019).
Drought tolerance has been reported as a polygenic trait involving several genes as observed in agronomic, morphological, and physiological traits (Beebe et al., 2013). The expression of these genes for some traits is variable as it is highly influenced by several environmental factors resulting in low heritability making the selection process complex (Beebe et al., 2013). The genetic

architecture of drought tolerance varies with species, races, traits used to evaluate it, the growth stage at which drought is induced, and environmental growing conditions (Farooq et al., 2009). Drought is a complex trait with several regions within the genome that contain genes associated with tolerance to it. The genomic regions associated with such complex quantitative traits are known as quantitative trait locus/loci (QTL). They appear to show a continuous range of variation in a population due to allelic differences that may occur in genes that change the gene action resulting in smaller phenotypic effects as they are quantitatively inherited. Therefore, the quantitative traits result from a combined effect of many genes (Collard, 2005).

The QTL mapping technique is a widely used method for the identification of genomic regions associated with complex traits by evaluation of genotypes for tolerance to drought and other biotic and abiotic stresses as well as traits of economic importance in plants (Collard, 2005). Asfaw et al. (2011), worked on the RIL population derived from the middle American gene pool parents and identified drought related QTL from traits attributed to the common bean roots. Diaz et al. (2020) on the other hand, mapped drought related QTL from a multi-parent advanced generation intercross (MAGIC population) derived from 8 middle American genotypes. A genome wide association study (GWAS) conducted on a subset of middle American genotypes comprised of 96 genotypes identified QTL for shoot biomass and lodging under drought stress (Hoyos-Villegas et al., 2017). Working on inter-gene pool RIL in common bean population, Mukeshimana et al. (2014) identified 13 QTL for tolerance to drought using seed yield, pod load, seed weight, pod harvest index, days to flowering and physiological maturity under drought stress conditions. When RILs were subjected to terminal drought at early pod filling, Briñez et al. (2017), identified 8 QTLs using chlorophyll content, fresh stem biomass, leaf temperature, seed load, seed weight, and pod dry weight derived from a population of middle American and an Andean parent. An Andean RIL population was subjected to intermittent drought at the flowering stage by Dramadri et al., (2019) and 12 QTL were detected on days to flowering, pod load, seed yield, harvest index, and pod partitioning index.

Most of the QTL analysis studies on drought tolerance in common bean have been conducted using the middle American gene pool populations enabling more progress in breeding for drought tolerance compared to the large-seeded Andeans, which are more popular in East and Southern Africa (Trapp et al., 2015; Berny Mier y Teran et al., 2019; Diaz et al., 2020). Identification of

Andean genotypes tolerant to drought and understanding their genetic mechanism of tolerance is important for supporting the genetic improvement of Andean beans for tolerance to drought.

CHAPTER THREE

EVALUATION OF MORPHOLOGICAL, AGRONOMIC AND PHYSIOLOGICAL TRAITS ASSOCIATED WITH DROUGHT TOLERANCE IN SELECTED ANDEAN GENOTYPES OF COMMON BEAN

ABSTRACT

Drought is one of the most important production risk of common bean (*P. vulgaris*) in Sub-Saharan Africa. The objectives of this study were (*i*) to evaluate the role and relative importance of agronomic, morphological and physiological traits in drought tolerance of Andean genotypes and (*ii*) identify genotypes with desirable combination of morpho-physiological traits for enhanced drought tolerance. A set of 20 common bean genotypes were evaluated under drought stress and non-stress conditions. Field trials laid in a randomized complete block design were conducted in Zambia from July to October 2021 in the dry season under irrigation. The sites included the University of Zambia (UNZA) research farm, Golden Valley Research Trust (GART) and Kabwe Agricultural Research Station. Drought induced reduction partitioning indices, seed yield and yield components i.e. total shoot biomass, number of pods per plant and 100 seed weight. Of all genotypes, Krimson, H9659-27-7, G 17913 and Pink Panther, were identified as tolerant to drought stress based on their superior performance on a number of traits under drought stress conditions. Based on relationship between seed yield and a measure of water use efficiency CID, 12 drought tolerant genotypes were identified where nine were classified as water spenders while the other three as water savers. This led to identification of suitable drought conditions under which the genotypes may be grown depending on the severity or type of drought prevalent in the area. A strong significant positive correlation between seed yield and partitioning indices as well as CID was observed across locations. This suggests that remobilization of photo-assimilates from the pod wall (PHI) and the rest of the plant (HI) was a major mechanism involved in the observed drought tolerance of the identified drought tolerant genotypes. Such traits can be used as surrogate traits for indirect selection of superior drought-tolerant cultivars under drought stress conditions.

3.1 INTRODUCTION

Common bean (*P. vulgaris*) is composed of two major gene pools known as Middle American and Andean which are further subdivided into seven races. The Middle American gene pool has four major races namely Durango, Jalisco, Mesoamerica, and Guatemala which consist of small seeded beans. The Andean gene pool has three major races that include Peru, Nueva Granada, and Chile are the ones mostly grown in Africa (Beebe et al., 2013).

African common bean production is concentrated in Eastern, Central, and Southern Africa (Katungi et al., 2009). Despite its economic importance, common bean yields obtained in sub Saharan Africa are only 30% of the yields obtained in the major bean producing countries of the world (Farrow and Muthoni-Andriatsitohaina, 2020). Among the major constraints in bean production is drought, which is a major abiotic stress of common beans responsible for significant yield losses after biotic stresses (Rodríguez De Luque and Creamer, 2014). In the past century, the world has experienced an increase in the frequency and severity of precipitation deficits leading to drought (Carrao et al., 2016). Areas of extensive bean production including Mexico, northeast Brazil, and Southern and Eastern Africa are expected to become progressively drier due to climate change (Yadav et al., 2011). The effect of drought due to climate change is likely to be more severe in Africa compared to other parts of the world as most common bean producers are resource poor farmers lacking means of mitigating drought challenges causing the reduction in seed yield. In addition, common bean production is being pushed to more marginal areas due to human population increase and competition for space with other crops (Katungi et al., 2009; Farrow and Muthoni-Andriatsitohaina, 2020).

In Zambia, common bean is a very important crop to consumers as well as a cash crop to small scale farmers, since 60% of the crop produced is marketed in urban areas, especially in the Copperbelt and Lusaka regions (Wortmann *et al.*, 1998). Despite the vast land resource available for agricultural production including bean production, the country is still a net importer of common beans (Sichilima et al., 2016). This is partly explained by the low productivity of 560 kg/ha in the country (Mulenga et al., 2021) compared to the continent's average of 943 kg/ha (FAO, 2019; Mulenga et al., 2021). Productivity and production is low partly due to factors attributed to the frequent *El nino* effects that lead to drought in the country as well as other weather related factors (Chapoto et al., 2019). The drought situation is expected to worsen in Zambia since common bean

production has been drifting from the traditional high rainfall (Agro-ecological region III) to agro-ecological region II which is prone to drought conditions due to population growth (Hamazakaza et al., 2014). In Zambia, like the rest of the Africa, common bean is mostly grown by small scale farmers. These resource-poor farmers lack irrigation facilities for farm irrigation to supplement the crop's rain-fed production in times of low rainfall. A deluge import of common bean from countries with high production may further exacerbate the poverty levels of the farmers as common bean is also an important economic crop. Hence, it is essential to develop drought tolerant varieties aimed at improving production as well as productivity that will improve the rural livelihood of farmers that engage in bean production.

Genetic variation in drought tolerance has been reported in common beans and within the *P. vulgaris* species (Beebe et al., 2013). The races, Durango and Mesoamerica, of the Middle American gene pool, Neuva Granada and Peru, of the Andean gene pool are tolerant to drought (Pérez-Vega et al., 2011; Beebe et al., 2013). Comparing the two common bean gene pools, more drought tolerance was found in the Middle American than in the Andean gene pool (Beebe et al., 2013). On the other hand, successful attempts to introgress drought tolerance from the related species of *Phaseolus acutifolius* to *P. vulgaris* have been made through congruity backcrossing (Mejia-Jimenez et al., 1993). Despite the successes, there has been a drawback in large seed size recovery when intercrosses are made between gene pools and their related species.

The role of morpho-physiological traits in drought tolerance and the underlying mechanisms is more understood in the Middle American genotypes than the Andean gene pool. Understanding the role and importance of morpho-physiological traits in drought tolerance of Andean genotypes could help to develop an integrated selection index for drought tolerance. This knowledge will also help in identifying genotypes that can be used to mitigate drought stress effects on bean production using the varieties that exist within the Andean common bean gene pool. The objectives of this study were to (*i*) evaluate the role and relative importance of morphological and physiological traits associated with drought tolerance in Andean common bean genotypes, (*ii*) identify genotypes with a desirable combination of morpho-physiological traits for enhanced drought tolerance.

3.2 MATERIALS AND METHODS

3.2.1 Study site

The field trials were conducted in the year 2021 at three locations: (i) the University of Zambia (UNZA) research farm, (ii) Golden Agricultural Valley Research Trust (GART), and (iii) Zambia Agricultural Research Institute - Kabwe Research Station. The three research sites have similar climatic conditions of temperature and humidity but have variable soil types (Table 3.1). All three sites are located in the Agro-ecological region II and receive an average annual rainfall between 800 and 1000mm from November to late April. Zambia experiences three climate weather seasons based on average humidity and temperature; warm rainy season, cold dry season, and hot dry season. All the three trials were conducted between August – November, which is a hot dry season in Zambia. The pot trial was conducted in a screen house at UNZA from January 2022 to February 2022.

Table 3.1. Experimental sites, their geographical location, and the soil types

Location	Longitude	Latitude	Elevation (masl)	Soil type/class
UNZA	28˚20’ E	15˚ 25’ S	1250	Fine loamy isohyperthermic paleustalf
GART	28” 10’ E	14” 50’ S	1139	very-fine, mixed isohyperthermic Udic Paleustoll
Kabwe	28° 50’ E	S14° 39’ S	1176	Sandy loam

UNZA: University of Zambia, GART: Golden Valley Research Trust, Kabwe: Kabwe Research Station, masl: meters above sea level

3.2.2 Common bean genotypes used in the study

A collection of 27 common bean genotypes (Table 3.2) was obtained from the University of Zambia bean breeding program. The 20 genotypes were selected from the Andean Diversity Panel based on previous published and unpublished reports of their drought tolerance (Cichy et al., 2015). Four Middle American genotypes SER16, SCR10, SCR16 and SCR44, were included in the trial as drought tolerant checks in the trial based on previous performance (Polania et al., 2016a; Traub et al., 2017). Also, the tepary bean (*Phaseolus acutifolius*) landrace G40001 was included as drought tolerant check (Mejia-Jimhnez et al., 1993: Polania et al., 2016a; Burbano-Erazo et al.,

2021). Two Andean Zambian landraces Kabulangeti and Lusaka were included as drought susceptible checks. The 20 genotypes and checks were evaluated for drought tolerance in three field trials and one pot experiment in Zambia.

Table 3.2. List of germplasm used, their source and phenotypic characteristics

Cultivar Name	Other ID	Market Class	Growth Habit	Country of origin
ADP Genotypes	**ADP ID**			
Gololi	ADP 16	Red	Type I	Tanzania
Kijivu	ADP 33	Purple speckled	Type I	Tanzania
Mrondo	ADP 41	Dark red	Type II	Tanzania
ADP 57	ADP 57	Dark red	Type II	Tanzania
Mshindi	ADP 107	Grey speckled	Type I	Tanzania
G6415	ADP 225	Light red	Type I	CIAT
G17913	ADP 303	Tan	Type I	CIAT
PR0737-1	ADP 434	Red mottled	Type I	Caribbean
Kibala	ADP 516	Yellow	Type I	CIAT Africa
RWR 10	ADP 549	Dark red	Type I	Rwanda
SEQ11	ADP 590	Purple mottled	Type IV	CIAT Africa
H9659-27-7	ADP 628	Light red kidney	Type III	North America
H9659-27-10	ADP 629	Light red kidney	Type III	North America
OAC Inferno	ADP 631	Light red kidney	Type III	Canada
TARS HT 1	ADP 632	Dark red	Type I	North America
Kardinal	ADP 657	Light red kidney	Type III	North America
Krimson	ADP 660	Cran berry	Type I	North America
VA-19	ADP 667	Red	Type III	North America
Pink Panther	ADP 687	Light red kidney	Type I	North America
PI638816	ADP 747	Dark red	Type III	East Africa

Drought susceptible controls				
Kabulangeti	Kabulangeti	Purple speckled	Type IV	Zambia
Lusaka	Lusaka	Yellow	Type I	Zambia
Drought tolerant positive controls				
SCR 10	SCR 10	Small red	Type III	CIAT
SCR 16	SCR 16	Small red	Type III	CIAT
SCR 44	SCR 44	Small red	Type III	CIAT
G40001	Tepary	White	Type IV	CIAT

Growth habit is described as; Type I: Determinate, Type II: Indeterminate upright, Type II: indeterminate prostrate, Type IV: Indeterminate with strong climbing ability (Singh, 1981)

3.2.3 Field Experimental procedure

3.2.3.1 Field preparation and planting

The land that was previously used for maize trial was disc ploughed and then the disc harrowed in order to achieve fine tilth soil suitable for bean planting. Each genotype was planted in a two-row plot that was 4 m long; with an inter-row spacing of 0.6 m per replication. Each plot measured 1.2 m by 4 m with 1m space between the plots. Granular compound D fertilizer (with the percentage proportions of 10, 20, and 10 for Nitrogen (N), Phosphorus (P), and Potassium (K) was broadcasted within each experimental unit at an equivalent rate of 200 kg ha^{-1}. The broadcasted fertilizer was then mixed with the soil before planting. The planting was done at a spacing of 0.05 m between seeds within a row and one seed per station.

A randomized complete block design (RCBD) with three replications was adopted in this study in all the three locations. Each trial had two water regimes; drought stress (DS) and non-stress (NS) as water regimes, separated by a buffer zone measuring 10 m wide where a common bean variety Mbereshi was grown. Each water regime had the 27 plots with three replicates which were separated by an alley measuring 1 m in width running perpendicular to the plots along the 27 plots.

3.2.3.2 Crop Management

Surface irrigation was used to supply sufficient water required for crop development. Weeds within the plots were controlled using a hand hoe at four weeks after planting when plants had started forming the flower primordia and had attained V4 growth stage. The second application of fertilizer was carried out after weeding at the same rate as at planting. Foliar fertilizer comprised of N = 77000, P = 172000, Mg = 36000, S = 48000, B = 880, Fe = 3480, Mg = 720, Zn 428, Cu =52, and Mo = 30 (all nutrients in mg/kg) was applied using the knapsack sprayer at the rate of 1.5Kg/ha at 8 weeks after planting to boost the plant growth vigor. Other optimal management practices such as disease and pest control were not done because they did not affect the crop as the trials were conducted in the off season.

Water was supplied sufficiently to both the DS and NS to avoid moisture stress to the crop until flowering when irrigation was withdrawn intermittently in the DS. During intermittent irrigation withdrawals, NS received twice more water as DS. Intermittent irrigation withdrawals continued until mid-pod fill when irrigation was permanently stopped from DS, but continued for the NS until physiological maturity. The amount of water supplied per irrigation was measured using the rain gauge. Rain gauges were placed to collect water per irrigation in an area of about $400m^2$ per rain gauge. The amount of water collected from each rain gauge was recorded to come up with the quantity supplied to the trial per irrigation.

3.2.4 Pot experiment

The purpose of this experiment was to measure electrolyte leakage. Genotypes used in this experiment are similar to the ones used in the field experiment. A Randomized Complete Block Design was used with 4 replications of 4 plants each and two water treatments (DS and NS).

3.2.4.1 Soil preparation and planting

Fine loamy (isohyperthermic paleustalf) with fine tilth was loaded in 5-L polyethelene pots and a total of eight seeds were planted (two seeds per station) at an approximate depth of 2-3cm at the University of Zambia green house. Compound D fertilizer was applied one week after planting to maintain the plant growth vigor. Thinning was done at the first trifoliate stage (growth stage V1) leaving one plant per station. The plants were supplied with water to field capacity daily until two days after thinning when water was withdrawn in the DS for 10 days while the NS plants were

watered regularly to field capacity. Plant leaf samples were then collected from both the DS and NS categories for electrolyte leakage analysis

3.2.4.2 Electrolyte Leakage Data Collection

Plant leaf tissues were excised using a 10mm diameter cork borer from the DS and NS plants. Deionized water was used to wash samples to remove electrolytes that adhered to the surface. Samples were then incubated at ambient temperature on a rotary shaker at 100 rpm for 24 hrs. The initial electrical conductivity (EC1) was determined using the WTW Cond 3310 SET 1 conductivity meter (Xylem Analytics Germany GmbH). Samples were autoclaved at 121℃ for 20 minutes and cooled to room temperature. After cooling, the final electrical conductivity (EC2) was determined. Electrolyte leakage was expressed as (EC1/EC2) x 100.

3.2.5 Data Collection

3.2.5.1 Agronomic and morphological traits

At growth stage R6 which is mid-pod fill, five above ground plant samples were randomly selected from the row of each genotype sub-plot, cut, and put in the dried for 72 hours in the oven at 60℃ to obtain a constant dry weight which was referred to as shoot dry weight.

At the physiological maturity stage, five plants were randomly selected again from the row of each genotype both in NS and DS. The temperature of 60℃ was used for 72 hours in the oven for samples to obtain a constant dry weight. After drying, the total above ground plant biomass referred to as total shoot dry weight (TSW) was determined then the number of pods and pod weight from each sample were determined. Lastly, threshing was done and seed weight was determined. Each total sample pod weight was obtained before the removal of the seeds and thereafter weight of the seed was estimated. The averages of TSW and pod number were determined by dividing sample TSW and pod number by five to determine the shoot dry weight (SDW) and the number of pods per plant (PN) respectively. The primary variables that were measured from the process described above included two sets of TSW samples collected at the mid-pod fill stage and physiological maturity respectively, PN, pod weight, and sample seed weight. These primary variables except PN and SDW collected at physiological maturity were then used to derive the physiological partitioning indices.

At harvest maturity, genotypes were harvested and put in the drier at 40 ℃ for three days and seed yield per plot was recorded and later converted into kilograms per hectare. A total of 100 seeds were weighed to obtain a Hundred seed weight (HSW).

3.2.5.2 Physiological traits

3.2.5.2.1 Partitioning Indices

Plant efficiency on remobilization of photo-assimilates from the source site (mostly leaves) to pods and later on from pod walls to the developing grain was determined by partitioning indices; pod harvest index (PHI) and harvest index (HI) which were calculated as shown in table 3.3 using some primary data from 3.2.3.3.

3.2.5.2.2 Carbon Isotope Discrimination (CID)

Carbon isotope discrimination measures the proportion of stable carbon isotopes of Carbon^{13} and Carbon^{12} ($^{13}C/^{12}C$) in the plant dry matter compared to the proportion in the atmosphere. The atmospheric ratio is estimated to be −8.0‰ (Farquhar et al., 1989). Oven dried seeds were ground with a Laboratory Grinding Mill (model MF 10 B S000) and sieved using a 1 mm sieve. About 5 mg of the ground seed powder for each genotype was carefully packed in aluminum tin capsules, tightly sealed, and shipped to the Stable Isotope Facility at the University of California, Davis, California, US for measurements of ^{13}Carbon. CID (‰) was calculated according to the following equation;

$$\Delta^{13}C(CID) = \frac{\Delta^{13}C_a - \Delta^{13}C_s}{[1 + (\Delta^{13}C_s/1000)]}$$

Where;

Δ^{13}Cs and Δ^{13}Ca are sample and atmospheric concentrations of 13C, respectively, and the carbon isotope composition of the atmosphere is assumed to be −8.0‰ (Farquhar et al., 1989).

The relationship between seed yield and CID was explored using a Quadrant plot. The 27 genotypes (ADP genotypes and check inclusive) were placed in a Quadrant based on their mean values for seed yield and CID under DS. Several previous studies that have used a quadrant plot analysis have classified genotypes that fall under the upper right and left top quadrants as “water spenders” and “water savers” respectively (Polania et al., 2016; Sanz-Saez et al., 2019). The water

spenders are those genotypes with higher values for seed yield and CID than the population means. The water savers have lower CID values than the population means but a higher value of seed yield than the population means.

3.2.5.3 Estimated Parameters

Other secondary variables used for selecting high yielding genotypes for both stressed and non-stressed environments were computed from seed yield per plot using the standard protocol described by Beebe et al., (2013). These included; Yield Geometric Mean (YGM), Yield Percentage Reduction (YPR), Yield Drought Intensity Index (DII), and Yield Drought Susceptibility Index (DSI). The formulae used to compute these listed secondary variables are provided in table 3.3 where Y_{DS} and Y_{NS} were the individual genotype's mean yields evaluated under drought stress and non-stress conditions, respectively, and X_{DS} and X_{NS} were the average seed yields for all genotypes evaluated under drought stress and non-stress conditions, respectively (Beebe et al., 2013; Darkwa et al., 2016).

Table 3.3. Formulae used to derive estimate selected secondary variable parameters

$$Harvest\ Index\ (HI) = \frac{Seed\ biomass\ dry\ weight\ at\ harvest}{Total\ shoot\ biomass\ dry\ weight\ at\ mid\ pod\ fill}\ x\ 100$$

$$Pod\ Harvest\ Index\ (PHI) = \frac{Seed\ biomass\ dry\ weight\ at\ harvest}{Pod\ biomass\ dry\ weight\ at\ harvest}\ x\ 100$$

$$Yield\ Geometric\ Mean\ (YGM) = (Y_{NS}xY_{DS})^{1/2}$$

$$Yield\ Percentage\ Reduction\ (YPR) = \frac{Y_{NS} - Y_{DS}}{Y_{NS}}\ x\ 100$$

$$Yield\ Drought\ Intensity\ Index\ (DII) = \frac{1 - X_{DS}}{X_{NS}}$$

$$Yield\ Drought\ Susceptibility\ Index\ (DSI) = \frac{1 - (Y_{DS}/Y_{NS})}{1 - (X_{DS}/X_{DS})}$$

3.2.6 Statistical Data Analysis

Statistical analyses on all traits measured in the current study were conducted in SAS 9.3 (SAS Institute, 2011). A t test was conducted between the drought stressed and non-stressed on electrolyte leakage. Normality tests were conducted on residuals for each trait using PROC UNIVARIATE NORMAL PLOT to determine if the data for each trait was normally distributed. Normality test results indicated that all traits were normally distributed. Initial ANOVA was conducted using PROC MIXED following the statistical model below:

$$Y = \mu + \alpha + k + \beta + l + \alpha*k + \alpha*l + k*l + \alpha*k*l + \mathscr{E}$$

Where: Y was the response variable e.g., Yield; μ is the population mean; α was the fixed effect of the genotype; k was the fixed effect of water regime (Non-drought Stressed (NS) or Drought Stressed (DS)); β was the random variable effect of a block; l was the fixed effect of the location; α*k was the random effect of the interaction between genotype and water regime; α*l was the random effect of interaction between genotype and location; k*l was the random effect of interaction between location and water regime; α*k*l the random effect of interaction between genotype, location and water regime; $\mathscr{E}$ was the residual (error) associated with replication and was considered as a random variable that was normally distributed with mean = 0. The above statistical model showed significant interaction between genotype and location for seed yield, pod number, shoot dry weight and hundred seed weight (Table 3.4 under results for objective 1 – Chapter three). Therefore, the data were analyzed according to individual location using PROC MIXED following statistical model:

$$Y = \mu + \alpha + k + \beta + \alpha*k + \mathscr{E}$$

Where: Y was the response variable e.g., Yield; μ is the population mean; α was the fixed effect of the genotype; k was the fixed effect of water regime (NS or DS); β was the random variable effect of a block; α*k was the random effect of the interaction between genotype and water regime; $\mathscr{E}$ was the residual (error) associated with replication and was considered as a random variable.

Genotypic correlation analysis between seed yield and other traits measured in the field was conducted using multivariate restricted maximum likelihood estimation with SAS PROC MIXED as described in Holland (2006).

3.2.6.1 Heritability Estimates

Broad sense heritability (H^2) was computed using data from all trials as they were conducted during the same period under similar weather conditions. The broad sense heritability estimate in a highly inbred crop such as common bean where the genotypes were evaluated is similar to narrow sense heritability (h^2) because dominance effects are assumed to be negligible. The variance component estimates (expected mean squares) from the ANOVA table were used to estimate broad-sense heritability using the following equation:

$$h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_{gl}^2/l + \sigma_e^2/rl}$$

where:

σ_g^2 is the genetic variance,

σ_{gl}^2 is the variance associated with genotype x location interaction,

σ_e^2 is the experimental error,

t is the number of environments, and

r is the number of replications.

3.3 RESULTS

3.3.1 Drought stress effect on the phenology of beans

There was no rainfall received in the area for the entire growing period of the field trials, and water was supplied through irrigation. There was a slight variation in the amount of water supplied to trials per site. The GART, Kabwe, and UNZA NS trials received about 150, 211, and 178mm of water more than the DS trials respectively after the induction of both the intermittent and terminal drought at flowering and mid-pod fill. Drought affected the days to physiological maturity of genotypes where genotypes subjected to DS took a fewer number of days from planting to physiological maturity compared to the same genotypes under NS (Figure 3.1).

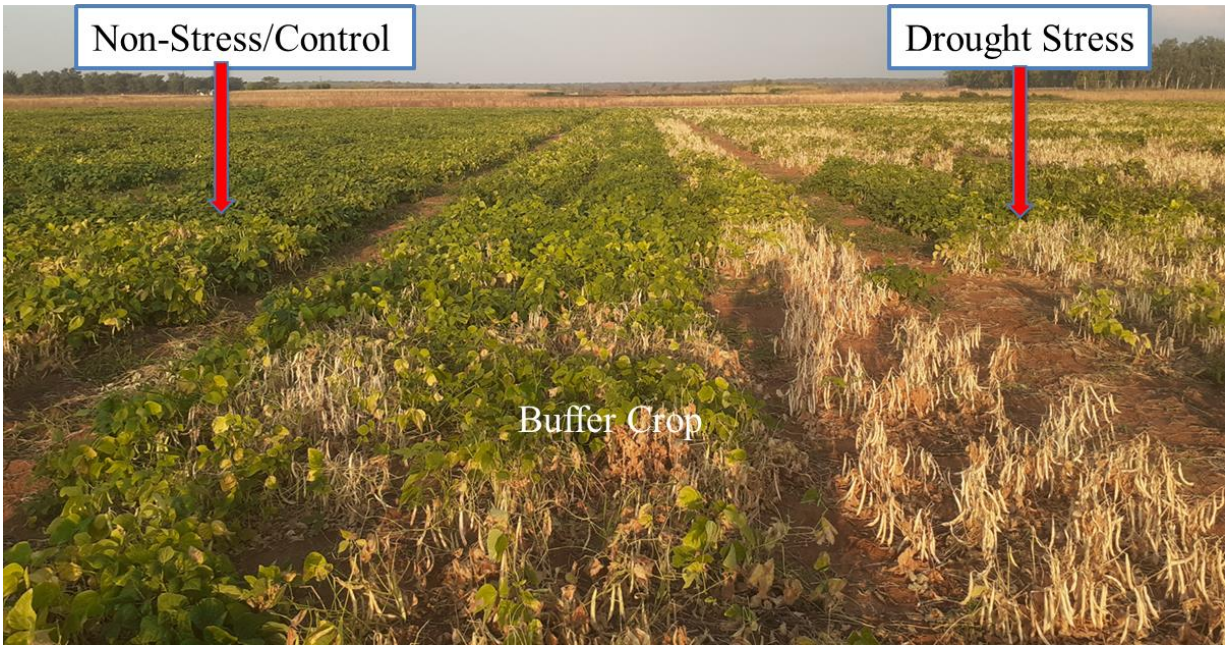

Figure 3.1: Field Trial three weeks after permanent termination of water supply in the drought stressed field at GART, Zambia in 2021

3.3.2 Across-site analysis of variance of agronomic, morphological, and physiological traits

The combined analysis of variance results across the sites (GART, Kabwe, and UNZA) indicated significant ($P<0.001$) effects due to genotypic, location, and treatment for Seed yield, PN, SDW, HSW, HI, and PHI (Table 3.4). The interaction for genotype x location was significantly different for seed yield ($p<0.001$), PN ($p<0.01$), SDW ($p<0.01$), and HSW ($p<0.01$) and not significant

(p>0.05) for partitioning indices. The interaction for Genotype x Treatment was significant for seed yield (p<0.05), HSW (p<0.001), and PHI (p<0.05). Effects due to the location x treatment interaction were significant for all traits. The overall interaction for genotype x treatment x location was not significant (p>0.05) for all traits. All traits were further re-analyzed according to individual locations (Tables 3.5 and 3.6).

Table 3.4. Mean squares from combined analysis of variance for common bean genotypes evaluated under drought-stress and non-stress treatments at GART, Kabwe and UNZA in Zambia.

SV	DF	Yield	PN	SDW	HSW	HI	PHI
Replication	2	186 507***	6361***	41709**	2.6***	0.067***	0.045***
Genotype	19	127201***	1766***	6682***	1100***	0.064***	0.031***
Location	2	6222739***	71675***	350700***	1001***	0.424***	0.116***
Treatment	1	11816353***	89615***	743018***	3797***	1.333***	0.766***
Genotype x Location	38	72487***	1394**	5128**	22**	0.008^{ns}	0.004^{ns}
Genotype x Treatment	19	50506*	1024^{ns}	3130^{ns}	25***	0.012^{ns}	0.008*
Location x Treatment	2	1849890**	11288***	68237***	59*	0.059**	0.005**
Genotype x Location x Treatment	38	31969^{ns}	456^{ns}	1997^{ns}	15ns	0.010^{ns}	0.006^{ns}
Residual	121	31172	751	3146	13	0.008	0.005

SV = Source of variation, DF=degrees of freedom, MS = mean square, ns = non-significant, * = significant at 5% ** = significant at 1% *** = significant at 0.1%. Yield=Seed yield, PN = pod load, HSW=hundred seed weight, SDW= Shoot Dry weight, HI=harvest index, PHI pod harvest index.

3.3.3 Agronomic and morphological traits of common Andean beans

3.3.3.1 Seed Yield

Significant differences between genotypes for seed yield were observed in GART, Kabwe, and UNZA (P<0.01 for all three locations) (Table 3.5). Seed yield for GART under NS ranged from 930 (G40001) kg ha^{-1} to 2946 (PI638816) kg ha^{-1} with an average yield of 1826 kg ha^{-1}. Under DS seed yield ranged from 277 kg ha^{-1} to 1160 kg ha^{-1} with an average yield of 791 kg ha^{-1}. Genotypes

Mshindi, G40001, and SCR 16 produced yields below Susceptible check Kabulangeti with 277, 350, and 458 kg ha^{-1} respectively. Genotypes including Pink Panther, and Kardinal. Krimson, Kijivu, H9659-27-10, OAC Inferno, G17913, H9659-27-7, Kibala, ADP 57, PI638816, and Mrondo produced higher yields compared to the drought tolerant checks. At Kabwe, G40001 and SER 16 were not planted because they did not have sufficient seed quantities. The seed yield range under NS was 575 – 2591 kg ha^{-1} with an average of 1417 kg ha^{-1}. Under DS seed yield range was 462-1165 kg ha^{-1} with an average of 795 kg ha^{-1}. The drought susceptible check *Lusaka* performed better than all the tolerant checks and only Kibala, Krimson, G17913, H9659-27-7, TARS HT1 and Kijivu had higher yields than it. At UNZA the seed yield range was 685 – 3076 kg ha^{-1} and 406– 1461 kg ha^{-1} under NS and DS, respectively. The average yields 1922 kg ha^{-1} and 1032 kg ha^{-1} were observed on NS and DS treatment respectively. The drought tolerant check SER 16 performed the best and Pink Panther was identified as the most drought tolerant genotype followed by G17913, PI638816, Krimson, and Mshindi respectively. The drought tolerant check G40001 performed worse than the susceptible check Kabulangeti (Appendix 1).

Genotype yield performance varied under the different conditions of watering regimes and locations. Under DS across the locations genotypes G17913, Pink Panther, Kijivu, PI638816, Krimson, Mshindi, Mrondo, and H9659-27-7 produced higher overall yield than all the checks (both susceptible and tolerant) while the checks G40001 (tolerant), SCR 16 (tolerant t) and Kabulangeti (Susceptible) performed poorly (Appendix 1).

3.3.3.2 Number of pods per plant (PN)

Pod load varied significantly among genotypes in the three locations due to treatments ($p<0.05$), and genotypes ($p<0.05$) and there were no significant ($p>0.05$) interactions among treatments and genotypes (Table 3.5). The PN in the three locations ranged between 9.3 and 14.3 under drought stress. The average PN in GART was 14.3 and 19.0 under DS and NS, respectively. At Kabwe, the PN average was 14.0 and 23.5, under DS and NS, respectively (Appendix 2). For UNZA the PN for DS and NS were 9.3 and 11.6, respectively. At GART under DS, TARS HT 1, H9659-27-10, Krimson, Kardinal, and H9659-27-7 had lower PN compared to the other genotypes while Kijivu, RWR 10, SCR 44, SEQ 11 and SER 16 had the higher PN compared to other genotypes. Under DS, RWR 10, H9659-27-7, G17913, SCR 16, and SEQ 11 had lower PN while LSK (Susceptible check), PI638816, TARS HT 1, Kardinal, and Kabulangeti (Susceptible check) had higher PN compared to other genotypes. ADP 57, PI638816, VA 19, Gololi, and SCR 10 had lower

PN while Mrondo, SER 16, Tepary, Mshindi, and SEQ 11 had higher PN compared to other genotypes at UNZA under DS. SER 16 and G40001 maintained high PN from the two locations they were grown with a percentage reduction of -0.03 and -0.08%. Genotypes G17913, Krimson, H9659-27-7, Pink Panther, RWR 10, VA 19, OAC Inferno, H9659-27-10, Kardinal, and ADP 57 had low PN compared to both categories of checks across locations under DS. Comparing PN to yield, genotypes G17913, Pink Panther, and H9659-27-7 had higher yields despite recording low PN compared to the checks (Appendices 1 and 2).

3.3.3.3 Shoot Dry Weight per plant (SDW)

Effects due to treatment were significant ($P<0.001$) for Shoot dry weight per plant among the genotypes grown at GART, Kabwe, and UNZA (Table 3.5). For GART, the average SDWs were 21.5 and 41.4 for DS and NS, respectively, representing a 48% reduction in SDW under DS compared to NS. The average SDWs for Kabwe were 26.9 and 48.1 for DS and NS, respectively. For UNZA it was 15.4 and 22.1 for DS and NS, respectively. The SDW reduction was 44 and 30% for Kabwe and UNZA respectively. G40001 had the lowest shoot dry weight at GART and UNZA both under DS and NS (Appendix 3). Kijivu, G17913, OAC Inferno, and PI638816 consistently had higher shoot dry weight across the locations under DS.

3.3.3.4 Hundred Seed Weight (HSW)

Significant ($P<0.001$) differences among genotypes for seed weight were observed in GART, Kabwe, and UNZA. Drought stress significantly ($p<0.001$) reduced HSW in all locations. The seed size reduction varied among genotypes (Table 3.5). The seed from the drought tolerant checks (Middle American genotypes and a *Phaseolus acutifolius*) generally weighed less than seed weight from the Andean genotypes. At GART under DS, seed weight ranged from 8.6 g (G40001) to 49.3 g (Pink Panther) with an average of 33.7 g. Genotype Pink Panther, Kijivu, G17913, OAC Inferno, and G6415 produced heavy seed weight compared to other genotypes (Appendix 4). For Kabwe, seed weight ranged from 24.2 g (SEQ 11) to 48.2 g (G17913) with an average of 34.9 g under drought stressed. Genotypes observed to have higher HSW compared to the others included G17913, Pink Panther, H9659-27-7, VA 19, and OAC Inferno in that order. The range for seed weight at UNZA under drought stressed was from 8.5 g (G40001) to 53.4g (G17913) with an average of 35.7 g. G17913, G6415, Pink Panther, VA 19, and OAC Inferno produced higher seed weight compared to other genotypes. Overall, among Andean genotypes G17913, Pink Panther,

G6415, VA 19, OAC Inferno, Kardinal, Krimson, H9659-27-7, Kijivu, and PI638816 consistently produced seed that were heavy HSW across the locations than both drought sensitive and tolerant checks. In this study, higher seed yield and seed weight were observed on genotypes G17913, Pink Panther, Kijivu, PI638816, Krimson, and H965927-7 compared to other genotypes (Appendices 1 and 4).

Table 3.5 Mean square for Single analysis on morpho-agronomic traits evaluated in the field on common bean genotypes grown under drought-stress and non-stress treatments at GART, Kabwe, and UNZA in Zambia.

GART	**DF**	Yield	PN	SDW	HSW
Replication	2	65406^{ns}	280.1*	1513.7***	51.4*
Genotype	19	105479**	49.5*	115.1^{ns}	339.7***
Treatment	1	12253803***	603.0***	13229.6***	5973.6***
Genotype: Treatment	19	74781^{ns}	30.9^{ns}	142.6^{ns}	37.6***
Error	78	47365	28.9	141.8	11.9
KABWE					
Replication	2	36837^{ns}	81.4	891.0**	50.9*
Genotype	19	42615**	63.8*	342.5*	256.4***
Treatment	1	644082***	2438.2***	12174.7***	1102.9***
Genotype: Treatment	19	29448^{ns}	44.0ns	127.7^{ns}	12.6ns
Error	78	18616	31.3	171.6	10.6
UNZA					
Replication	2	14713$_{ns}$	47.9**	141.5**	51.3*
Genotype	19	63527***	33.1***	46.6**	4.0.4***
Treatment	1	1403134***	42.0*	908.3***	3711.6***
Genotype: Treatment	19	16661^{ns}	6.3^{ns}	36.5^{ns}	36.8*
Error	78	16634	7.2	21.5	13.2

ns = non-significant, * = significant at 5% ** = significant at 1% *** = significant at 0.1%. Yield=Seed yield, PN = Number of pods per plant, HSW=hundred seed weight, SDW= Shoot Dry weight

3.3.4 Physiological Traits

3.3.4.1 Pod Harvest Index

Genotypic and treatment effects were significant ($p < 0.001$) for pod harvest index (PHI) at the three locations (Table 3.6). For GART, under NS the PHI ranged from 54 to 79% with an average of 72%. Under DS, the range for PHI was 51 – 73% with an average of 65%. In Kabwe, the average PHI was 60 and 66% for DS and NS, respectively. For UNZA, the averages for PHI were 62 and 72% DS and NS, respectively. The genotypes G17913, Pink Panther, and Krimson showed superior partitioning efficiency under drought stress across the three locations. The lowest PHI was observed on drought-susceptible susceptible check Kabulangeti (Appendix 5).

3.3.4.2 Harvest Index

The harvest Index was significantly ($p < 0.001$) different among genotypes and between treatments in GART, Kabwe, and UNZA (Table 3.6). The average HI for GART was 58% and 50% under NS and DS, respectively. For Kabwe the HI averages were 50 and 41% for NS and DS, respectively. For UNZA, HI averages were 73 and 63% for NS and DS, respectively (Appendix 6). The genotypes Gololi, G17913, Pink Panther, and Krimson showed superior HI across the three locations.

3.3.4.3 Carbon Isotope Discrimination (CID)

Carbon Isotope Discrimination, which was measured only in GART, was significantly ($p<0.001$) different among genotypes. The water treatment effects on CID were also significant ($p<0.001$). Further, the Genotype x Water treatment interaction effect on CID was also significant ($p<0.05$). The average CID for NS and DS were 19.6 and 18.6, respectively, which were statistically different (t-test; $p<0.01$). The CID range under DS was 17.5 (Kabulangeti) – 19.3‰ H9659-27-7. Under NS, CID ranged from 18.7 (Pink Panther) – 20.5‰ (SCR 44).

3.3.4.4 Electrolyte Leakage

A t-test result showed a significant difference in electrolyte leakage between NS and DS conditions ($P<0.001$). The average electrolyte leakage for the genotypes under NS and DS was 16.7 and 91.0, respectively. Genotypic effects on electrolyte leakage were significant ($p<0.01$). Treatment effects were highly significant ($p<0.001$) (Table 3.6), The range for electrolyte leakage was 71.8 (G40001) – 100.0% (ADP 57) under DS while under NS was 9.9 (G40001) - 32.7% (H9659-27-7). High

electrolyte leakage was observed on genotypes PI638816, TARS HT1, Mshindi, PR0737-1, ADP 57, and G6415 and this was a difference of more than 80% between DS and NS (Appendix 7).

Under DS, drought tolerant check G40001 (Tepary) had the lowest electrolyte leakage value followed by another drought tolerant check SCR16 under DS. Among the 20 Andeans genotypes, three genotypes Krimson, SEQ11, and OAC-Inferno ranked first, second, and third, respectively, in low electrolyte leakage levels, and the electrolyte leakages of these three genotypes were lower than the tolerant check SER16. Higher electrolyte leakage was observed on genotypes ADP 57, H9659-27-7, G6415, Mrondo, Mshindi, Kibala, and PR0737-1 compared to other genotypes (Appendix 7).

Table 3.6. Mean square for Single location analysis on physiological traits evaluated on common bean genotypes grown under drought-stress and non-stress treatments at GART, Kabwe, and UNZA in Zambia

Location	Source of Variation	DF	PHI	HI	CID	EL
GART	Replication	2	30.2^{ns}	231.9***	1.22**	
	Genotype	19	83.2***	166.7***	0.68***	
	Treatment	1	1230.3***	1500.9***	31.8***	
	Genotype: Treatment	19	16.5^{ns}	41.6^{ns}	0.35*	
	Error	78	12.6	29.7	0.2	
Kabwe	Replication	2	4.5^{ns}	164.8		
	Genotype	19	120.2***	318.3***		
	Treatment	1	743.5***	2425.6***		
	Genotype: Treatment	19	64.2**	142.9*		
	Error	78	28.9	76.5		
UNZA	Replication	2	95.0*	164.4**		131*
	Genotype	19	81.0***	122.3***		83**
	Treatment	1	1934.5***	3424.8***		174325***
	Genotype: Treatment	19	13.3^{ns}	30.0^{ns}		63^{ns}
	Error	78	20.4	31.6		38

ns = non-significant, * = significant at 5% ** = significant at 1% *** = significant at 0.1%. PHI pod harvest index, HI=harvest index, CID= carbon isotope discrimination, EL= electrolyte leakage

3.3.5 Effect of drought stress on seed yield

3.3.5.1 Drought Intensity Index (DII)

The drought intensity index for GART, Kabwe, and UNZA were 0.61, 0.55, and 0.53, respectively. Drought severity for UNZA and Kabwe were similar and significantly less than that for GART.

3.3.5.2 Drought Susceptibility Index and Percentage Reduction in Seed Yield

The yield Drought Susceptibility Index (DSI) differed between genotypes at all three locations. DSI at GART, Kabwe, and UNZA ranged from 0.20 – 1.46, 0.3-1.4, and 0.4- 1.4 respectively. Genotypes including SER 16, G40001, Kijivu, Krimson, and Pink Panther had lower DSI and were considered drought tolerant. These genotypes also had relatively low seed yield percentage reduction (YPR). The genotypes including Mshindi, Kabulangeti, SCR 16, and TARS HT1 had high DSI and were considered more sensitive to drought. The average yield percentage reduction for GART, Kabwe, and UNZA was 56, 41, and 45 respectively (Table 3.7).

3.3.5.3 Yield Geometric Mean (GM)

Significant genotypic differences ($p<0.05$) were observed for the yield geometric mean. The yield geometric mean range for GART was 570 (G40001) – 1702 (Pink Panther) kg ha^{-1}, with an average of 1191 kg ha^{-1} OAC Inferno, Mrondo, Kardinal, PI638816 and Pink Panther had higher GM than the drought tolerant checks. The GM for Kabwe ranged from 566 - 1457 kg ha^{-1} and the average was 1049 kg ha^{-1}, which was significantly ($p<0.05$) lower than GMs for GART or UNZA. The GM for UNZA ranged from 528 (G40001) – 2013 (SER 16) kg ha^{-1}, with the average being 1396 kg ha^{-1}. Concerning GM, genotype OAC Inferno was considered to be drought tolerant compared to other genotypes at UNZA (Table 3.7).

Based on the drought stress tolerance indices, DSI, YPR, and GM, drought stress generally reduced the yield of bean genotypes in DS conditions as compared to NS conditions across the locations. Genotypes Kabulangeti, SCR 16, and SCR 44 had high DSI and YPR values while Gololi, G17913, Kijivu, H9659-27-7, Kardinal, Krimson, and Pink Panther had low DSI and YPR values. The drought tolerant checks SER 16 and G40001 were among the genotypes with low DSI. Concerning GM G40001, SCR 16 and Kabulangeti had low GM while PI638816, SER 16, OAC Inferno, G17913, and Pink Panther had high GM across locations.

Table 3.7 Drought tolerance indices of genotypes grown under drought stress (DS) and non-drought stress (NS) conditions over one season at GART, Kabwe, and UNZA.

	Drought tolerance indices								
	GART			Kabwe			UNZA		
GENOTYPE	GM	DSI	YPR	GM	DSI	YPR	GM	DSI	YPR
Gololi	1058	1.1	62.4	1121	0.3	44.6	1422	0.7	37.0
Kijivu	1136	0.8	46.1	1301	0.3	46.4	1479	0.9	30.7
Mrondo	1523	1.1	60.4	1118	0.6	38.7	1565	1.0	40.3
ADP 57	1472	0.9	56.9	944	0.7	60.4	1217	1.2	55.5
Mshindi	710	1.5	84.7	1457	0.8	36.0	1513	0.9	25.7
G6415	1189	1.0	60.3	955	0.8	51.5	1268	0.4	21.7
G17913	1435	1.0	58.0	1421	0.8	47.3	1483	0.5	14.4
PR0737-1	1238	1.0	48.8	880	0.8	56.7	1145	1.1	55.3
Kibala	1242	1.0	45.3	1138	0.8	26.0	1607	1.2	59.2
RWR 10	1098	1.0	47.0	864	0.8	11.6	1721	1.2	62.1
SEQ11	1045	1.1	52.5	693	0.8	55.6	1500	1.3	59.3
H9659-27-7	1218	0.9	46.3	1025	0.9	1.5	1344	0.6	34.0
H9659-27-10	1285	0.5	42.0	931	1.0	63.6	994	1.0	43.0
OAC Inferno	1505	1.0	58.4	1425	1.0	43.2	1496	1.1	50.8
TARS HT 1	1171	1.0	61.2	1152	1.1	19.1	1371	1.2	53.5
Kardinal	1542	0.8	49.9	566	1.1	3.3	1497	0.9	44.0
Krimson	1268	0.9	50.8	1152	1.1	22.7	1517	0.7	32.8
VA-19	1265	1.2	70.5	1087	1.2	38.6	1422	1.0	40.0
Pink Panther	1702	0.9	53.5	981	1.2	44.0	1569	0.7	13.2
PI638816	1678	1.1	67.5	926	1.2	29.9	1911	0.8	42.3
Kabulangeti	758	1.0	59.9	871	1.2	69.7	1200	1.4	70.9
Lusaka	988	0.7	45.4	1223	1.3	35.9	1321	1.0	52.8
SCR_10	1219	1.0	63.8	1106	1.3	51.9	1094	1.3	53.6
SCR_16	839	1.2	70.3	719	1.3	53.6	1180	1.1	54.8
SCR_44	1055	0.9	57.3	1181	1.4	64.9	1327	1.2	58.1
SER_16	956	0.2	53.1	-	-	-	2013	1.1	57.2
G40001	570	0.3	62.4	-	-	-	528	0.9	40.8
Genotype Mean	1191	1	56	1049	1	41	1396	1	45

GM=Seed yield geometric mean, DSI=Seed yield drought susceptibility index and YPR=Seed yield percentage reduction.

3.3.6 Correlations between seed yield and other Morpho-agronomic and Physiological traits

The results of the correlations between seed yield and other traits under both DS and NS are presented in table 3.7. Shoot dry weight was significantly correlated with seed yield (r=0.35***) under drought stress, but not under NS in GART. In Kabwe, there was no significant correlation between seed yield and shoot dry weight under both DS and NS. For the UNZA trial, there was a significant and positive correlation between seed yield and shoot dry weight under both NS (r=0.30**) and DS (r=0.25**).

There was no significant correlation between seed yield and the number of pods per plant in the Kabwe and UNZA trials under both NS and DS. For the GART trial, the correlation between seed yield and the number of pods per plant was significant and under both NS (r=-0.20**) and DS (r=0.19*).

No significant correlation between seed weight (HSW) and seed yield except for Significant positive correlations was observed for GART under NS (r=0.35**) and UNZA under DS (r=0.63***). No significant correlations were observed for Kabwe (NS and DS), UNZA (NS), and GART (DS).

A strong positive correlation between seed yield and HI under DS in GART (r=0.45***), Kabwe (r=0.42***), and UNZA (r=0.2*) was observed. Significant positive correlations between seed yield and HI (r = 0.35, 0.37 and 0.35 for GART, Kabwe and UNZA respectively) were also observed under NS, but with lesser coefficients of determinations (r = 0.45, 0.42 and 0.20 fpr GART, Kabwe and UNZA respectively) than those under DS except for UNZA. A strong positive correlation between PHI and seed yield was observed under DS in GART (r=0.46**), Kabwe (r=0.38***), and UNZA (r=0.20*). The correlation between PHI and seed yield was also significant and positive (Table 3.8).

A significant strong positive correlation (r=0.57***) was observed between seed yield and carbon isotope discrimination under DS in GART. Under NS, the correlation between seed yield and CID was not significant.

Table 3.8 Correlation coefficients between seed yield and shoot dry weight, pod number, seed weight, harvest index, Pod harvest index, and carbon isotope discrimination for 27 common bean genotypes grown under non-drought stress (NS) and drought stress (DS) conditions at GART, Kabwe, and UNZA in Zambia.

	GART		KABWE		UNZA	
Trait	NS	DS	NS	DS	NS	DS
Shoot Dry Weight (g)	-0.05^{ns}	0.35***	0.21^{ns}	-0.06^{ns}	0.30**	0.25**
Number of Pods per plant	-0.20**	0.19*	0.08^{ns}	0.06^{ns}	-0.06^{ns}	0.09^{ns}
100 Seed Weight (g)	0.38**	0.05^{ns}	0.05^{ns}	0.11^{ns}	0.18^{ns}	0.63**
Harvest Index	0.35**	0.45***	0.37**	0.42***	0.35**	0.20*
Pod Harvest Index	0.32**	0.46***	0.37**	0.38**	0.37**	0.20*
Carbon Isotope Discrimination	0.07^{ns}	0.57***	-	-	-	-

DS = drought stress, NS = Non stress, ns = non-significant, * = significanct at 5% ** = significant at 1% *** = significant at 0.1%

3.3.7 Relationship between seed sield and Carbon Isotope Discrimination (CID)

The relationship between seed yield and CID under drought stress was explored using a quadrant plot (Figure 3.2). the quadrant plot helped to classify drought tolerant genotypes into two categories i.e. the ones that fall under the upper right and left top quadrants as “water spenders” and “water savers” respectively. Based on these criteria nine genotypes were placed in the upper right quadrant and classified as water spenders while three genotypes were placed in the top left quadrant and classified as water savers. The ones on the bottom left and right quadrants were classified as drought susceptible because they had their yield below the average under DS.

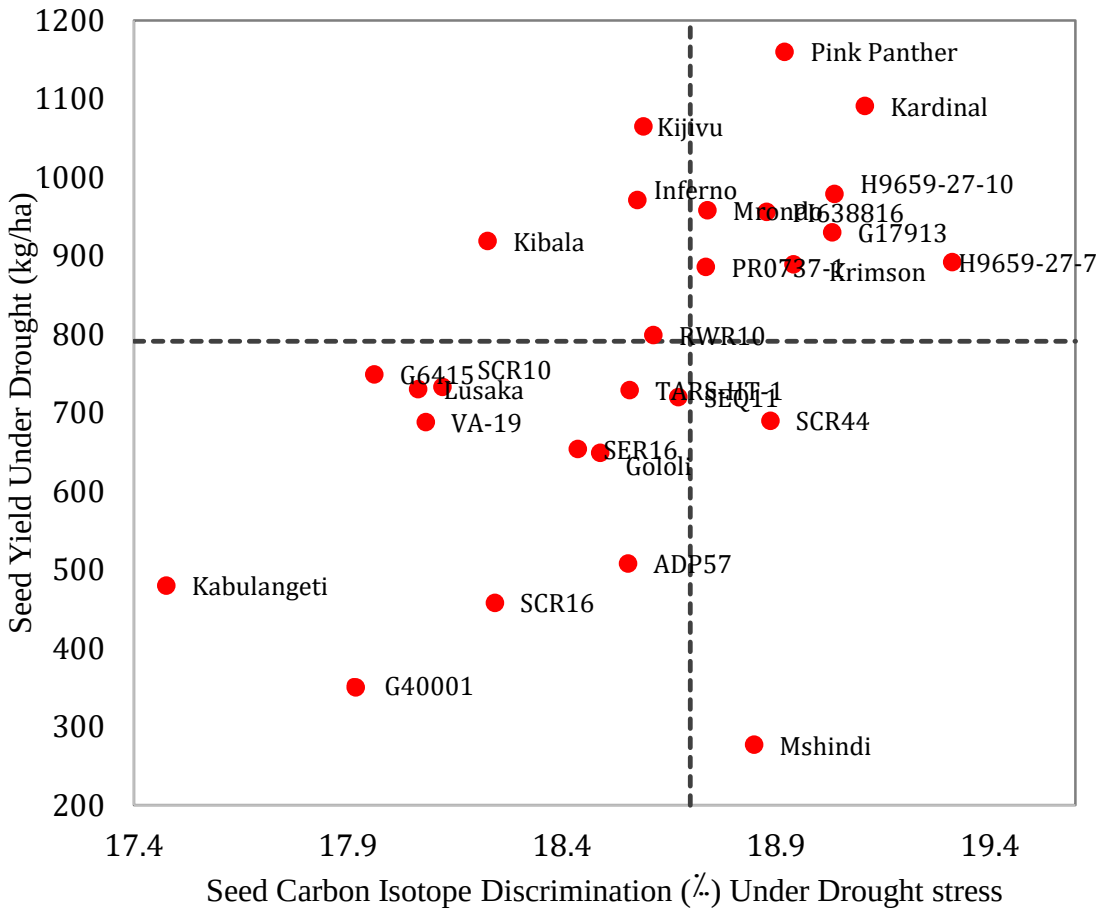

Figure 3.2: Relationship between grain yield and grain carbon isotope discrimination (CID) under drought stress. Water spenders with higher grain yield and greater values of CID were identified in the upper, right-hand quadrant. Water savers with higher grain yield and lower values of CID were identified in the upper, left-hand quadrant.

3.3.8 Heritability Estimates

Broad sense heritability (H^2) estimates were computed for the morpho-physiological traits across locations using the estimated mean squares from the ANOVA table (Tables 3.4 and 3.6). The broad sense heritability for (seed yield, pod load, shoot dry weight, hundred seed weight, harvest index and pod harvest index) from all locations and carbon isotope discrimination (GART only) were estimated at 0.43, 0.21, 0.23, 0.96, 0.88, 0.87, and 0.27 respectively.

3.4. DISCUSSION

In this study, the role and relative importance of agronomic and morpho-physiological traits to drought was investigated using 20 Andean genotypes selected based on previous knowledge/reports of their tolerance to drought. This revealed the complexity of the response of common beans to drought.

Drought significantly reduced seed yield, seed yield components, and partitioning indices, similar observations have been reported (Rezene et al., 2014, Darkwa et al., 2016, Polania et al., 2016a, b). The drought intensity index for GART, Kabwe, and UNZA were 0.61, 0.55, and 0.53, respectively. These figures indicate high drought severity at all three locations to identify the agronomic and morpho-physiological traits for the 20 Andean genotypes. The percentage reduction in seed yield at these three locations followed a similar trend for DII. These percentage reductions are similar to previously reported percentage reductions and highlight the damaging effect of drought on seed yield. Across the three locations, six genotypes including Kijivu, H9659-27-7, G 17913, Kardinal, Krimson, and Pink Panther showed much lower yield percentage reductions (<40%) compared to the tolerant check SER16 whose yield percentage reduction was 55.1% (Table 3.7). These six genotypes were less sensitive to drought than the other 14 Andean genotypes and the tolerant checks. The genotypes Krimson, Kardinal, OAC Inferno, and G17913 showed superior seed yield under both DS and NS. The genotypes would be recommended for genetic improvement of common beans not only for the drought but also for non-drought conditions because of their positive yield response to irrigation.

Drought tolerant checks performed below the average across locations, the poor performance could be attributed lack of adaptability of these genotypes to the environment. The drought susceptible checks showed higher PN despite having low yield, this could be because they have relatively low seed weight and also shorter pods accommodating less seed per pod. For genotypes that had higher yield, low PN, and higher seed weight such as H9659-27-7, G 17913, and Pink Panther, could be due to longer pods and high partitioning efficiency of photo-assimilates from the rest of the plant to the seed (Appendices 1, 2 and 4). The genotypes could also have exhibited high total shoot biomass to enable higher photosynthesis capacity (Polania et al., 2016).

Pod partitioning indices (HI and PHI), which represent the ability of a genotype to mobilize photo-assimilates to the seed from the rest of the plant, were significantly correlated with seed yield under both NS and DS across all the locations (Table 3.8). However, coefficient correlations for HI and PHI under DS were higher than under NS, suggesting a stronger relationship between the two partitioning indices under DS than under NS conditions. The strong relationship between partitioning indices suggests that efficient remobilization of photo-assimilates played a significant role in the observed drought tolerance of some of the genotypes in the current study. In addition, heritability estimates for PHI (0.87) and HI (0.88) were significantly higher than for seed yield (0.43). The low seed yield heritability was not a surprise because is a quantitative trait. PHI has previously been reported to be strongly associated with drought tolerance and has been suggested for use to indirectly select for drought tolerance because of its strong correlation with seed yield and its higher heritability than seed yield under drought stress (Ramírez-Vallejo and Kelly, 1998; Rosales et al., 2012; Beebe et al., 2013; Beebe et al., 2014; Polania et al., 2016a, 2016b).

Drought causes damage to the cell membrane, and the extent of this damage can be assessed using electrolyte leakage. Under drought stress, the stability and integrity of the cell membrane are compromised resulting in stress induced leakage of electrolytes from the cell. Drought sensitive genotypes tend to have higher electrolyte leakage than drought tolerant genotypes. In this study, electrolyte leakage for 27 genotypes was evaluated under NS and DS conditions in a greenhouse pot experiment. The genotypic effect on electrolyte leakage was not significant under NS but was highly significant under DS. This suggests that under NS, the 27 genotypes had little damage to their cell membranes and the electrolyte leakage was minimal and not significantly different between genotypes. However, under DS where there was induced oxidative damage to the cell membrane caused by drought, which could have led to increased electrolyte leakage, and the extent of cell membrane damage and leakage differed significantly between genotypes. The tepary bean genotype G40001, which was used as a drought tolerant check in this study, showed the least electrolyte leakage suggesting that it had the least damage to its cell membrane. This can be attributed to the well-developed fine root system that could have enabled it to effectively extract more water under drought stress conditions than the other genotypes. However, given that the electrolyte leakage experiment was a pot experiment where all genotypes had their root system constrained in equal soil volume, it is plausible that there could have been shoot traits including that which contributed to reducing damage to the cell membrane and the subsequent low

electrolyte leakage. The genotype G40001 has been used for improving drought tolerance in P. vulgaris (Mejia-Jimhnez et al., 1993) and has been previously reported Polania et al. (2016a); Burbano-Erazo et al. (2021) as a drought tolerant genotype. Its poor performance in this study could be attributed to it not being adapted to the environment it was grown under. Four genotypes Krimson, SEQ11, Lusaka, and OAC-Inferno had lower electrolyte leakage than the tolerant check SER16. Interestingly, Krimson was also among the six genotypes with the least percentage reduction in seed yield across the three locations, suggesting the potential usefulness of electrolyte leakage in selection for drought tolerance in common beans. França et al., (2000) also identified cell membrane stability under drought stress assessed based on electrolyte leakage as a useful physiological trait for the identification of drought- tolerant common bean genotypes.

Based on the CID (measurement for WUE) relationship with seed yield under DS, nine genotypes in this study were classified as water "spenders". These nine water "spenders" genotypes with high CID values under drought stress conditions may suggest that they had access to water from lower soil profile after the onset of drought stress, which could have enabled these genotypes to maintain higher transpiration rates, open stomata, healthy gaseous exchange, and carbon assimilation through sustained photosynthesis. The high access to soil moisture may be due to the extensive root system for the water "spenders" genotypes. The lower CID values for the water "savers" may be due to stomata closure in response to drought, which could have limited gaseous exchange and reduced carbon assimilation.

Water Use efficiency plays an important role in drought tolerance as it is directly related to the amount of dry matter produced per unit volume of water. Under drought conditions, genotypes with high WUE will transpire less, have a lower water use rate, and produce more dry matter for less amount of water. CID, which represents an integrated measurement of leaf gaseous exchange between the plant and atmosphere over time is inversely related to WUE and is used as a surrogate measure of WUE. In this study, genotypic differences for CID were identified under both DS and NS conditions suggesting differences in WUE and carbon assimilation between genotypes. Genotypic differences in CID reflect genetic differences in leaf gaseous exchange (hence photosynthesis) and plausibly genotypic differences in root systems (White et al., 1990). Under drought conditions, genotypes with deeper roots are likely to continue extracting water from lower soil profiles that cannot be accessed by shallow-rooted plants, thereby maintaining healthy leaf

gaseous exchange and high CID. Additionally, CID was highly correlated with seed yield under drought stress (r=0.57***). Genotypes that were identified in this study as drought tolerant (based on CID) were classified into two i.e., "water savers" and "Water Spenders". This classification was based on genotypic WUE inferred from seed yield relationship with CID under DS (Figure 3.2) (Polania et al., 2016). Nine drought tolerant genotypes (top right quadrant of Figure 3.2) which had higher seed yield and CID than averages, were classified as "water spenders". Some of these genotypes including H9659-27-7, G17913, and Krimson were previously reported as drought tolerant (Dramadri et al., 2021). These nine drought tolerant genotypes could probably have depended on their deeper root system to extract more water from the lower soil profile to maintain transpiration, and normal gaseous exchange through open stomata, which could have allowed higher carbon production and assimilation. Subsequent partition of these assimilates to the seed could have resulted in higher seed yields under drought conditions. In common beans, a strong correlation (r=0.84) between CID and root length was previously reported. Under drought, genotypes with deeper roots are likely to extract more water from lower soil profiles than shallow-rooted genotypes which helps them maintain higher transpiration, photosynthesis, and carbon discrimination (White 1993).

The other group of drought tolerant lines, which had high seed yield, but low CID under DS were classified as "water savers". The three genotypes in this group including Kijivu, OAC Inferno, and Kibala could have transpired less water and were probably more efficient at utilizing their water to produce assimilates. Also, these three genotypes could have been efficient at partitioning assimilates to the seed based on the higher partitioning indices than the other genotypes. Among the water savers identified in this study, Kijivu has previously been identified as a drought tolerant genotype (Mndolwa et al., 2018).

Classification of drought tolerant genotypes into water savers and water spenders has previously been used to make recommendations on the suitability of the drought tolerant genotypes to specific agro-ecological zones that experience either terminal or intermittent drought (Polania et al 2016). The nine drought tolerant genotypes classified as "water spenders" would be suited to less severe drought conditions and/or intermittent drought as these genotypes could use their efficient water extraction ability from the deeper soil profile. On the other hand, the three "water savers" drought tolerant genotypes identified in this study may be suited to agro-ecological zones characterized by

shorter growing seasons and prone to terminal drought because these genotypes have high WUE and conserve water under drought but are still able to produce dry matter and partition to seed resulting in sustained seed yield under drought.

3.5 CONCLUSION

Significant genotypic differences were observed in morpho-physiological traits measured under drought and non-drought stress conditions. The genotypic effect on electrolyte leakage under DS was significant suggesting differences between genotypes in their susceptibility to cell membrane damage caused by drought. Pod harvest index, harvest index, and carbon isotope discrimination were strongly correlated with seed yield under drought stress. Additionally, PHI and HI had higher heritability than the other morpho-physiological traits and can be used to indirectly select for drought tolerance. Based on the relationship between seed yield and CID under drought conditions, 14 genotypes were identified as drought tolerant and of these, three were classified as water savers while nine as water spenders. This study has highlighted the complex interplay of agronomic and morpho-physiological traits in the adaptation of Andean genotypes to drought. This study has also identified the traits and genotypes that can be used in the genetic enhancement of common bean adaptation to drought through breeding.

CHAPTER FOUR

IDENTIFICATION OF QUANTITATIVE TRAIT LOCI ASSOCIATED WITH DROUGHT TOLERANCE IN RECOMBINANT INBRED LINE POPULATION OF ANDEAN COMMON BEAN (*Phaseolus vulgaris* L.) IN ZAMBIA

ABSTRACT

Drought is a major production constraint of common bean worldwide. The objective of this study was to identify the QTL for drought tolerance in an Andean population of Recombinant Inbred Lines (RILs). A total of 155 F4:5 RILs derived from a cross between drought tolerant genotype Kijivu and drought susceptible genotype Bukoba were evaluated for drought tolerance in field experiments. A total of four field experiments were conducted at three locations in 2020 and 2021. The 155 RILs were genotyped with 12, 000 single nucleotide polymorphism (SNPs) markers chip and composite interval mapping was conducted to identify QTL for drought tolerance. Seed yield for Kijivu under drought stress condition was consistently higher than for Bukoba across all four field trials. In this study, 71 QTL were identified for morphological, agronomic and physiological traits under both drought stress and non-stress conditions. However, majority of these QTL were specific to drought stress. QTL "hotspots" for drought tolerance were identified on chromosomes Pv06, Pv07 and Pv10. Extensive co-localizations for morpho-agronomic traits under drought stress were observed at the three drought tolerance QTL "hotspots". In addition, these three QTL hotspots overlapped with previously identified QTL for drought tolerance. Some of the identified QTL are novel. If validated further, the three identified QTL hotspots could be used in Marker-assisted selection for drought tolerance in common bean.

4.1. INTRODUCTION

Drought is a major source of seed yield losses in common beans (*Phaseolus vulgaris*) worldwide. About 60% of common bean production worldwide is in environments that are prone to drought. Drought is one of the contributing factors to low bean yields in Africa as production of bean is extended to marginal areas, which often have poor soil fertility and/or are prone to drought (Beebe et al., 2013).

In Southern Africa, there was a significant drop in yield between the years 1998 and 2018 (Farrow and Muthoni-Andriatsitohaina, 2020), and this drop was partly attributed to low soil moisture regimes caused by drought. Climate change is likely to exacerbate drought episodes as nearly 73% of bean producing areas in Africa are projected to be affected by increased drought frequency and severity caused by climate change. Unfortunately, bean farmers in Africa, who are predominantly small-scale farmers mostly women who lack access to irrigation infrastructure to mitigate drought effects on crop productivity.

Genotypes belonging to race Durango from the Middle American gene pool have the highest levels of drought tolerance and have been used as sources of tolerance to improve other market classes, especially those in the Middle American gene pool. Drought-tolerant Middle American genotypes from races Durango and Mesoamerican have been identified, and crosses between drought tolerant genotypes from these two races have produced progenies with superior drought tolerance. However, breeding efforts to transfer tolerance from races Durango or Mesoamerican to large-seeded Andean genotypes have been hampered by poor agronomic traits of progenies from inter-gene pool crosses.

Several agronomic, morphological, and physiological traits involved in drought tolerance have been identified (Beebe et al., 2013; Rezene et al., 2014; Polania et al 2016; Dramadri et al 2021). Genetic enhancement of common bean for drought tolerance requires characterization of these traits and how they relate to seed yield under drought stress. Photo-assimilate partitioning indices such as harvest index (HI) and pod harvest index (PHI) have been identified as important target physiological traits in breeding for drought tolerance (Assefa et al., 2013; Mukeshmana et al., 2014; Dramadri et al 2021). Common bean genotypes that are efficient in remobilizing assimilates to the seed during drought stress tend to be high yielding. Because of the high heritability of PHI

and its strong correlation with seed yield, PHI has been recommended for use to indirectly select for drought tolerance (Mukeshmana et al., 2014; Assefa et al., 2013). Photosynthesis is highly sensitive to drought stress and under drought stress, photosynthetic activity and subsequently production of photo-assimilates is significantly diminished resulting in reduced biomass accumulation, pod filling, grain filling, and ultimately seed yield. Variation in photosynthetic activity under drought stress has been reported in the Andean gene pool of common beans (Dramadri et al., 2021). Identification of genotypes with higher photosynthetic performance and an understanding of their genetic basis can support the genetic enhancement of drought tolerance in common beans.

Drought tolerance is a genetically complex trait involving several genes for agronomic, morphological, and physiological traits. The expression of genes for some of these traits is highly influenced by the environment resulting in lower heritability. Quantitative trait loci analyses and GWAS studies have been used to identify genomic regions significantly associated with drought tolerance by assessing different traits (Mukeshimana et al., 2014; Berny Mier y Teran et al., 2019; Sedlar et al., 2020; Berny Mier y Teran et al., 2020; Dramadri et al., 2020; Valdisser et al., 2020). These traits include seed yield and its components, partitioning indices, and shoot dry weight among others. QTL for these traits have been reported on all eleven chromosomes of beans using mapping populations of RILs evaluated under drought-stress and non-stress conditions field trials (Schneider et al., 1997; Acosta-Díaz et al., 2004; Beebe et al., 2006; Mukeshimana et al., 2014; Sedlar et al., 2020). Most of the RIL populations used in these studies were derived from Middle American parents or both gene pools. GWAS studies using diversity panels with Andean and Middle American genotypes have been used to identify genomic regions and candidate genes associated with traits for drought tolerance (Hoyos-Villegas et al., 2017; Valdisser et al., 2020; Dramadri et al., 2021). As expected the percentage of variation explained by these QTL have generally been low for seed yield, but high for traits such as partitioning indices, and plant biomass measured under moisture stress. Co-localizations have been reported on some of the identified QTL for traits associated with drought tolerance. For example, seed yield QTL under drought stress was consistently identified on chromosome *Pv06* (18 – 25 Mb) in previous QTL studies (Berny Mier y Teran et al., (2019) and GWAS (Dramadri et al., 2021). Identification of QTL in different environments and populations suggests that these QTL are stable and may have the potential to be used as markers in molecular breeding against drought stress.

Most of the QTL analysis studies on drought tolerance in common beans have been conducted using Middle American populations (Asfaw and Blair., 2011; Hoyos-Villegas et al., 2017; Diaz et al., 2020), and these results have provided important insights into the genetic architecture of drought tolerance in common bean. These insights have enabled more progress in breeding for drought tolerance in the small-seeded Middle American gene pool in comparison to the large-seeded Andeans, which are more popular in East and Southern Africa.

Identification of Andean genotypes tolerant to drought, and understanding the genetic basis of that tolerance is important for supporting the genetic improvement of Andean beans for tolerance to drought. QTL analysis studies using Andean germplasm are important for the identification of drought tolerance QTL alleles from the Andean gene pool. Additionally, such studies are important for determining the stability in the Andean genetic background of drought QTL identified from the Middle American gene pool (Mukeshimana et al., 2014; Dramadri et al., 2019). The objective of this study was to identify quantitative trait loci for drought tolerance in an Andean population of recombinant inbred lines derived from two Andean genotypes with contrasting responses to drought.

4.2. MATERIALS AND METHODS

4.2.1 Experimental sites

The study sites in this study were as outlined in section 3.2.1 of Chapter III of this thesis. The trials were conducted in two dry seasons i. e. hot dry season in the years 2020 and 2021 at three locations. One trial was conducted in 2020 at Kabwe (hereafter known as Kabwe_2020). The other three trials were conducted in the hot dry season of 2021 at GART, Kabwe, and UNZA hereafter known as GART, Kabwe_2021, and UNZA respectively.

4.2.2. Genotypes

A population of 155 $F_{4:5}$ RILs derived from a cross of Andean parents Bukoba (ADP 7) and Kijivu (ADP 33) was used in the current study. Bukoba has a mayocoba yellow large seeded and Kijivu is purple speckled large-seeded. Both parents have a type I (bushy) growth habit and were developed in Tanzania. Genotypes with purple seed color are a popular market class in Tanzania and Zambia where they are known as "Kabulanketi" and "Kabulangeti", respectively. Previous studies (Mndolwa et al., 2018) identified Kijivu as being drought tolerant and Bukoba susceptible. A Middle American genotype SER16 with known drought tolerance (Polania et al., 2016a) was used as a drought tolerant check while a local landrace Kabulangeti as a drought susceptible check.

4.2.3. Field Experiment Procedure

Land preparation and fertilizer application (granular at planting at four weeks after planting, and foliar fertilizer) were done as described in chapter three. A total of 159 genotypes composed of 155 RILs, their parents, and the checks SER16 and Kabulangeti were evaluated in the field for drought tolerance. Each genotype in replication was planted in a single row plot that was 4 m long; with the spacing of 0.6m and 0.05m for inter-row and intra row spacing respectively. The randomized complete block design (RCBD) was used with three replications for each treatment (DS and NS) as in chapter three. The four trials were conducted during the hot dry season in August to November 2020 as of Kabwe_2020 while the rest were carried out in 2021. The water supply for these trials was similar to the one described in chapter III of this thesis for both NS and DS.

4.2.4 Pot Experiment Procedure

A single pot experiment was conducted during the dry season (no rainfall) between August – November 2021. Three seeds of each genotype were planted in a 20cm diameter 5-L polyethelene pots filled with loamy clay soil (Altisols) collected from the University of Zambia Research Farm. The pots were arranged in RCBD with three replications. The pots were placed in the Middle of an open field secured with a wire fence at the University of Zambia Research Farm. Half of the total number of pots was designated for drought-stress (DS) while the other half was for non-stress (NS). When seedlings had reached the first trifoliate stage, thinning was conducted to leave only two plants in each pot. Plants in both DS and NS were treated similarly until the start of flowering when water was intermittently withdrawn from the DS trial to simulate intermittent drought. Each water stress episode under DS lasted for a variable number of days depending on temperature and heat in the field, but water stress was up to the point when plants were getting close to wilting point.

4.2.5 Data Collection

4.2.5.1 Field Trials Data Collection

Data was collected on primary variables including total shoot biomass (TSB), pod load (PN), seed yield, and seed weight (HSW). The secondary variables included the partitioning indices and the estimated variables (Table 3.3) using data collected as described in section 3.2.5.1, 3.2.5.2.1, and 3.2.5.3 where the only difference was the materials used.

4.2.5.2 Pot Experiment Data Collection

Photosynthetic parameters were measured using the MultispeQ device (Figure 4.1) (Kuhlgert et al., 2016) in the DS experiments on the fifth day after each water-withdrawal while in the NS about a day after watering. The photosynthetic parameters focused on included LEF, PhiNO, and PhiNPQ. No additional photosynthetic data apart from the listed above were analyzed from the pot experiments.

Figure 4.1: Multispeq (Photosynq) device used to collect photosynthesis related data

4.2.6 Genotyping

Leaf tissue was collected from an individual plant for each RIL and parents grown in the USDA-ARS greenhouses at Prosser, WA, United States. Genomic DNA was isolated from 20 mg of leaf tissue using a QIAGEN DNeasy 96 Plant Kit (Hilden, Germany). The population was then genotyped with 12,000 SNP markers using the Illumina BeadChip modified from Song et al. (2015), at the USDA-ARS, Soybean Genetics and Improvement Laboratory, Beltsville, MD. The SNPs were aligned with the v2.1 reference genome assembly of G19833. Further filtering was perfomed, as SNP markers with > 20% missing data, significant deviation from the expected Mendelian segregation ratios, or redundant SNPs in complete linkage disequilibrium, were removed. 1,838 SNPs were retained for linkage map construction using MapDisto version 1.8.1 (Lorieux, 2012) with an r_{max} of 0.24, LOD_{min} of 3.0, and the Kosambi function. Of the 11,292 SNPs, 1838 were polymorphic between parents and were used to build a linkage map using JoinMap. A total of 11 linkage groups corresponding to the 11 chromosomes were built and span a genetic distance of about 909 cM (Table 1.1). The number of markers per linkage group ranged from 53 (chromosome Pv06) to 262 (chromosome Pv10) with an average size of 167 SNPs per linkage group. The linkage group size ranged from 45 cM (Pv06) to 107 cM (Pv01 and Pvo7) with an average size of 83 cM. The distance between markers ranged from 0.23 cM (Pv10) to 0.98 (Pv07) with an average of 0.55 cM.

Table 4.1 Distribution and distance on individual chromosomes of the genetic linkage map of Bukoba/Kijivu recombinant inbred line (RIL) population of common bean

Linkage group	No. of markers	Linkage group length (cM)	Average distance between markers (cM)
Pv01	154	107	0.69
Pv02	227	101	0.44
Pv03	209	99	0.47
Pv04	182	82	0.45
Pv05	165	72	0.44
Pv06	53	45	0.85
Pv07	109	107	0.98
Pv08	191	89	0.47
Pv09	175	86	0.49
Pv10	262	61	0.23
Pv11	111	60	0.54

4.2.7 Statistical Data Analysis

4.2.7.1 Phenotypic Data Analysis

Statistical analyses on phenotypic data were conducted in SAS 9.3 (SAS Institute, 2011). A ***t***-test was conducted between the parents for both primary and secondary traits. The assumption for normally distributed residuals required for analysis of variance (ANOVA) was checked for all traits measured. Normality tests were conducted on the combined residuals of all treatments for each trait using PROC UNIVARIATE NORMAL PLOT. Analysis of variance (ANOVA) for each trial was conducted using PROC MIXED on all the traits based on the following statistical model:

$$y = \mu + G + L + Y + G * L + G * Y + G * L * Y + B + E$$

Where: y was the response variable e.g., Yield; μ was the population mean; G was the fixed variable effect of the genotype (RIL); L was the fixed effect of the location, Y was the fixed effect of the year; G*L was the genotype by location interaction effect; G*Y was the genotype by year interaction; G*L*Y was the genotype by location by year interaction effect; B was the random effect of a block within a location; E was the residual (error), which was assumed to be normally distributed with mean =0. For the pot experiment, ANOVA was conducted on photosynthetic traits using a mixed model, where genotype and water treatment were considered to have fixed effects while replication and error had random effects.

Genotypic correlation analysis between traits measured in the field was conducted as described in section 1.5.1 of this thesis.

4.2.7.2 QTL Analysis

QTL analysis was conducted using composite interval mapping in the software QTL Cartographer (Wang et al. 2011). In composite interval mapping the following control parameters were used: (i) model 6 (Standard model), (ii) 5 control/background markers, (iii) 10 cM window size, (iv) forward and backward multiple regression model, and (V) 1 cM walk speed (genome scan interval). A permutation test (1,000 permutations) was used to determine the LOD threshold of 3.0 (Churchill and Doerge 1994), which was used to determine QTL significance. The software MapChart (v. 2.30, Wageningen University and Research, Wageningen, Netherlands) was used to display the linkage maps with QTL on them. The coefficient of determination (R^2) was used to estimate the proportion of variation explained by a QTL.

The QTL physical positions identified in the current study were based on *Phaseolus vulgaris* v2.1. The QTL physical positions of the previously reported QTL based on *Phaseolus vulgaris* v1.0 were adjusted to positions based on *Phaseolus vulgaris* v2.1. Thus, all the QTL physical positions reported in the current study and those previously reported (referenced in the discussion) were based on *Phaseolus vulgaris* v2.1.

4.3. RESULTS

4.3.1 Phenotypic Analyses

4.3.1.1 Drought tolerance Indices

The drought stress index based on seed yield was computed for all four field trials. The highest drought stress index of 0.71 was observed at Kabwe-2021 while UNZA had the lowest index of 0.45.

Significant ($p<0.05$) differences between parents for Yield Geometric Mean (YGM) were observed. In this study, genotype Kijivu showed higher YGM than Bukoba in all four trials. Significant ($p<0.05$) differences were observed between RILs for YGM in all four experiments. YGM ranged from 381 (Kabwe_2020) to 1782 kg^{-1} (GART) with an average of 967 kg^{-1} across locations.

The drought susceptibility index (DSI) was computed for the RILs, parents, and the checks based on seed yield. Significant ($p<0.05$) differences between parents were detected for DSI. Bukoba had a higher DSI than Kijivu for the four trials. The DSI for the check *SER 16* was higher than the two parents in all four trials. Significant ($p<0.05$) differences between RILs for DSI were observed in all four trials. The average population DSI for GART, UNZA, Kabwe_2020, and Kabwe_2021 were 0.99, 0.51, 0.52, and 0.38, respectively.

4.3.1.2 Seed Yield

The two parents (Kijivu and Bukoba) were significantly (t-test; $p<0.05$) different for yield at all four locations under DS conditions Table 4.2. Under the DS condition, Kijivu had a higher seed yield than Bukoba for the GART, UNZA, Kabwe_2020, and Kabwe_2021 trials (Table 4.2). The average yield of Kijivu across the four experiments was 1123 kg ha^{-1} while for Bukoba it was 593 kg ha^{-1}. Under the NS condition, the average yields across the four experiments were 1671 kg ha^{-1} and 1148 kg ha^{-1} for Kijivu and Bukoba, respectively. Overall, Kijivu showed superior seed yield performance to Bukoba under both DS and NS conditions. The seed yield percentage reduction for Kijivu was 55, 34, 40, and 32% for GART, UNZA, Kabwe_2020, and Kabwe_2021 trials, respectively. For Bukoba, yield percentage reduction in yield were 69, 52, 55, and 36% for GART, UNZA, Kabwe_2020, and Kabwe_2021 trials. These results showed on average a smaller yield percentage reduction for Kijivu than for Bukoba.

RILs were significantly ($P < 0.01$) different for seed yield Table 4.1). Population average yields under DS conditions were 1471, 686, 480, and 236 kg $ha^{-1}$for the GART, UNZA, Kabwe_2020, and Kabwe_2021 trials respectively. Under the NS condition, the population average seed yields were 2226, 1110, 1623, and 659 kg ha^{-1} for GART, UNZA, Kabwe_2020, and Kabwe_2021 trials respectively. These yields represented percentage reductions of 57, 42, 44, and 61% for GART, UNZA, Kabwe_2020, and Kabwe_2021, respectively, in seed yield due to drought stress. The biggest reduction was observed for GART while the smallest reduction was observed for Kabwe_2020.

4.3.1.3 Pod Harvest Index (PHI) and Harvest Index (HI)

Parents showed a significant ($P < 0.05$) difference between PHI and HI. Kijivu had higher PHI and HI in all trials. Significant differences ($P < 0.05$) for PHI and HI were observed among the RILs. PHI average under DS ranged from 56% (Kabwe_2020) to 68% (UNZA) while under NS the range was 63 to 74%. HI under DS ranged from 41% (Kabwe_2020) to 57% (UNZA) while under NS the range was 46 to 62 % for Kabwe_2021 and GART respectively. The means for PHI and HI for the population under DS and NS are shown in Table 4.2.

4.3.1.4 Pod Number per plant (PN)

There were significant differences between the parents in pod number (PN). Under DS, Kijivu had higher PN than Bukoba while under NS, the two parents did not vary very much. RILs were significantly ($P < 0.05$) different in PN for all four trials and the averages for PN for the RILs under DS and NS in the four experiments are shown in Table 4.2.

4.3.1.5 Hundred Seed Weight (HSW)

Results for HSW showed significant differences among parents for both DS and NS experiments. Kijivu had higher HSW than Bukoba in all four trials under trial conditions (NS and DS). Results among RILs showed significant differences in HSW under both DS and NS in all four experiments (Table 4.2). Percentage reductions in HSW were minimal in all four trials.

4.3.1.6 Total shoot dry weight (SDW)

Total shoot dry weight (SDW) was significantly different between parents under DS conditions at GART, Kabwe_2021 and Kabwe_2020. The results showed that Kijivu had higher SDW than Bukoba in all trials. Under NS conditions, significant differences between parents were only

observed at GART and Kabwe_2020. Significant differences among RILs were observed for SDW under both DS and NS at GART trials, there were no significant ($p>0.05$) differences among RILs for for UNZA DS, Kabwe_2021 NS and Kabwe_2020 NS conditions (Table 4.2).

Table 4.2 Means (±SE) and ranges for water treatment effects on yield, yield components and partitioning indices of parents (Bukoba and Kijivu) checks (SER 16 [tolerant] and Kabulangeti [susceptible]), and 155 recombinant inbred lines (RILs) grown under drought stress and non-drought stress in Zambia in 2020 and 2021.

	Parental Means		t-test	Checks		RILs (n = 155)		
	Bukoba	Kijivu		Kabulangeti	SER 16	Mean	Range	ANOVA
GART Stressed 2021								
Yield	969 ±31.2	2125±12.0	***	842±65.28	1222±6.33	1470±45.5	144-2677	**
Biomass	94.7±2.32	117.6±0.17	*	104.69±24.0	344.47±8.55	96.74±2.04	9.48-219.89	*
PHI	65.4±1.1	73.0±4.9	***	51.6±7.6	67.2±1.7	64.4±0.5	22.3-84.9	**
HI	47.6±2.3	63.3±1.4	***	36.9±8.5	54.3±2.1	51.3±1.3	0.0-70.3	***
HSW	32.8±2.7	45.2±2.9	***	35.88±1.81	22.06±2.65	35.59±0.38	21.12-56.46	***
PL	16.9±0.73	15.8±1.26	*	13.36±2.30	18.47±1.66	17.00±1.94	Jan-45	***
GART Control 2021								
Yield	1939±35.8	2975±48.1	*	2121±28.11	2448±15.61	2226±22.17	813-3020	***
Biomass	309.9±34.3	271.3±38.9	*	258.43±4.76	734.37±71.13	206.64±4.05	14.34-448.72	**
PHI	76.8±2.5	74.9±1.6	ns	68.5±0.4	73.8±1.4	74.7±0.3	40.3-89.4	ns
HI	65.4±2.3	62.7±1.5	ns	0.55±2.3	61.7±1.3	62.4±0.4	15.2-80.5	***
HSW	36.0±1.6	52.0±2.4	**	27.78±4.30	31±3.87	44.27±0.44	25.26-82.94	***
PL	32.3±2.65	21.8±1.73	*	18.3±1.48	24.8±2.09	23.42±1.98	Aug-60	***
UNZA Stressed 2021								
Yield	512±41.2	749±18.3	*	711±67.99	910±22.98	686.5±5.76	272-1463	***
Biomass	61.7±10.4	55.0±7.9	ns	55.05±8.93	107.02±7.66	41.47±0.84	6.89-104.23	ns
PHI	69.2±3.1	69.4±1.7	ns	63.2±4.7	71.6±1.2	68.4±2.3	25.4-83.7	***
HI	60.9±3.8	61.3±1.0	*	39.3±13.1	61.2±1.3	57.8±2.0	16.6-67.9	*
HSW	32.7±0.9	42.7±0.4	***	43.2±0.50	20.15±0.98	34.54±0.35	21.20-58.30	***
PL	-	-	-	5.4±1.81	15.2±1.80	-	-	
UNZA Control 2021								
Yield	1053±48.3	1185±94.0	ns	1090±89.81	1585±57.29	1110±26.8	439-2631	***

Biomass	62.3±9.9	77.1±18.2	ns	122.77±12.74	173.11±14.73	94.05±1.79	30.77-220.29	**
PHI	74.1±1.2	66.8±9.2	ns	72.5±1.3	76.8±2.4	71.3±0.4	34.6-86.9	ns
HI	64.8±2.5	57.3±1.8	ns	61.7±1.5	67.4±2.2	60.8±0.4	21.3-77.8	**
HSW	33.7±0.7	45.3±0.2	*	46.67±1.44	32.41±2.29	38.29±0.46	24.00-86.07	***
PL	-	-		13.89±0.89	20.6±2.92	-	-	
Kabwe Stressed 2021								
Yield	518±41.0	1090±61.9	*	657±47.97	-	480±34.97	75-1062	***
Biomass	63.7±2.1	119.6±16.2	*	181.64±66.68	-	78.29±2.14	27.75-288.48	*
PHI	49.8±1.3	66.7±3.4	*	54.3±4.3	-	58.7±1.3	10.3-76.5	***
HI	30.8±11.4	54.6±3.8	*	35.4±5.2	-	37.3±1.9	1.8-65.4	***
HSW	21.9±0.81	37.6±0.2	*	32.51±1.89	-	30.92±0.31	15.58-53.38	***
PL	10.6±0.51	12±1.35	*	11.8±0.61	-	10.62±1.61	Apr-22	***
Kabwe Control 2021								
Yield	978±58.3	1803±93.7	ns	1156±75.96	-	1623±47.56	436-2985	*
Biomass	78.7±12.9	121.3±16.3	ns	326.73±57.24	-	128.05±2.95	35.83-348.15	ns
PHI	67.5±1.8	64.6±1.4	ns	61.4±1.6	-	62.4±0.4	32.4-76.4	**
HI	15.2±1.4	15.2±2.3	*	39.6±2.1	-	46.6±0.1	17.2-67.8	**
HSW	34.9±1.1	44.7±0.1	**	37.13±0.55	-	36.10±0.54	22.7-63.74	***
PL	13.6±0.88	15.2±0.65	*	28.6±0.98	-	20.67±0.46	Jun-36	**
Kabwe Stressed 2020								
Yield	372±11.33	526±18.67	*	283±12.44	-	236±2.32	30-544	*
Biomass	83.4±1.3	135.1±2.4	**	23.67±5.72	-	30.48±0.82	4.44-87.20	*
PHI	51.5±0.1	61.4±1.4	*	46.2±6.4	-	56.2±1.3	2.5-74.3	*
HI	44.3±1.2	43.3±6.4	*	24.9±7.2	-	41.2±0.9	1.3-63.4	*
HSW	32.8±0.3	43.5±0.9	**			36.17±0.38	23.44-61.67	***
Kabwe Control 2020								
Yield	623±33.3	724±34.6	ns	482±27.73	-	660±16.63	183-1496	*
Biomass	75.4±2.12	179.8±9.0	*	27.89±2.12	-	53.68±2.42	8.61-186.64	ns
PHI	68.5±2.3	60.7±2.4	*	51.4±4.3	-	62.8±1.4	27.3-80.3	**
HI	57.8±0.3	51.4±1.3	*	36.1±6.5	-	48.7±1.6	9.6-68.9	***
HSW	38.6±1.7	64.89±1.4	**	37.91±0.93	-	49.57±0.52	29.37-89.75	***

t-test represent the level of significance for the *p*-value of a *t*-test between parental means, ANOVA represents the level of significance among RILs. *=significant, **=highly significant, *** = very highly significant ns= non-significant at p< 0.05, 0.01 and 0.001 respectively.

4.3.2 Genetic Correlation Analysis

Correlation coefficients were computed from average values of three locations. Under DS, seed yield was significantly and highly positively correlated with HI (r^2=0.16), HSW (r^2=0.16), SDW (0.51), and PN (r^2=0.40) $p < 0.01$, but was not significant with PHI. Other correlations between traits measured under DS are shown in Table 4.3. All correlations were positive except correlations between PHI and SDW (*r*=-0.02), HI and SDW (*r*=-0.04), and HSW and PN (*r*=-0.07). all negative correlations were also non-significant.

Table 4.3. Correlations between seed yield and morpho-agronomic and physiological traits on 155 recombinant Inbred lines grown under field drought stress conditions

Trait	Mean	Yield	PHI	HI	HSW	SDW	PN
Yield	232.02	1					
PHI	65	0.03ns	1				
HI	48	0.16**	0.85**	1			
HSW	33.91	0.16**	0.02ns	0.08**	1		
SDW	65.58	0.51**	-0.02ns	-0.04ns	0.05ns	1	
PN	13.99	0.40**	0.08*	0.11**	-0.07*	0.28**	1

*=significant, **=highly significant, ns= non-significant at p< 0.05 and 0.01 respectively. Yield=Seed yield, PHI = Pod Harvest Index; HI = Harvest Index; HSW = Hundred Seed Weight, SDW= Shoot Dry weight, PN = Pods Per Plant.

4.3.3 QTL Analysis

A total of 71 QTL for yield, biomass, PN, HI, PHI, GM, DSI, HSW, and Yield Percentage Reduction were identified under both DS and NS conditions. There were 22 QTLs for the NS while 35 were identified in the DS trials and 14 were not specific to water treatment (Table 4.4). The results showed large number of QTL associated with drought identified in the DS than in NS. The QTL percentage of variation also known as coefficient of determination (R^2) varied from 3.1 to 42.7% indicating that the identified QTL were comprised of both major and minor QTL.

4.3.3.1 Seed yield

A total of 12 seed yield QTL were identified from the four field trials under both DS and NS conditions (Table 4.4). Of these twelve, six were specific to DS while six were specific to NS. Under DS, the identified QTLs were on chromosomes *Pv01* (SY01.1), *Pv03* (SY03.1), *Pv06* (SY06.1), *Pv07* (SY07.1), and *Pv10* (SY10.1). The QTL SY01.1 (47.5 Mbp) that was identified from the UNZA trial explained 5.8% of the variation in seed yield and the drought tolerant parent

Kijivu contributed the positive allele. The QTL SY03.1 (3.1 Mbp) was only identified from the Kabwe_2020 trial. This QTL explained 6.8% of seed yield variation and Kijivu, the drought tolerant parent, contributed the positive allele. The yield QTL SY06.1 (23 Mbp; R^2 =8.0%) and SY06.2 (29.1 Mbp; R^2 =8.2), were identified from UNZA and Kabwe_2020 trial, respectively, and Bukoba contributed the positive allele at both loci. The QTL SY07.1 (24.3 Mbp; R^2 =8.2%) was identified from the UNZA trial and the parent Kijivu contributed the positive allele. The QTL SY10.1 (38.7 Mbp - 40.4 Mbp) was identified under DS in GART trial (R^2 =8.3%), Kabwe_2021 trial (R^2 =9.2%), UNZA trial (R^2 =5.5%) and Kabwe_2020 trial (R^2 =6.5%). The drought tolerant parent Kijivu contributed the positive allele at SY10.1, which had an additive effect that ranged from 8.5 – 55.2 kg ha -1.

Under NS, six yield QTL were identified on chromosomes *Pv03*, *Pv05*, *Pv08*, and *Pv10* from the four trials (Table 4.4). The proportion of variation explained by these QTL ranged from 6.8% - 10.3%. Both parents contributed the positive alleles at the QTL identified under NS. In general, the additive effects of positive alleles at the seed yield identified under NS were higher than those under DS (Table 4.4).

4.3.3.2 Drought tolerance indices

A total of eight QTL were identified for drought tolerance indices (Table 4.4). A total of five QTL for GM were identified on chromosomes *Pv03, Pv04, Pv05, Pv06*, and *Pv08*. These QTLs were identified from GART, Kabwe_2020, and UNZA trials. The percentage of variation explained by these QTLs ranged from 6.3 – 11.8%. Both parents contributed positive alleles. The QTL GM06.1, which explained 11.8% of the variation in geometric mean overlapped with the seed yield QTL SY06.2.

The QTL DSI10.1 (36.2 Mbp – 42.1 Mbp) for drought susceptibility index was identified on chromosome *Pv10* from GART and UNZA trials. DSI10.1 overlapped with seed yield QTL SY10.1 identified under DS in GART, UNZA, Kabwe_2020, and Kabwe_2021 trials. The R^2 for DSI10.1 were 8.1% and 9.7% for GART and UNZA trials, respectively. Kijivu the drought tolerant parent contributed the positive allele at DSI10.1.

Two QTLs YPR8.1 and YPR10.1 for yield percentage reduction were identified on chromosomes *Pv08* and *Pv10*, respectively. The QTL YPR10.1 (39.8 Mbp – 40.9 Mbp) was identified from

GART and UNZA trials. YPR10.1 overlapped with QTL for seed yield (SY10.1) and DSI (DSI10.1).

4.3.3.3 Pod harvest index

A total of 12 QTLs for PHI (Table 4.4) were identified from the four trials. Of these 12 QTL, six (PHI02.1, PHI03.1, PHI04.1, PHI04.2, PHI06.1, and PHI08.1) were specific to DS, four (PHI01.1, PHI03.2, PHI10.1, and PHI10.2) were specific to NS, and two (PHI05.1 and PHI07.1) were identified under both DS and NS. The percentage of variation in PHI explained by individual QTLs identified under DS, ranged from 7.6% - 19.4%. Under DS, the QTL PHI4.1 was identified from both Kabwe_2021, and UNZA trials. The QTL PHI06.1 (23.6 Mbp) overlapped with QTL for seed yield (SY06.1), HI (HI06.1), HSW (HSW06.1), and TB (TB06.1).

4.3.3.4 Harvest index

A total of 10 QTLs for HI were identified from the four field trials under DS and NS conditions. Of these 10 QTL, three (HI03.1, HI06.1, and HI10.1) were specific to DS, three (HI03.2, HI08.1, and HI09.1) were specific to NS, and four (HI04.1, HI05.1, HI07.1, and HI07.2) were non-specific to water treatment. The R^2 for individual QTLs identified under DS ranged from 5.2% - 15.9%. The QTL HI06.1, and HI10.1 overlapped with the seed yield QTL SY06.1, and SY10.1, respectively, identified under DS.

4.3.3.5 Shoot Dry weight

A total of eight QTLs were identified for shoot dry weight from the four trials. Among the eight QTL, TB02.1, TB03.1, TB05.1, TB06.1, TB07.1, and TB09.1 were specific for DS while QTL TB07.2 and TB08.1 were specific to NS. The QTL TB05.1 was identified in both GART and Kabwe_2020. The QTL TB06.1 (24.2 – 26.5 Mbp) was identified in Kabwe_2021 and GART. TB06.1 overlapped with QTL for seed yield (SY06.1), PHI (PHI06.1), and HSW (HSW06.1), which were identified under DS. The QTL TB09.1 was identified in both UNZA and Kabwe_2020. The QTL TBM5.1, which was identified in both GART and Kabwe_2020 trials, had the highest R^2 (11%) for all QTLs for TB identified under DS from the four locations. TB3.1 and TB7.1 overlapped with seed yield QTL SY03.1 and SY07.1, respectively.

4.3.3.6 Pod number per plant

In this study, five QTL for pod number per plant were identified from GART and Kabwe_2021 trials. Among these QTL, PN07.1, PN09.1, and PN10.1 were specific to DS, one PN09.2 was

specific NS, and PN08.1 was identified under both DS and NS. The QTL PN07.1 (5.2 – 9.2 Mbp; R^2 =9.3% - 9.5%) was identified in both GART and Kabwe_2021. PN07.1 overlapped with QTL for PHI (PHI07.1), HI (HI07.1), and HSW (HSW07.1) identified under DS. PN08.1 overlapped with PHI QTL PHI8.1 under DS. The QTL PN10.1 identified under DS in GART co-localized with the seed yield QTL SY10.1.

4.3.3.7 Hundred Seed Weight

A total of 10 QTL were identified for seed weight on chromosomes *Pv02* (HSW2.1 and HSW2.2), *Pv03* (HSW3.1 and HSW3.2), *Pv04* (HSW4.1), *Pv06* (HSW6.1), *Pv07* (HSW7.1), *Pv08* (HSW8.1 and HSW8.2), *Pv09* (HSW9.1) and *Pv10* (HSW10.1). Of these 10 QTL, two QTL (HSW2.2 and HSW6.1) were specific to DS while two QTL (HSW3.2 and HSW10.1) were specific to NS. The other six QTL were identified under both DS and NS in multiple locations.

The QTL HSW7.1 (2.0 Mbp – 11 Mbp) was identified under DS in GART, UNZA, and Kabwe_2020 trials. This QTL was also identified under NS in GART, UNZA, and Kabwe_2020 trials. The percentage of variation in seed weight explained by HSW7.1 ranged from 21.0% - 42.7% under DS, while under NS it ranged from 16.4% - 26.9%. The parent Kijivu contributed the positive allele at HSW7.1. HSW7.1 overlapped with QTL for seed yield (SY7.1), PHI (PHI7.1), HI (HI7.1), TB (TB7.1), and PL (PL7.1). The QTL HSW8.1 (0 – 1 Mbp) was identified under both DS (GART and Kabwe_2021 trials) and NS (GART, UNZA, and Kabwe_2020). The QTL HSW8.2 (61.0 -63.0 Mbp) was also identified under both DS (UNZA, Kabwe_2020, and Kabwe_2021) and NS (GART, UNZA, Kabwe_2020, and Kabwe_2021). Kijivu contributed the positive allele at both HSW8.1 and HSW8.2. The QTL HSW9.1 (32 Mbp – 34 Mbp) was detected under DS (GART and Kabwe_2021) and NS (GART and UNZA). The parent Bukoba contributed the positive allele at HSW9.1.

4.3.3.8 Photosynthetic Parameters

A total of six QTL for photosynthesis traits including Phi2, PhiNPQ, and LEF. The first QTL for Phi2 (Phi2_5.1) was detected on chromosome *Pv05* (30.3 Mbp – 34.9 Mbp) with R^2 of 7.2%. This QTL was detected under NS and the parent Bukoba contributed the positive allele. The second QTL for Phi2 (Phi2_10) was detected on chromosome *Pv10* (39 Mbp – 40.9 Mbp; R^2=8.9%) under NS. One QTL for PhiNPQ (PhiNPQ4.1) was detected on Pv04 (43.7 Mbp; R^2=9.4%). Three QTL

for LEF were detected on *Pv05* (LEF5.1), *Pv07* (LEF7.1) and *Pv10* (LEF10.1). The QTL LEF5.2 (34 Mbp; R^2=11.8%) was detected under both DS and NS. The drought tolerant parent Kijivu contributed the positive allele at LEF5.1. LEF5.1 overlapped with HI5.1. LEF7.1 (1.3 Mbp); R^2=10.7%) was detected under DS and Kijivu contributed the positive allele. LEF10.1 (40.4 Mbp; R^2=10.4%) was detected under NS and Bukoba contributed the positive allele. LEF10.1 co-localized with QTL for seed yield (SY10.1), DSI (DSI10.1), yield percentage reduction (YPR10.1), HI (HI10.1) and PL (PL10.1).

Table 4.4 Quantitative trait loci for drought stress and non-stress conditions identified using 155 recombinant inbred lines grown in Zambia in the field at UNZA, GART, and Zambia Agricultural Research Institute (Kabwe_2020 and Kabwe_2021) in the Hot Dry Seasons of 2020 and 2021.

Trait	QTL Name	Trial Name	Treatment	Chr[a]	QTL Peak Position (Mbp)	QTL Interval (Mbp)	LOD[b]	R^2 (%)[c]	ADD[d]
Seed Yield	SY01.1	UNZA	DS	1	47.5	47.5-47.7	2.8	5.8	-14.85
	SY03.1	Kabwe_2020	DS	3	3.1	3.0-3.2	2.8	6.8	-8.76
	SY06.1	UNZA	DS	6	23.3	21.2-25.4	3.8	8	17.4
	SY06.2	Kabwe_2020	DS	6	29.1	28.5-29.9	3.4	8.2	9.24
	SY07.1	UNZA	DS	7	24.3	9.2-30.8	3.9	8.2	-17.4
	SY10.1	GART	DS	10	40	3.8-40.2	3.6	8.3	-55.19
		Kabwe_2021	DS	10	39.8	7.4-41.3	4.4	9.2	-18.1
		Kabwe_2020	DS	10	38.7	38.0-39.9	2.7	6.5	-8.53
		UNZA	DS	10	40.4	40.2-40.4	2.7	5.5	-14.12
	SY03.2	GART	NS	3	40.1	37.1-40.4	2.8	6.3	66
	SY03.3	UNZA	NS	3	30	3.7-33.5	4.2	10.3	31.42
	SY05.1	Kabwe_2021	NS	5	2.9	2.9-3.6	3.2	7.6	-53.98
	SY08.1	GART	NS	8	59.2	58.7-60.0	3.5	8	75
	SY08.2	Kabwe_2021	NS	8	7.8	5.8-47.0	2.9	6.9	50.23
	SY10.2	Kabwe_2020	NS	10	2.1	0.5-2.4	3.6	8.1	-19.31
Yield Geometric Mean	GM03.1	Kabwe_2020	-	3	2.6	1.3-2.6	3.7	9.3	-11.3
	GM04.1	UNZA	-	4	2.3	0.4-3.3	4	9.3	20.3
	GM05.1	GART	-	5	0.4	0.2-0.82	4	9	-49.2
	GM06.1	Kabwe_2020	-	6	29.5	27.9-29.9	4.9	11.8	12.4
	GM08.1	UNZA	-	8	10.2	8.2-43.3	2.7	6.3	16.62
Yield Drought Susceptibility Index	DSI10.1	GART	-	10	40.5	36.2-42.8	3.7	8.1	-0.07

		UNZA	-	10	42.1	40.2-42.3	4.3	9.7	-0.53
Yield Percentage Reduction	YPR08.1	UNZA	-	8	0.8	0.5-0.9	3.6	7.6	-13.69
	YPR10.1	GART	-	10	39.8	6.2-40.8	4.1	9.8	4.56
		UNZA	-	10	40.9	39.9-42.3	4.8	10.3	-16.23
Pod Harvest Index	PHI02.1	UNZA	DS	2	3.8	2.3-22.0	7.3	19.4	0.04
	PHI03.1	GART	DS	3	50.7	47.6-52.3	5	12	-0.02
	PHI04.1	Kabwe_2021	DS	4	7.5	4.2-42.8	4.2	7.7	-0.02
	PHI04.1	UNZA	DS	4	7.3	7.2-42.3	3.1	7.6	-0.03
	PHI04.2	Kabwe_2021	DS	4	47.5	45.7-47.7	6	11.1	0.03
	PHI05.1	Kabwe_2021	DS	5	34.9	4.9-38.0	7.8	14.9	-0.03
	PHI06.1	Kabwe_2021	DS	6	23.6	0-26.4	6.7	12.4	0.03
	PHI07.1	GART	DS	7	5.9	4.7-7.2	3.4	8	0.01
	PHI08.1	UNZA	DS	8	60.8	59.6-61.4	3.7	8	-0.02
	PHI01.1	Kabwe_2020	NS	1	51.2	51.1-51.3	3.1	6.9	0.02
	PHI03.2	Kabwe_2021	NS	3	2.6	2.4-2.8	3.7	8.2	-0.02
	PHI05.1	Kabwe_2021	NS	5	3.9	2.1-34.9	5.6	12.8	-0.02
	PHI07.1	Kabwe_2020	NS	7	3.5	3.2-4.4	2.8	6.1	0.02
	PHI10.1	Kabwe_2020	NS	10	43.4	42.7-43.6	5	11.1	0.02
		UNZA	NS	10	44.2	43.9-44.2	3.3	8	0.01
	PHI10.2	Kabwe_2021	NS	10	37.2	6.2-38.6	4	8.8	0.02
Harvest Index	HI03.1	GART	DS	3	50.6	50.1-51.6	3.1	6.5	-0.02
	HI04.1	Kabwe_2021	DS	4	39.9	6.5-41.7	3.9	7.4	-0.03
	HI05.1	Kabwe_2021	DS	5	35.5	5.8-38.8	7.9	15.9	-0.04
	HI06.1	Kabwe_2021	DS	6	21.3	21.3-23.6	2.8	5.2	0.02
	HI07.1	GART	DS	7	5.9	4.1-9.1	4.5	10.1	0.03
	HI07.2	Kabwe_2021	DS	7	36.8	36.1-37.4	3.1	5.8	0.02
	HI10.1	GART	DS	10	38.9	5.8-40.2	3.9	8	-0.02
	HI03.2	Kabwe_2020	NS	3	45.9	37.1-46.0	3	6.8	-0.02
	HI04.1	GART	NS	4	32.7	4.3-42.8	5.1	11	-0.02
	HI05.1	Kabwe_2020	NS	5	36.1	34.7-37.4	3.1	7.1	-0.02
		Kabwe_2021	NS	5	35.5	5.8-38.8	4.9	11.3	-0.02
	HI07.1	GART	NS	7	5.9	3.5-9.1	4.3	9.7	0.02
		Kabwe_2020	NS	7	3.2	3.2-3.6	2.8	6.3	0.02
	HI07.2	GART	NS	7	38.6	38.2-39.4	3.8	8.5	0.012
	HI08.1	Kabwe_2020	NS	8	45.9	6.2-49.6	3.1	7.2	0.02
	HI09.1	Kabwe_2021	NS	9	30.1	29.4-30.5	2.7	6.4	-0.02
Total shoot dry weight	TB02.1	Kabwe_2021	DS	2	3.2	2.4-3.7	3	7.1	-5.61
	TB03.1	GART	DS	3	5.3	3.6-31.2	4.3	9.1	-6.9
	TB05.1	GART	DS	5	0.5	0.4-0.7	4.1	9.1	-6.59
	TB05.1	Kabwe_2020	DS	5	0.3	0.2-0.5	3.7	11	6.27
	TB06.1	GART	DS	6	26.5	25.9-27.2	3.6	7.7	6.1
	TB06.1	Kabwe_2021	DS	6	24.2	23.3-26	3.5	8.5	6.19

	TB07.1	UNZA	DS	7	29.9	13.7-30.2	3.6	7.9	-2.12
	TB09.1	Kabwe_2020	DS	9	37.2	37.2-37.3	2.6	7.1	4.1
		UNZA	DS	9	37.2	36.7-37.8	2.8	6.4	1.91
	TB07.2	UNZA	NS	7	5.4	5.4-7.2	3.5	7.9	-4.21
	TB08.1	Kabwe_2021	NS	8	50.8	7.3-54.5	3.2	7.8	10.73
Pod Load	PN07.1	GART	DS	7	5.9	2.6-7.5	5.6	9.5	6.51
	PN07.1	Kabwe_2021	DS	7	9.2	5.4-11.7	3.8	9.3	3.57
	PN08.1	GART	DS	8	62.2	61.4-62.9	5.3	10.8	6.79
	PN09.1	Kabwe_2021	DS	9	22.8	22.7-23.1	2.9	6.4	-2.91
	PN10.1	GART	DS	10	39.8	37.2-40.4	3	6.2	-5.13
	PN08.1	GART	NS	8	58.8	57.3-62	4.6	11.5	9.8
	PN09.2	Kabwe_2021	NS	9	33.1	30.0-33.5	3.2	7.5	-4.55
Hundred Seed Weight	HSW02.1	UNZA	DS	2	36.7	31.5-39.6	3.6	3.1	-1.02
	HSW02.2	Kabwe_2020	DS	2	49.6	48.3-49.7	3.8	6.2	-1.58
	HSW03.1	GART	DS	3	40.9)	36.5-46.0	3.7	6.2	0.73
	HSW06.1	Kabwe_2021	DS	6	25.4	23.6-27.2	3.5	5.6	0.93
	HSW07.1	GART	DS	7	5.9	2.7-11.7	11	21	-1.37
		Kabwe_2020	DS	7	5.4	2.5-9.9	19	36.3	-3.75
		Kabwe_2021	DS	7	5.9	2.5-9.9	13.2	24.7	-1.95
		UNZA	DS	7	5.9	2.5-9.9	26.6	42.7	-3.7
	HSW08.1	GART	DS	8	0.5	0.5-0.6	3.1	5.1	-0.66
		Kabwe_2021	DS	8	1.3	0.7-2.6	4.7	7.7	1.06
	HSW08.2	Kabwe_2020	DS	8	62.8	60.9-62.9	6.4	9.7	-1.3
		Kabwe_2021	DS	8	61.2	61.2-62.9	4.2	6.8	-1.01
		UNZA	DS	8	62.2	60.5-63.9	9.3	10	-1.76
	HSW09.1	Kabwe_2021	DS	9	34.1	32.5-34.2	2.8	4.5	0.81
		UNZA	DS	9	34.1	24.6-35.1	5.9	6	1.37
	HSW02.1	UNZA	NS	2	35.8	34.1-36.5	3.2	4	-1.56
	HSW03.1	GART	NS	3	36.8	35.4-41.4	4.4	6.4	0.91
	HSW03.1	UNZA	NS	3	4.3	3.3-37	4.4	6.1	1.94
	HSW04.1	Kabwe_2020	NS	4	47.7	47.5-47.7	4.7	6.5	-2.15
	HSW07.1	GART	NS	7	5.9	2.5-11.7	10.3	16.4	-1.49
		Kabwe_2020	NS	7	9.2	2.5-9.9	15.4	30.3	-4.66
		Kabwe_2021	NS	7	9.2	4.8-31.1	6.9	17.4	-3.78
		UNZA	NS	7	5.6	2.5-11.7	16.1	26.9	-4.1
	HSW08.1	GART	NS	8	3.2	1.5-5.1	3.6	5.1	-0.82
		Kabwe_2020	NS	8	1.7	1.0-2.5	3.7	6.5	-2.14
		UNZA	NS	8	4.9	3.2-8.2	4.6	6.4	-1.96
	HSW08.2	GART	NS	8	61.2	60.1-62.9	8	12.1	-1.25
		Kabwe_2020	NS	8	62.6	60.9-62.9	9	13.3	
		Kabwe_2021	NS	8	62.7	62.4-62.7	3.2	7	-2.37
		UNZA	NS	8	62.6	60.9-62.9	5	7	-2.03
	HSW09.1	GART	NS	9	34.1	24.5-35.2	7.4	11.1	1.2
		UNZA	NS	9	33.3	27.5-34.6	3.8	5.1	1.74

	HSW10.1	GART	NS	10	40.1	39.9-40.5	2.8	3.7	0.7
Photosynthetic parameters	LEF05.1	UNZA	DS	5	34.5	7.3-37.4	5.9	11.8	-0.03
	LEF07.1	UNZA	DS	7	1.3	37.7-46.5 (3)	3.2	10.7	-0.02
	LEF05.1	UNZA	NS	5	42.0 (34.5)	6.8-35.5	4.8	11.1	8.35
	LEF10.1	UNZA	NS	10	40.4	39.2-42.0	4.6	10.4	7.12
	NPQ04.1	UNZA	NS	4	43.7	9.8-44.9	3.9	9.4	1.7
	Phi2_05.1	UNZA	NS	5	34.7	30.3-34.9	3.3	7.2	0.8
	Phi2_10.1	UNZA	NS	10	40.4	39.6-40.9	4	8.9	0.9

[a]Chr = Chromosome, [b]LOD = Logarithm od odds, [c]R^2 = Proportion of phenotypic variance explained by the QTL. [d]ADD, additive effects on the alternative allele of the QTL where a negative is from Kijivu and a positive is from Bukoba.

4.3.4. QTL "Hotspots" under Drought stress

Three QTL hotspots for drought tolerance were identified on *Pv06*, *Pv07*, and *Pv10* where the QTL for seed yield co-localized with the other traits QTL (Figures 4.2, 4.3 and, 4.4). A seed yield QTL SY6.1 was identified on chromosome *Pv06*. SY6.1 overlapped with QTL for PHI (PHI6.1), HI (HI6.1) SDW (TBM6.2), and HSW (HSW6.1), all identified under drought stress. Chromosome *Pv07* was identified as a QTL hotspot for drought tolerance as many QTL including HSW7.1 (HSW found under both DS and NS), TBM7.1 (SDW), HI7.1 (HI), PHI7.1 (PHI), and SY07.1 (seed yield) all identified under DS, co-localized at this genomic region. Another major QTL hotspot for drought was identified on chromosome *Pv10*. The QTL for seed yield SY10.1 was identified from all four locations. This QTL was specific to DS condition. Given that this QTL was consistently identified from four field experiments suggests that it is major and stable across the environment and whose expression is specific to drought stress. Other QTL that were identified in this genomic region include those for PN (PN10.1), drought susceptibility index (DSI10.1), yield percentage reduction (YPR10.1), and HI (HI10.1). In addition to the five QTL identified under DS two photosynthesis-related QTL LEF10.1 and Phi2_10.1 were identified under NS (Figure 4.4).

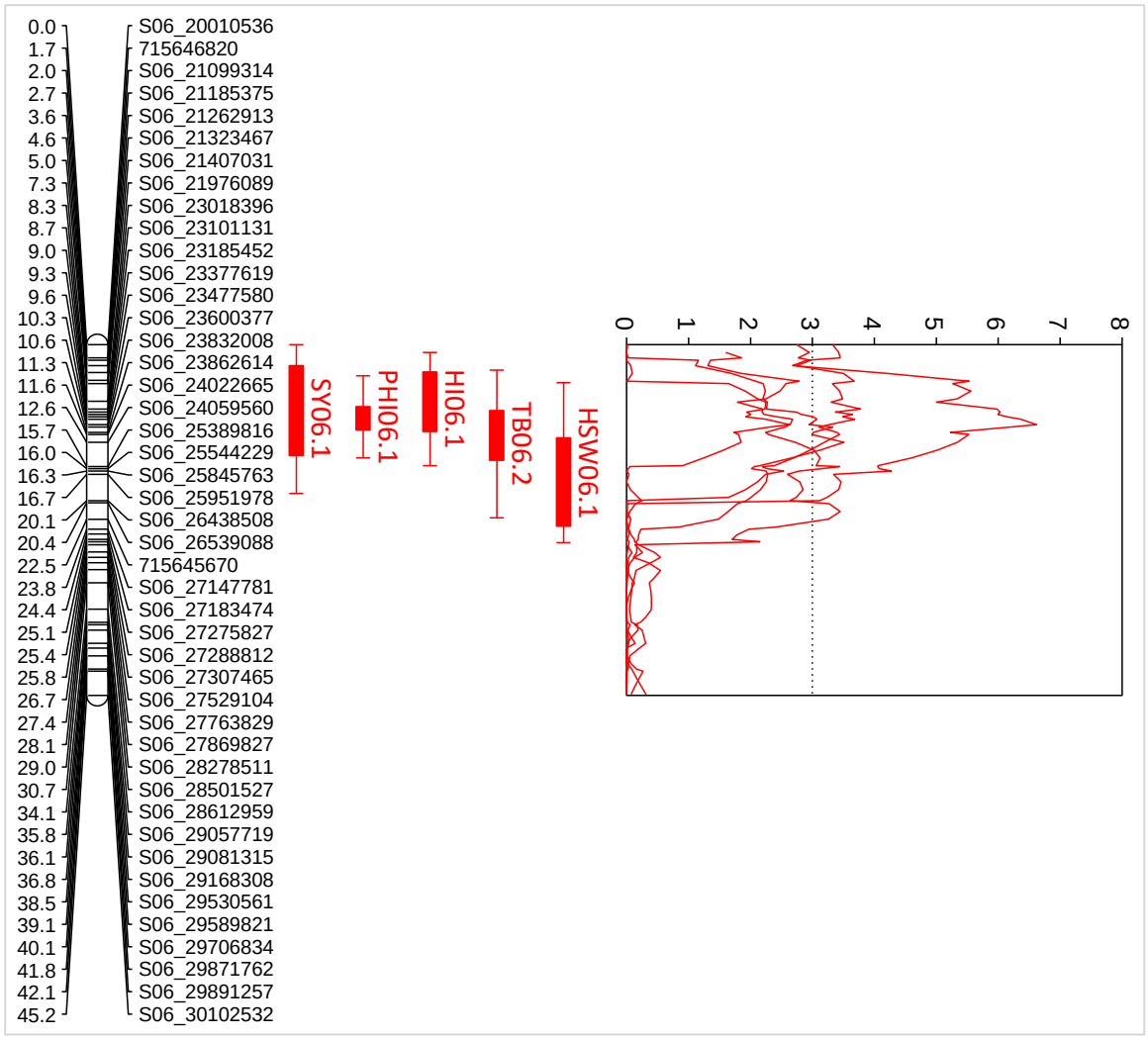

Figure 4.2. Linkage map for chromosome *Pv06* showing drought tolerance QTL "hotspot" of co-localized QTL for seed yield (SY06.1), harvest index (HI06.1), pod harvest index (PHI06.1), shoot dry weight (TB06.1) and hundred seed weight (HSW06.1) in Bukoba x Kijivu mapping population.

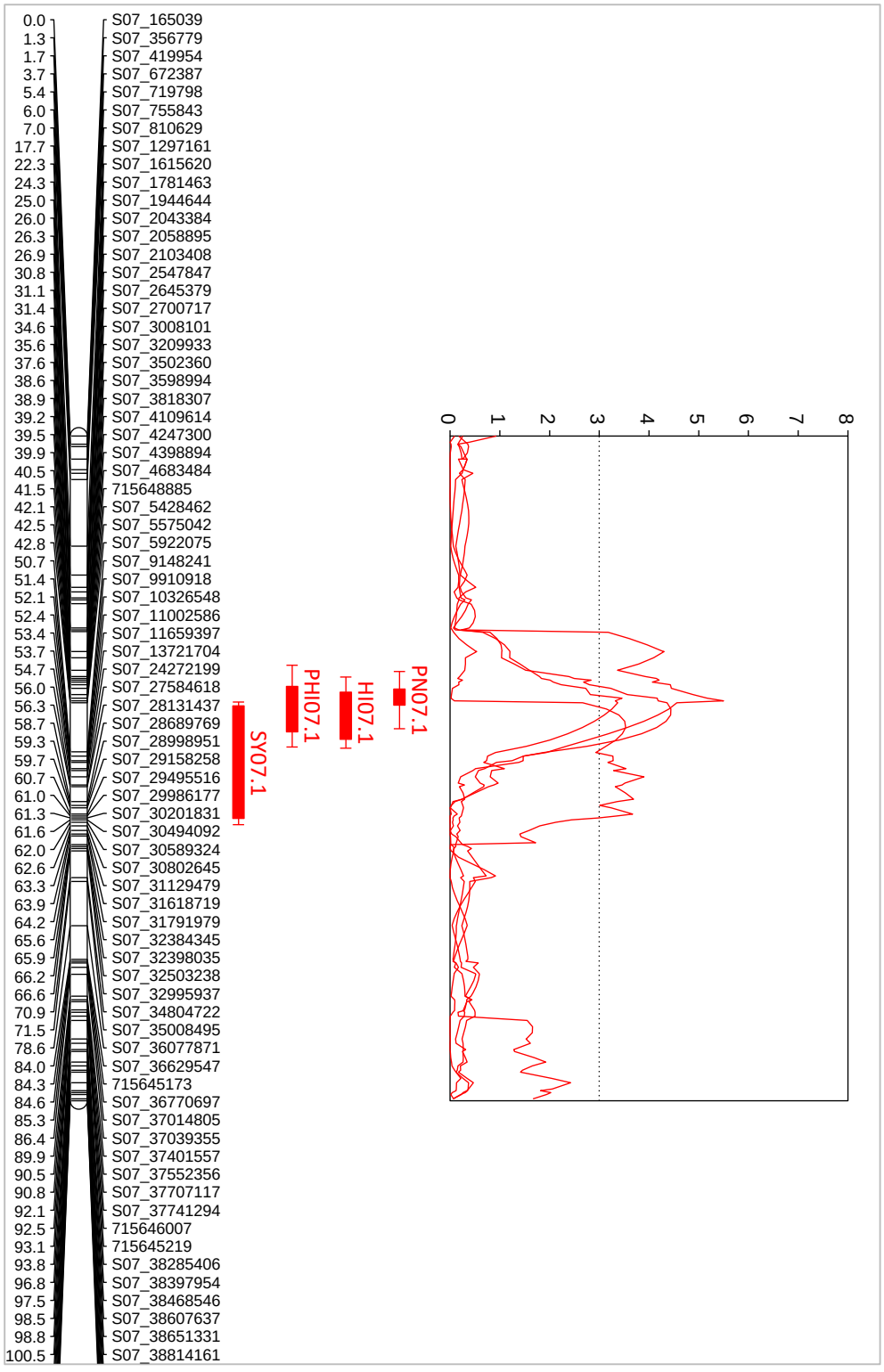

Figure 4.3. Linkage map for chromosome *Pv07* showing drought tolerance QTL "hotspot" of co-localized QTL for seed yield (SY07.1), harvest index (HI07.1), pod harvest index (PHI07.1), pod number (PN07.1) in Bukoba x Kijivu mapping population.

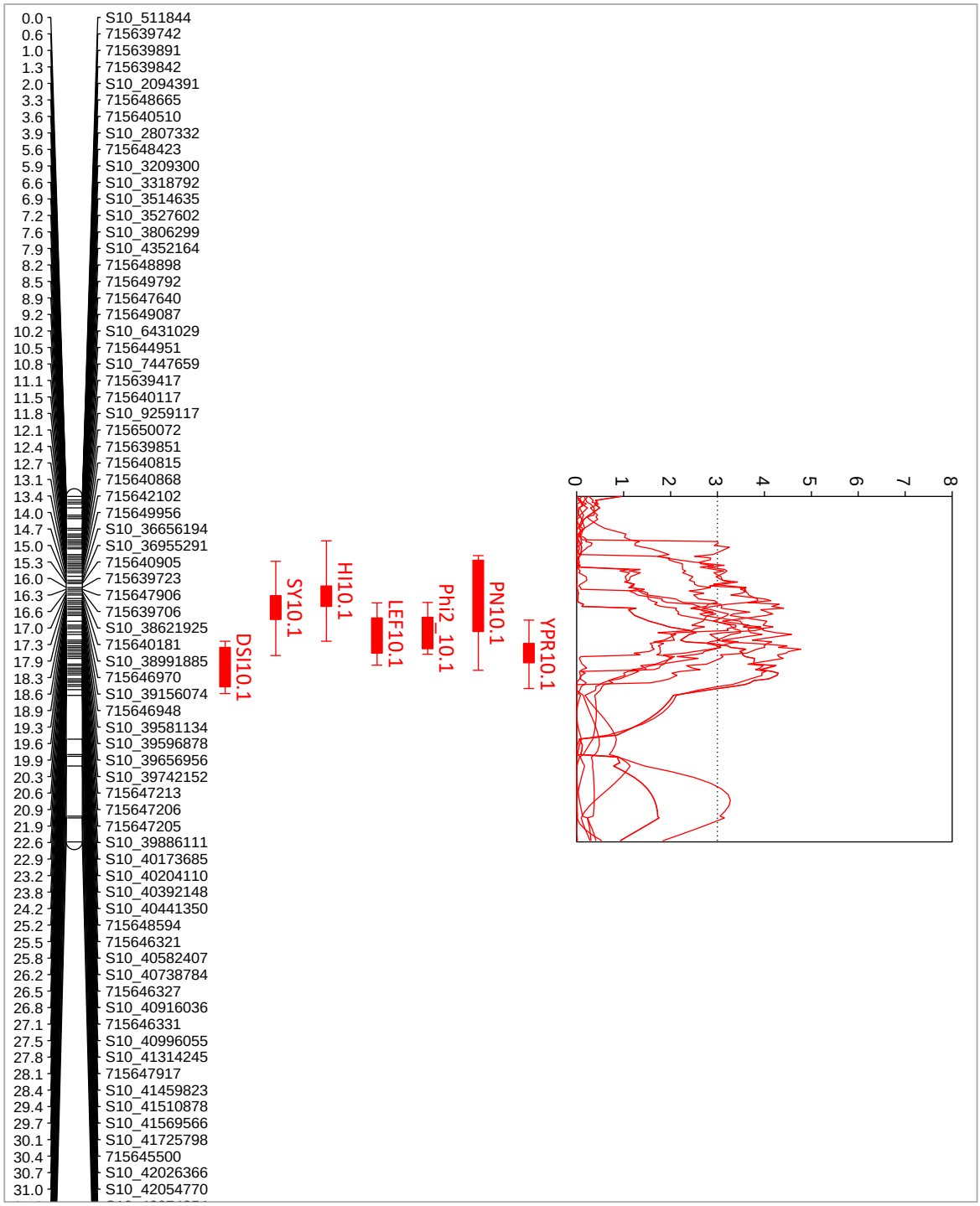

Figure 4.4. Linkage map for chromosome *Pv10* showing drought tolerance QTL "hotspot" of co-localized QTL for drought susceptibility index (DSI10.1), seed yield (SY10.1), harvest index (HI10.1), linear electron flow (LEF10.1), quantum yield of photosystem II (Phi2_10.1), pod number (PN10.1) and yield percentage reduction (YPR10.1) in Bukoba x Kijivu mapping population.

4.4 DISCUSSION

Drought stress is a major cause of seed yield losses in common beans worldwide. This study evaluated the drought tolerance of 155 RILs and their parents in field and pot experiments. In addition, QTL analyses were conducted to identify genomic regions for drought tolerance. Significant differences in seed yield between the parents Kijivu and Bukoba under DS across all four field trials were observed. However, there were no significant seed yield differences between Kijivu and Bukoba under NS except for the GART trial. Under DS, the drought tolerant parent Kijivu had a significantly higher seed yield than Bukoba across all four trials. These results confirmed that Kijivu is more tolerant to drought than Bukoba, which is consistent with previous studies that identified Kijivu as drought tolerant.

Drought enlists strong agronomic, morphological, and physiological responses. In the current study, significant differences were observed among RILs for all measured traits except shoot biomass under DS across all four field trials suggesting significant genetic variation between RILs in response to drought stress. Transgressive segregation was observed for all traits, suggesting that though Bukoba is drought susceptible, it may have valuable alleles for drought tolerance. RILs with Kabulangeti seed type and higher seed yield than the drought tolerant parent Kijivu under drought stress could potentially be used as parents in breeding for drought tolerance in the Kabulangeti seed type, which is a major market class in Tanzania and Zambia.

In this study, seed yield under drought stress was significantly correlated with total shoot biomass. This indicates the potential usefulness of shoot biomass in addition to seed yield to select for drought tolerance. It is important though that selection for biomass is coupled with the selection for partitioning efficiency. Significant correlations between seed yield and partitioning indices have been observed in beans (Polania et al., 2016a; 2016b; Dramadri et al., 2019; Dramadri et al., 2021).

QTL Analysis

To gain insights into the genetic architecture of drought tolerance in the Andean gene pool, QTL analyses were conducted for all traits measured under DS and NS conditions in this study. A large number (71) of QTL for traits related to drought were identified in the current study. The majority of the identified QTL in this study were under DS conditions. A large number of identified QTL for drought tolerance suggest that response to drought stress is genetically complex and involves

a large set of genes for different morphological, agronomic, and physiological traits. The majority of the identified QTL explained less than 10% of the variation for their respective traits suggesting that genes mainly with minor and additive gene action control drought tolerance. This is consistent with the previous description of the genetic architecture of drought tolerance in common beans and other crops. Drought tolerant parent Kijivu contributed the positive alleles at the majority of the identified seed yield QTL under drought stress. However, Bukoba the drought susceptible parent did contribute positive alleles at a few QTLs, which could explain the transgressive segregation observed in the population for all traits measured in the current study.

A seed yield QTL SY01.1 was identified from UNZA under DS conditions. This QTL located at 47.5 Mbp overlapped with previously identified QTL for seed yield $SY1.1^{BR}$ located at 47.7 Mbp, which was identified by Trapp et al. (2015) under both DS and NS. Additionally, SY01.1 is near the seed yield QTL located at 48.5Mbp identified under DS by (Berny Mier y Teran et al., 2019). A seed yield QTL SY03.1 located at 3.1 Mbp co-localized with the previously identified QTL for pod number ($PN3.1^{SC}$) located at 2.3- 3.7 Mbp (Mukeshimana et al., 2014).

A seed yield QTL SY6.1 was identified on chromosome *Pv06*, and this QTL explained 8% of the variation in seed yield under drought conditions for the UNZA experiment. SY6.1 overlapped with QTL for PHI (PHI6.1), HI (HI6.1), SDW (TBM6.2), and HSW (HSW6.1), all identified under drought stress. This was another important region associated with drought tolerance given the various co-localizations of QTLs for variable traits identified under drought conditions. In this study, the peak of seed yield QTL SY6.1, which was at 23.3 Mbp overlapped with a seed yield QTL identified by Diaz et al (2020) at 24.1 Mbp using the Middle American MAGIC population evaluated for drought tolerance. In addition, SY6.1 overlapped with the genomic region on 22.95 Mbp – 24.94 Mbp on chromosome *Pv06* that is associated with seed yield under drought stress from a genome-wide association analysis using the Andean Diversity Panel (Dramadri et al., 2020). Furthermore, Dramadri et al., (2019) reported a seed yield QTL under drought stress whose peak was at 17.92 Mbp, which falls within the QTL SY6.1. This overlap with the previously identified QTL in both the Andean and Middle American population demonstrates the stability of this QTL across genetic backgrounds including those between gene pools. QTL hotspots for drought tolerance have been identified on chromosomes *Pv06, Pv07*, and *Pv10*. These QTL

hotspots if validated further could be targeted for Marker-assisted selection for drought tolerance in common beans.

The genomic region 9.0 Mbp - 30.6 Mbp on chromosome *Pv07* was identified as a QTL hotspot for drought tolerance as many QTLs including HSW7.1 (seed weight), TB7.1 (Total shoot dry weight), PN07.1 (PN), HI7.1 (HI), PHI7.1 (PHI) and SY07.1 (seed yield) all identified under DS, co-localized at this genomic region. This co-localization could suggest a pleiotropic effect of the underlying gene/s for multiple agronomic and physiological traits involved in drought tolerance. It could also suggest a linkage of genes for the traits. The QTL that were consistently identified from both UNZA and GART trials, which could suggest stability in the expression of the underlying genes across environments. This QTL hotspot on chromosome *Pv07* for drought tolerance overlapped with previously identified QTL for seed yield under drought conditions. The peak for the QTL SY7.1 in the current study was at 27.6 Mpb (9.0 Mbp - 30.6 Mbp), which overlapped with seed yield QTL under drought identified at 28.25 Mbp using GWAS (Valdisser et al., 2020) and another seed yield QTL under drought condition identified on 29.1 Mbp using a MAGIC population (Diaz et al., 2020). The seed QTL SY07.1 identified in the current study also co-localized with the previously identified QTL (11.5 Mbp - 36.7 Mbp) for seed yield and PHI identified under drought conditions by (Berny Mier y Teran et al., 2019) using the population of RILs derived from the Middle American parents, ICA Bunsi and SXB40. The extensive co-localizations of various traits under drought stress from different locations within the current study and with QTLs for drought tolerance from previous studies could suggest that the QTL hotspot on Pv07 is stable across environments, genetic backgrounds within and across gene pools with very diverse genetic structures.

Another major QTL hotspot for drought tolerance was identified on chromosome *Pv10* where the QTL SY10.1 (38.7 Mbp - 40.4 Mbp) for seed yield under DS was located. Other QTL that were identified in this genomic region included those for pod number (PN10.1), drought susceptibility index (DSI10.1), harvest index (HI10.1), yield percentage reduction (YPR10.1), and QTL for photosynthetic performance (LEF10.1 and Phi2_10.1). Given that SY10.1 was consistently identified from four field trials under DS conditions suggests that it is a major QTL and was stable across environments. The QTL SY10.1 identified in this study co-localizes with previously identified seed yield QTL $SY10.1^{BR}$ under DS (Trapp et al., 2015).

4.5 CONCLUSION

The seed yield under DS for genotype Kijivu (drought tolerant parent) was consistently higher than for genotype Bukoba (drought susceptible) across all four field trials. A total of 71 QTL for agronomic, morphological, and physiological traits were identified under DS and NS. From this study, 35 and 22 QTL were specific to DS and NS, respectively, while 14 were identified under both DS and NS. Genomic regions with extensive QTL co-localization of seed yield and other traits under DS were observed on chromosomes *Pv06, Pv07*, and *Pv10*. Some of the identified QTLs for drought tolerance were novel while others overlapped with previously reported QTLs. Lack of stability across environments and genetic backgrounds of the identified QTL for drought tolerance has been an obstacle to the application of marker-assisted selection to drought tolerance. This study has demonstrated stability across genetic backgrounds and environments for some of the identified QTLs for drought tolerance. These stable QTLs could be targeted for use in molecular breeding to enhance selection efficiency for common bean drought tolerance.

CHAPTER FIVE

GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1. GENERAL DISCUSSION

Drought tolerance in common beans is both phenotypically and genetically complex. From the phenotypic perspective several agronomic, and morpho-physiological traits are involved in drought tolerance mechanisms including drought escape, and avoidance that plants use to adapt to drought. The role and relative importance of these traits is not well understood in the Andean gene pool of common bean. Further, the genomic regions associated with the agronomic and morpho-physiological response to drought stress in the Andean gene pool is not well understood.

In the first study, a total of 20 Andean genotypes were evaluated in field and pot experiments for agronomic and morpho-physiological traits including seed yield, seed weight, carbon isotope discrimination, partitioning indices (PHI and HI), canopy biomass, pod load and electrolyte leakage under drought and non-drought. Significant genotypic differences were observed in these traits. Partitioning indices (PHI) and carbon isotope discrimination were highly correlated with seed yield under drought. The strong correlation between seed yield and partitioning indices is indicative of the important role of photo-assimilate remobilization from all plant tissues to the seed in the adaptation of Andean beans to drought. PHI and HI showed high broad sense heritability (H^2) than the other agronomic and morpho-physiological traits. The strong correlation of PHI and seed yield under drought stress coupled with high heritability suggests that PHI and HI could be used in addition to seed yield to enhance phenotypic selection for drought tolerance. The 20 Andean genotypes were significantly variable in their water use efficiency, which was measured based on CID.

The relationship between WUE and seed yield was explored. Drought tolerant genotypes with higher CID and yield were identified and classified as “water spenders” while genotypes with low CID but higher seed yield were classified as “water savers”. The “water-savers” are recommended for severe/or terminal drought while the “water-spenders” are recommended for moderate to intermittent drought. Drought stress damages the cell membrane resulting in the leakage of electrolytes from the cell. Therefore, low electrolyte leakage has been associated with drought tolerance. In this study, drought tolerant genotypes Krimson, OAC Inferno, and SEQ 11 showed

lower electrolyte leakage than the drought tolerant check. This result demonstrated the usefulness of electrolyte leakage in the identification of drought tolerant common bean genotypes.

From study one, the genotype Kijivu was identified as having superior drought tolerance based on its lowest drought susceptibility index among all 20 Andean genotypes and the drought tolerant checks as well as having high WUE under drought stress as demonstrated in CID. A population of RILs derived from Kijivu (drought tolerant) and Bukoba (drought susceptible) was used to understand the genetic basis of the observed superior drought tolerance of Kijivu. The RILs were phenotyped for agronomic and morpho-physiological traits under drought and non-drought stress conditions. Three QTL "hotspots" for morpho-physiological traits were identified on chromosomes *Pv06, Pv07,* and *Pv10*. These three QTL hot spots were identified mainly under DS and Kijivu contributed the majority of the positive alleles at these three QTL. The results of study 2 indicated that the superior tolerance of Kijivu observed in study 1 was controlled mainly by the additive effect of genes underlying the three QTL hotspots on chromosomes *Pv06, Pv07,* and *Pv10* in addition to the minor effect QTL identified on other chromosomes.

5.2. CONCLUSIONS AND RECOMMENDATIONS

5.2.1. Conclusions

The physiological traits CID and partitioning indices were closely associated with yield under drought stress suggesting the important role that water use efficiency and partitioning efficiency played in the observed variable response of the 20 Andean genotypes to drought. Another physiological trait, electrolyte leakage, was effective in identifying genotypes with superior drought tolerance. These three physiological traits can be used as selection indices for drought tolerance in addition to seed yield. The genotype Kijivu was identified as having superior drought tolerance. QTL analysis attributed this drought tolerance to the additive effect of genes underlying the QTL hotspots identified on chromosomes 6, 7, and 10. Together, results from studies 1 and 2 have highlighted a complex interplay of agronomic and morpho-physiological traits in drought tolerance and the genetic complexity of drought tolerance in the Andean gene pool of common beans.

5.2.2. Recommendations

- The partitioning indices (PHI and HI) can be used together with seed yield to enhance selection efficiency for drought tolerance in common beans.
- The genotypes identified as water savers can be used as parents to breed for drought tolerance for genotypes targeted for regions that experience terminal drought.
- The drought QTL hotspots identified on chromosomes *Pv06, Pv07,* and *Pv10* if validated in different environments and genetic backgrounds could potentially be used in molecular breeding through marker-assisted selection for drought tolerance to enhance selection efficiency for drought and accelerate the development of drought tolerant common bean genotypes.

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APPENDICES

Appendix 1: Grain yield of genotypes grown under drought stress (DS) and non-drought stress (NS) conditions over one seasons at GART, Kabwe and UNZA.

	Grain yield Kg ha^{-1}								
	GART		Kabwe		UNZA				
GENOTYPE	DS	NS	DS	NS	DS	NS	Mean DS	Mean NS	Overall mean
Gololi	649	1726	834	1506	1129	1791	768	1402	1085
Kijivu	1065	1211	953	1778	1231	1777	915	1397	1156
Mrondo	958	2422	876	1428	1209	2025	863	1576	1219
ADP 57	966	2241	594	1500	812	1824	638	1501	1070
Mshindi	277	1817	1165	1822	1304	1755	872	1511	1191
G6415	749	1887	665	1372	1123	1433	727	1266	997
G17913	930	2214	1032	1958	1372	1603	965	1575	1270
PR0737-1	886	1729	579	1337	765	1713	603	1320	962
Kibala	919	1680	979	1323	1026	2517	830	1575	1202
RWR 10	799	1509	812	919	1060	2793	764	1502	1133
SEQ11	720	1515	462	1039	956	2352	599	1396	998
H9659-27-7	892	1662	1017	1032	1092	1654	859	1187	1023
H9659-27-10	979	1687	561	1543	751	1317	609	1249	929
OAC Inferno	971	2334	784	2591	1050	2134	782	1984	1383
TARS HT 1	729	1880	1036	1280	935	2011	785	1427	1106
Kardinal	1091	2179	556	575	1120	2000	750	1241	995
Krimson	889	1809	1013	1309	1244	1851	908	1371	1139
VA-19	688	2328	852	1386	1102	1836	772	1482	1127
Pink Panther	1160	2496	734	1311	1461	1684	935	1436	1186
PI638816	956	2946	775	1107	1451	2516	910	1724	1317
KAB	480	1197	657	1156	647	2224	519	1337	928
LSK	730	1337	979	1528	907	1923	757	1385	1071
SCR_10	733	2027	767	1594	745	1607	633	1423	1028
SCR_16	458	1540	489	1056	793	1755	508	1207	857
SCR_44	690	1615	700	1993	858	2050	640	1631	1136
SER_16	654	1395	-	-	1317	3076	831	2236	1368
G40001	350	930	-	-	406	686	295	808	441
Genotype Mean	791	1826	795	1418	1032	1922	742	1450	1086
LSD	227	418	160	263	146	231			

Appendix 2: Number of pods per plant of genotypes grown under drought stress (DS) and non-drought stress (NS) conditions over one seasons at GART, Kabwe and UNZA

	Number of pods per plant								
	GART		Kabwe		UNZA				
GENOTYPE	DS	NS	DS	NS	DS	NS	Mean DS	Mean NS	Overall mean
Gololi	16.1	16.8	16.9	19.2	9.3	9.8	14.1	15.3	14.7
Kijivu	19.9	20.6	13.9	17.3	8.0	9.4	13.9	15.8	14.9
Mrondo	26.2	13.2	11.8	29.9	15.6	14.1	17.8	19.1	18.5
ADP 57	18.9	13.0	12.3	31.8	9.0	12.2	13.4	19.0	16.2
Mshindi	19.9	12.0	12.0	24.2	12.5	12.3	14.8	16.2	15.5
G6415	25.3	14.4	10.8	17.8	9.5	8.8	15.2	13.7	14.4
G17913	17.9	13.8	8.3	16.7	7.3	8.7	11.2	13.0	12.1
PR0737-1	20.3	16.1	10.9	21.6	11.4	13.3	14.2	17.0	15.6
Kibala	20.0	15.4	16.4	25.1	7.6	13.4	14.7	18.0	16.3
RWR 10	22.1	20.3	7.4	19.1	8.9	15.1	12.8	18.1	15.5
SEQ11	20.9	19.1	9.7	22.2	12.0	17.5	14.2	19.6	16.9
H9659-27-7	19.0	11.1	8.2	21.5	7.9	8.5	11.7	13.7	12.7
H9659-27-10	15.5	9.2	17.4	20.0	6.5	6.4	13.1	11.9	12.5
OAC Inferno	15.9	15.7	15.4	22.7	8.0	11.9	13.1	16.8	14.9
TARS HT 1	22.8	8.7	19.0	26.6	8.0	12.5	16.6	15.9	16.2
Kardinal	10.9	10.9	18.2	19.6	10.5	11.7	13.2	14.0	13.6
Krimson	15.7	10.2	11.1	25.1	7.4	8.5	11.4	14.6	13.0
VA-19	17.8	16.5	11.8	23.9	9.2	9.3	13.0	16.6	14.8
Pink Panther	15.4	11.5	11.8	14.3	8.8	8.4	12.0	11.4	11.7
PI638816	12.9	14.8	22.6	25.4	9.2	12.2	14.9	17.5	16.2
KAB	19.7	15.6	18.0	28.7	5.6	15.3	14.4	19.8	17.1
LSK	21.2	13.8	27.8	35.2	8.1	13.1	19.0	20.7	19.9
SCR_10	19.1	12.1	14.8	27.4	9.3	11.3	14.4	17.0	15.7
SCR_16	21.7	13.3	9.5	29.7	10.0	13.3	13.7	18.8	16.2
SCR_44	20.9	19.5	15.5	23.8	11.0	14.2	15.8	19.2	17.5
SER_16	25.4	18.3	-	-	13.5	19.3	19.5	18.8	19.2
G40001	21.3	15.7	-	-	13.1	16.3	17.2	16.0	16.6
Genotypes Mean	19.4	14.5	14.1	23.5	9.5	12.1	14.4	16.6	15.5
LSD	8.2	10.2	8.5	ns	4.96	5.76			

Appendix 3: Total shoot biomass per plant of genotypes grown under drought stress (DS) and non-drought stress (NS) conditions over one seasons at GART, Kabwe and UNZA

	Total shoot biomass per plant								
	GART		Kabwe		UNZA				
GENOTYPE	DS	NS	DS	NS	DS	NS	Mean DS	Mean NS	Overall mean
Gololi	14.1	33.8	20.4	43.0	12.8	19.8	15.8	32.2	24.0
Kijivu	18.7	42.3	17.5	33.3	14.2	22.5	16.8	32.7	24.8
Mrondo	20.0	41.4	16.6	49.8	15.2	20.1	17.3	37.1	27.2
ADP 57	12.2	50.1	28.2	45.3	11.9	22.5	17.4	39.3	28.4
Mshindi	19.3	57.5	20.6	44.3	14.9	16.2	18.3	39.3	28.8
G6415	17.5	35.7	23.7	55.5	13.7	20.1	18.3	37.1	27.7
G17913	20.0	36.4	-	-	18.1	34.6	19.0	35.5	27.3
PR0737-1	19.3	41.5	23.6	60.5	14.3	23.7	19.1	41.9	30.5
Kibala	20.4	52.4	21.5	44.9	15.5	20.7	19.2	39.3	29.3
RWR 10	18.5	49.7	22.6	48.7	16.3	21.1	19.2	39.8	29.5
SEQ11	24.9	33.8	16.4	38.3	16.5	26.7	19.3	32.9	26.1
H9659-27-7	21.7	43.0	23.8	39.6	13.8	27.5	19.8	36.7	28.2
H9659-27-10	18.3	40.1	25.3	37.5	18.2	18.7	20.6	32.1	26.3
OAC Inferno	17.0	43.5	28.7	52.8	16.2	20.5	20.6	38.9	29.8
TARS HT 1	25.5	32.1	23.7	45.6	13.0	24.6	20.7	34.1	27.4
Kardinal	28.4	50.5	21.9	38.8	14.2	22.9	21.5	37.4	29.4
Krimson	18.9	28.5	32.6	39.2	14.9	24.1	22.1	30.6	26.4
VA-19	23.8	36.9	29.2	45.8	13.7	18.5	22.3	33.7	28.0
Pink Panther	32.0	49.5	18.9	29.5	16.5	33.3	22.5	37.4	29.9
PI638816	18.4	38.5	37.4	50.5	13.6	17.5	23.2	35.5	29.3
KAB	28.2	50.7	27.4	66.3	17.8	17.6	24.5	44.8	34.7
LSK	26.7	39.1	31.6	57.3	17.7	23.8	25.4	40.0	32.7
SCR_10	24.1	49.2	43.7	57.9	13.7	22.5	27.2	43.2	35.2
SCR_16	26.5	37.5	42.4	61.1	17.8	26.1	28.9	41.6	35.2
SCR_44	20.9	51.7	36.3	65.3	29.5	24.6	28.9	47.2	38.1
SER_16	36.0	37.3	39.0	50.8	16.2	16.9	30.4	35.0	32.7
G40001	8.5	13.6	-	-	6.5	11.1	7.5	12.3	9.9
Genotype Mean	22.0	42.4	26.9	48.1	15.8	22.6	21.5	37.5	29.5
LSD	59.3	116.6	86.7	130.8	41.1	43.8			

Appendix 4: Hundred Seed Weight of genotypes grown under drought stress (DS) and non-drought stress (NS) conditions over one seasons at GART, Kabwe and UNZA

	Hundred Seed Weight (g)								
	GART		Kabwe		UNZA				
GENOTYPE	DS	NS	DS	NS	DS	NS	Mean DS	Mean NS	Overall mean
Gololi	34.9	46.4	33.8	37.7	34.4	47.3	34.4	43.8	39.1
Kijivu	40.6	49.5	38.8	48.9	38.3	54.5	39.2	51.0	45.1
Mrondo	26.5	48.3	28.7	35.0	29.1	41.4	28.1	41.6	34.8
ADP 57	31.3	47.9	32.4	40.4	38.1	47.5	33.9	45.2	39.6
Mshindi	28.9	39.7	29.2	36.0	28.7	42.1	28.9	39.3	34.1
G6415	42.7	60.5	40.0	49.9	49.3	54.0	44.0	54.8	49.4
G17913	48.9	63.8	48.2	56.2	53.4	66.6	50.2	62.2	56.2
PR0737-1	30.6	41.9	31.0	33.7	26.3	46.7	29.3	40.8	35.0
Kibala	35.1	44.5	27.7	38.2	35.7	42.5	32.8	41.7	37.3
RWR 10	33.4	47.8	32.3	37.9	36.3	52.5	34.0	46.1	40.0
SEQ11	23.9	28.0	24.2	25.6	20.5	30.3	22.9	28.0	25.4
H9659-27-7	35.6	53.3	41.6	42.4	43.4	51.4	40.2	49.1	44.6
H9659-27-10	35.8	50.0	39.0	46.1	36.7	53.3	37.2	49.8	43.5
OAC Inferno	44.3	51.5	40.1	42.8	44.5	55.3	43.0	49.8	46.4
TARS HT 1	36.9	50.7	37.3	43.2	36.9	47.6	37.0	47.2	42.1
Kardinal	40.5	50.2	39.8	44.3	42.9	52.3	41.1	48.9	45.0
Krimson	38.8	55.6	39.7	46.6	42.3	53.0	40.3	51.7	46.0
VA-19	41.5	64.9	40.7	50.2	48.9	63.0	43.7	59.4	51.5
Pink Panther	49.3	63.6	45.2	50.5	49.1	60.2	47.9	58.1	53.0
PI638816	37.0	58.9	38.2	43.3	41.3	46.7	38.8	49.6	44.2
KAB	35.9	27.8	32.5	37.1	43.2	46.7	37.2	37.2	37.2
LSK	31.1	27.3	30.7	36.4	37.5	42.8	33.1	35.5	34.3
SCR_10	25.1	33.6	26.9	30.3	25.9	30.5	26.0	31.4	28.7
SCR_16	24.0	35.3	26.6	29.0	25.0	33.9	25.2	32.7	29.0
SCR_44	26.2	35.8	26.9	28.9	27.3	34.5	26.8	33.1	29.9
SER_16	23.2	34.0	-	-	20.1	32.4	21.7	33.2	27.4
G40001	8.6	10.4	-	-	8.5	9.9	8.6	10.1	9.4
Genotype Mean	33.7	45.2	34.9	40.4	35.7	45.9	34.3	43.4	38.8
LSD	3.7	7.65	4.5	5.3	5.5	6.2			

Appendix 5: Pod harvest index of genotypes grown under drought stress (DS) and non-drought stress (NS) conditions over one seasons at GART, Kabwe and UNZA

	Pod Harvest Index								
	GART		Kabwe		UNZA				
GENOTYPE	DS	NS	DS	NS	DS	NS	Mean DS	Mean NS	Overall mean
Gololi	71	79	68	70	71	75	70	75	72
Kijivu	67	75	67	65	66	74	67	71	69
Mrondo	71	78	63	67	66	75	67	73	70
ADP 57	68	74	58	67	51	71	59	71	65
Mshindi	67	75	49	72	68	73	61	74	68
G6415	65	73	63	67	63	71	64	70	67
G17913	73	78	69	71	69	76	71	75	73
PR0737-1	54	65	55	59	52	66	54	63	59
Kibala	69	77	71	68	68	78	69	74	72
RWR 10	68	72	49	59	60	72	59	67	63
SEQ11	66	67	55	67	54	68	58	68	63
H9659-27-7	66	73	57	63	66	71	63	69	66
H9659-27-10	66	58	58	66	59	70	61	65	63
OAC Inferno	66	71	52	68	56	72	58	70	64
TARS HT 1	67	75	68	71	69	74	68	73	71
Kardinal	67	73	64	64	62	73	64	70	67
Krimson	66	72	68	69	69	75	68	72	70
VA-19	68	75	63	65	63	72	65	71	68
Pink Panther	66	76	68	69	69	76	68	74	71
PI638816	66	76	67	65	67	74	67	72	69
KAB	51	68	54	61	63	72	56	67	62
LSK	65	71	68	66	65	74	66	71	68
SCR_10	59	71	57	62	59	73	58	69	64
SCR_16	57	66	63	62	51	73	57	67	62
SCR_44	59	66	29	64	52	73	46	68	57
SER_16	67	74	-	-	58	76	63	75	69
G40001	55	69	-	-	58	64	56	67	62
Genotype Mean	65	72	60	66	62	73	62	70	66
LSD	8	6	14	6	18	6			

Appendix 6: Harvest index of genotypes grown under drought stress (DS) and non-drought stress (NS) conditions over one seasons at GART, Kabwe and UNZA

	Harvest index								
	GART		Kabwe		UNZA				
GENOTYPE	DS	NS	DS	NS	DS	NS	Mean DS	Mean NS	Overall mean
Gololi	62	67	53	60	62	65	59	64	61
Kijivu	57	68	55	53	59	65	57	62	59
Mrondo	52	68	41	47	53	66	49	60	54
ADP 57	54	62	32	51	33	61	40	58	49
Mshindi	49	63	28	60	59	65	45	63	54
G6415	53	60	50	51	53	62	52	58	55
G17913	64	70	56	61	59	69	60	67	63
PR0737-1	42	51	35	44	39	57	39	51	45
Kibala	54	59	56	50	46	68	52	59	56
RWR 10	51	58	24	38	40	62	38	53	46
SEQ11	48	48	29	51	37	56	38	52	45
H9659-27-7	50	61	29	47	51	60	43	56	50
H9659-27-10	51	43	42	50	44	60	46	51	48
OAC Inferno	56	58	29	56	41	64	42	59	51
TARS HT 1	57	63	51	60	58	67	55	63	59
Kardinal	56	59	46	49	48	62	50	57	53
Krimson	54	62	52	56	60	68	55	62	59
VA-19	55	63	35	49	50	59	47	57	52
Pink Panther	53	63	50	48	57	67	53	59	56
PI638816	53	65	53	49	57	66	54	60	57
KAB	36	53	35	39	39	62	37	51	44
LSK	49	56	54	48	49	64	50	56	53
SCR_10	41	51	34	42	34	60	37	51	44
SCR_16	34	48	42	44	29	62	35	51	43
SCR_44	41	51	15	48	35	63	30	54	42
SER_16	54	62	-	-	42	67	48	64	56
G40001	37	42	-	-	44	49	41	45	43
Genotypes Mean	50	58	41	50	47	63	46	57	52
LSD	10	9	16	15	19	8			

Appendix 7: Carbon Isotope Discrimination (CID) and Electrolyte Leakage (EL) of genotypes grown under drought stress (DS) and non-drought stress (NS) conditions over one seasons at GART, Kabwe and UNZA

	CID			EL		
GENOTYPE	DS	NS	CID Overall mean	DS	NS	EL Overall mean
Gololi	18.5	18.9	18.7	92.8	15.1	53.9
Kijivu	18.6	19.5	19.1	92.7	18.8	55.7
Mrondo	18.7	20.0	19.4	96.1	22.4	59.2
ADP 57	18.6	20.2	19.4	100.0	14.3	57.1
Mshindi	18.8	18.8	18.8	95.8	11.6	53.7
G6415	18.0	19.4	18.7	96.6	10.3	53.5
G17913	19.0	19.6	19.3	89.0	13.3	51.1
PR0737-1	18.7	20.1	19.4	95.3	9.9	52.6
Kibala	18.2	19.2	18.7	95.5	15.8	55.6
RWR 10	18.6	19.7	19.2	92.4	14.4	53.4
SEQ11	18.7	19.8	19.2	86.6	19.3	53.0
H9659-27-7	19.3	20.2	19.7	97.7	32.7	65.2
H9659-27-10	19.0	20.0	19.5	94.3	25.4	59.8
OAC Inferno	18.6	20.4	19.5	87.5	14.7	51.1
TARS HT 1	18.6	19.8	19.2	91.1	11.0	51.0
Kardinal	19.1	20.0	19.6	90.0	12.7	51.4
Krimson	18.9	20.4	19.7	86.5	13.9	50.2
VA-19	18.1	19.2	18.6	89.1	23.7	56.4
Pink Panther	18.9	18.7	18.8	94.8	19.9	57.3
PI638816	18.9	19.7	19.3	92.2	12.3	52.3
KAB	17.5	19.7	18.6	90.7	17.5	54.1
LSK	18.1	20.1	19.1	81.8	11.0	46.4
SCR_10	18.1	20.0	19.1	87.7	15.7	51.7
SCR_16	18.2	19.9	19.1	85.7	17.9	51.8
SCR_44	18.9	20.5	19.7	94.6	14.8	54.7
SER_16	18.4	19.3	18.9	88.1	13.4	50.8
G40001	17.4	18.4	17.9	71.8	29.5	50.7
Genotype mean	18.5	19.7	19.1	91.0	16.7	53.8
LSD	0.9	0.7		12.8	15.0	