Physiological analysis of responses of lateral branching of the primary root for tolerance to phosphorus stress and drought in the common bean (*Phaseolus vulgaris* L.)

by

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A THESIS SUBMITTED IN FULFILMENT OF THE ACADEMIC REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PhD) IN CROP SCIENCE

School of Agricultural, Earth and Environmental Sciences

University of KwaZulu-Natal (UKZN)

Pietermaritzburg, Republic of South Africa

January 2021

GENERAL ABSTRACT

Common bean (*Phaseolus vulgaris* L.) is essential to the food security of millions of people in developing nations. However, inadequate precipitation and low soil fertility, mainly phosphorus (P) deficiency, tend to limit its production in smallholder systems. Drought stress severely restricts root growth, binding the capacity of soil water exploration in deep horizons, while phosphorus (P) limitation increases the root capacity of foraging for nutrients in the top soil. Therefore, the development of traits associated with drought resistance and phosphorus stress will contribute to common bean improvement for lines suited for these environments. Thus, the present study aimed to evaluate the physiological response of lateral branching of the primary roots for tolerance to low phosphorus and drought stress in the common bean. To achieve this, pot and field experiments were established at the Agricultural Research Institute of Mozambique (IIAM) - Chókwè Research Station to: (1) determine the effect of drought stress on crop performance at different bean growth stages in the field and pot trials, (2) evaluate phosphorus use efficiency on grain yield efficiency index (GYEI) and P concentration in the plant tissues of selected genotypes in a pot study, and (3) assess the contribution of root phenes to shoot biomass and grain yield under combined stress (drought and low P) in the field and pot study. Eight genotypes were tested for shoot biomass, root biomass, shoot P concentration and uptake assessment in the pot study. For the field experiments, a total of 24 bean lines were used to assess phenology, yield components and total yield per unit. All pot studies were set using a randomized complete block design in a factorial arrangement with four replications. The first study had two levels of water (water stress and irrigated- no water stress); while the second study had two treatments combining phosphorus levels x genotypes, grown in a system of stratified phosphorus, 0.025 g P kg^{-1} of soil as low rate and 0.2 g P kg^{-1} of soil as high P rate. The last pot experiment combined both water and phosphorus levels and, in all cases, eight genotypes were tested. The field experiment was set as a split plot design with four replications for the drought and phosphorus studies, while for the combined study it was set as a split-split plot.

Water stress lowered substantially the leaf relative water content, leaf water potential and all growth parameters in the pot experiment, decreasing shoot biomass by 47%, leaflet growth rates by 49% and number of leaflets by 53%. In spite of significant reduction, these variables were highly and positively correlated, and can be recommended for early selection of genotypes grown under limited water conditions. Phosphorus levels also significantly affected shoot and root biomass, shoot phosphorus concentration, phosphorus uptake and phosphorus leachate under high P levels. However, genotypes responded differently to phosphorus levels in term of root biomass, shoot biomass and P uptake; genotypes BFS 81, SEQ342-87 and IBC 301-204 performing better in terms of root and shoot biomass as well as P uptake. These genotypes can be recommended for early selection under low soil fertility, especially in soils with P deficiency. Under field conditions, drought stress had a significant effect ($p<0.05$) on yield components. Among all the genotypes evaluated, SEF 16, SX 14825- 7-1, TARS MST-1, SEN 52, BRT103-182, FBN1211-66, IBC 301-204, SER 125 and MHR 311-17 were the most adapted and showed the best yield performances under drought stress, and therefore can be incorporated in breeding programs particularly in drought prone areas. Meanwhile, high phosphorus treatment significantly increased all yield components (pods per plant, 100-seed weight, and grain yield), and variability among genotypes was also observed for yield and yield components. Based on the grain yield efficiency index (GYEI), 10 bean lines out of the 24 were categorized as P use efficient genotypes and therefore, they can be used in phosphorus deficient soils as well as crop improvement program.

Grain yield from field data under drought stress and low P were positively correlated with the pot data on root traits. Response of root phene to drought and phosphorus stress appeared to be related to the phenotype traits of water stress and P use efficiency (that is, deep and shallow rooted systems, respectively). Deeper rooted genotypes produced more total root biomass, high taproot lateral branching density, which resulted in high total root length under drought and low P stress. On the other hand, shallow rooted genotypes allocated relatively low total root biomass and less allocation of taproot lateral branching. Increase in shoot biomass and seed yield in drought and low P stress was associated with higher mean values of taproot lateral branching density and total taproot length. Genotypes SER 125, BFS 81, FBN12111-66 and MER 22 11-28 showed greater score of taproots branching density in the pot study with the highest grain yield in the field under low P and drought stress. Therefore, these genotypes can be used in phosphorus deficient soils and drought stress environments or serve as parents for improving phosphorus use efficiency and drought tolerance in common bean. An increased total taproot lateral branching and length observed under stressful conditions (drought stress), contributed for plant performance, and could be considered as an important trait for selecting cultivars.

Although no previous study has explicitly explored the utility of taproot lateral length and lateral branching for plant performance under edaphic stress, results from the present study suggest that an increased taproot lateral branching and length provide benefits under multiple environments. Genotypes that exhibited higher scores of taproot lateral branching in the field such as SER 125, BFS 81, FBN12111- 66 and MER 22 11-28, combining with higher grain yield under low P and drought were ranked as deeper rooted and suited to environments where water is limiting. In contrast, genotypes INB 814, SEN52, BIOF 2-06 and SEQ342-87 had relatively low scores of tap root branching density, but with better yield under low P and drought, and were classified as shallow rooted and suited to environments where P is limiting.

Keywords*: common bean, taproot lateral branching, drought, phosphorus use efficient*

PREFACE

The research contained in this thesis was completed by the candidate while based in the Discipline of Crop Science, School of Agricultural, Earth and Environmental Sciences of the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Pietermaritzburg Campus, South Africa. The research was financially supported by Agricultural Productivity Program for Southern Africa (APPSA) and the Alliance for a Green Revolution in Africa (AGRA).

The contents of this work have not been submitted in any form to another university and, except where the work of others is acknowledged in the text, the results reported are due to investigations by the candidate.

[redacted]

Signed: Prof.

Julia Sibiya

Date: 04/02/2021

DECLARATION - PLAGIARISM

I, **Samuel Alves Camilo declares** that:

(i) The research reported in this thesis, except where otherwise indicated or acknowledged, is my original work;

(ii) This thesis has not been submitted in full or in part for any degree or examination to any other university;

(iii) This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons;

(iv) This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

  a) Their words have been re-written but the general information attributed to them has been referenced;

  b) Where their exact words have been used, their writing has been placed inside quotation marks, and referenced;

(v) Where I have used material for which publications followed, I have indicated in detail my role in the work;

(vi) This thesis does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the thesis and in the References sections.

Signed:
[redacted]

Samuel Alves
Camilo Date:
04/02/2021

As the candidate's supervisors we agree to the submission of this thesis

[redacted]

Prof. Julia Sibiya (Supervisor)

……………[redacted]………………………………………..

Prof Alfred Odindo (Co-Supervisor)

ACKNOWLEDGEMENTS

Thanks to the Agricultural Productivity Program for Southern Africa (APPSA) for the Scholarship, the Alliance for a Green Revolution in Africa (AGRA) for financial support on my tuition, USAID-CRIB for the seeds used in this research, my Institution, Agricultural Research Institute of Mozambique (IIAM), for allowing me to continue with my studies.

Thanks to Professors Julia Sibiya and Alfred Odindo for their mentorship in this work, for sharing their knowledge; for their dedication and attention during my academic training and execution of the thesis.

Special thanks to Dr. Aleck Kondwakwenda for his readiness because this work would not have been possible without his help in editing this thesis.

Thanks to all the “Bean Team”, Dr. Celestina Jochua, Eng. Isabel Mogovo, Dr. Manuel Amane, Januário Coutinho, Eng. William for all the support from planning to harvest of trials, particularly during the excavation and scoring of root crowns.

I also thank my colleagues Bento Filipe and Egas Nhamucho for their friendly support throughout the entire study period.

I am deeply indebted to my wife Dulcilia Nhate, and my little Keylon for their unwavering love, support, encouragement and patience throughout the study period.

DEDICATION

This work is dedicated to:

My late brother Leonardo Alegria Mario Camilo who always inspired me to never give up.

My dad Mario Camillo and mom Bernardete José Alves for their unconditional support every time I needed it.

My Sisters Justina Camilo, Ravieza Camilo, Rosalina Camilo, Mariana do Rosário, and my brother Celso Camilo for their support and encouragement throughout the study and always believing in me.

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LIST OF ABBREVIATIONS

ARN	Adventitious root number
BRN	Basal root number
BRWN	Basal root whorls number
BRGA	Basal root growth angle
DAP	Days after planting
DII	Drought Intensity Index
DS	Drought stress
DSI	Drought susceptibility
GM	Geometrical mean
GYEI	Grain yield efficiency index
LGR	Leaf growth rate
LW	Low watered
NS	Non-stress
P	Phosphorus
PHI	Pod harvest index
PR	Percent reduction
SB	Shoot biomass
SRL	Specific root length
TD	Taproot diameter
TDR	Time domain refractometer
TRB	Taproot biomass
tRB	Total root biomass
TRL	Taproot Lateral
TRLB	Taproot lateral branching
VWC	Volumetric water content
WW	Well-watered

1. CHAPTER ONE

General Introduction

1.1 Overview of common bean

Common bean (*Phaseolus vulgaris* L.) plays an important role as a food legume for food security in most developing countries in Africa and Latin America (Androcioli et al., 2020; Beebe et al., 2013). It is a cheap source of protein than meat, contains fibre, carbohydrates, and trace minerals (Hummel et al., 2018; Ramirez-vallejo & Kelly, 2014) and is affordable to smallholder farmers in regions with endemic poverty (Arruda et al., 2018).

Common bean belongs to the Fabaceae family, and has two gene pools – Mesoamerican originating from Central America and Andean from South America (Polania et al., 2016). The gene pools differ in seed size, color, phaseolin protein, morphological and molecular background (Blair et al., 2006). Common bean is mainly cultivated by smallholder farmers in marginal lands, with less inputs (Beebe et al., 2013; Broughton et al., 2003) and crop yields are constantly affected by a series of abiotic factors, especially water scarcity (Androcioli et al., 2020; Assefa et al., 2019). The growing period of common bean can be up to 80 days and during that period it requires between 350-500 mm of water depending on the climate, soil depth and variety (Beebe et al., 2013). It is estimated that water scarcity in the world affects about 60% of the land used for bean production with prolonged drought cycles (Beebe et al., 2013).

Drought stress is an important factor that reduces yields, disrupting the security of production systems in developing countries (Asfaw & Blair, 2012). It is one of the yield-limiting factor in the smallholder production systems (Androcioli et al., 2020; Hageman et al., 2019) especially under dry land conditions and low inputs (Hummel et al., 2018; Manjeru et al., 2007) resulting in yield reductions of between 10 to 100%. In addition, climate change has altered rainfall patterns and reduced the availability and supply of water (Androcioli et al., 2020; Cortés & Blair, 2018). The decrease in rainfall affects available soil moisture and has negative consequences on roots and consequently crop productivity, since roots are the main organs responsible for water absorption and maintaining crop growth under drought conditions.

Phosphorus (P) distribution and availability is highly heterogeneous in the soil and is another major cause of low been seed yield. Its availability to the plant is determined by chemical and biological reactions among the several elements present in the soil causing only a small amount of phosphorus in the soil to be present as orthophosphate (Pi) (Suriyagoda et al., 2014). Compared to other macronutrients, P is the least mobile in the soil and therefore the least available to plants (Hinsinger, 2001; Namugwanya et al., 2014). The reactivity of P with iron and alumina oxide in soil results in compounds that are not available to plants (Lynch & Brown, 2008). Due to these factors, the recorded bean yields in Southern Africa have only averaged 600 kg ha^{-1} compared to the attainable yields of more than 1.500 kg ha^{-1} (Namayanja et al., 2014). On the other hand, high costs of fertilizers and lack of capital prevent many farmers from obtaining commercially available P fertilizer.

Mourice and Tryphone (2012) reported that beans extract from the soil approximately about 12.5 kg P/ha, which is considered high compared to what is added in terms of phosphorus fertilization by low-income farmers. It has been documented that in Africa, where most bean production occurs, soils have very low concentrations of extractable phosphorus, that is, from 1.6 to 3.1 mg P/kg soil (Giller et al., 1998). Fertilizer use is very low with an average application dosage of 1.9 kg P/ha of used land (Buruchara et al., 2011). This is attributed to high costs of agricultural inputs and transport (Hudgens, 1996), erratic rainfall patterns, inadequate supply and untimely, availability of fertilizer, and limited credit opportunities

(Wallace, 1997). Therefore, phosphorus deficiency can be addressed with corrective soil fertility improvement practices such as use of phosphorus-based fertilizers and liming.

Use of P-efficient genotypes capable of utilizing available phosphorous from limiting soil environments helps to enhance productivity and lowers the cost of fertilizers. Therefore, identification of bean cultivars efficient in P utilization would be helpful in the establishment of a breeding program to target high P use efficient cultivars, which are able to thrive in soils with P deficiencies. The development of new varieties with high P use efficiency, in addition to the best agricultural practices, has been linked to a good root system that is able to explore resources when P is limiting. While shoots have an important role in water and nutrient utilization when these resources are limited, the root system is strictly responsible for the acquisition of the resources (Strock et al., 2018). Roots are important for crop adaptation where nutrient and water are limited, and this is explained by the increment on root growth in relation to shoot growth under edaphic stress (Ho et al., 2005; Strock et al., 2018) and it has been proven that the root systems have a significant influence on bean yield under drought stress (Adams et al., 2002; Kaeppler et al., 2011).

According to York et al. (2013), root phenotypes are composed of phenes that are the fundamental elements of the phenotype, whose heritability are quantitatively controlled, and thus influenced by environmental interactions. Root phenotypes have an important role in enhancing crop yield in harsh environments by increasing the metabolic efficiency of soil exploitation and distributing roots into the soil profile where limited soil resources are most available (Jaramillo et al., 2013; Lynch, 2018). Roots have an important role in nutrients and water absorption by plants. Phenotypic assessment of root traits of common bean in drought stress conditions, showed the influence of varied rooting patterns, including a deep rooting system that provides water from deeper soil horizons (Beebe et al., 2013, 2014; Burridge et al., 2016). Distinct ideotypes of root systems were investigated and suggested for superior crop adaptation, either for a single or combined abiotic stress (Polania et al., 2016; Zheng et al., 2020).

In *Phaseolus vulgaris*, root phenes changes with soil foraging depths based on basal root growth angle (Liao et al., 2001; Miguel et al., 2013; Miller et al., 2003; Ochoa et al., 2006; Rangarajan et al., 2018; Walk et al., 2006), basal root whorl number (Miguel et al., 2013; Miller et al., 2003; Rangarajan et al., 2018; Walk et al., 2006), adventitious root abundance (Miller et al., 2003; Ochoa et al., 2006; Rangarajan et al., 2018), and lateral root branching density (Rangarajan et al., 2018). According to Basu et al. (2007), the basal roots emerge from the base of the hypocotyl and reach either the surface or the lower soil horizons depending on the growth angle. In combination with the lateral roots arising from them, basal roots constitute the largest portion of the total root length. Basal root gravitropism is important for determination of the overall shallowness of the root system, because they generate a framework where most of the bean root system develops (Liao et al., 2001; Rubio et al., 2003). The spread of basal roots relative to the gravity over time determines if this class of root system penetrate rapidly into the subsoil or remains on the soil surface (Lynch & Brown, 2008).

Basal root gravitropism can be determined through the basal root growth angle (BRGA) of the root crown or by the rate of basal roots in the soil surface relative to the total amount of basal roots (Bonser et al., 1996; Liao et al., 2001). Topsoil exploration is mostly guaranteed through basal root gravitropism, even though in common bean and other legumes, variation in basal root gravitropism exists among genotypes, with P availability controlling basal root gravitropism in a genotype-dependent way (Bonser et al., 1996).

The BRWN is a primary part of the root architecture of a bean root system and refers to the number of whorls a genotype has, most commonly ranging between one, two, and three and up to four whorls. Genotypes with a BRWN of one have the potential for four basal roots, a BRWN of two for eight, and a BRWN of three for a total of 12 basal roots (Widrig 2005). The basal whorl position, vertically on the hypocotyl, can determine its growth angle (Basu et al., 2007). Greater BRWN improves shoot growth of common bean in phosphorus deficient soils (Miguel et al., 2013). Basal whorls nearest to the shoot have smaller angles relative to horizontal (i. e. shallower growth) and whorls farther from the shoot have larger basal root angles for deeper rooting.

Adventitious rooting is another important element of topsoil exploration. In common bean, adventitious roots arise from the lower part of the hypocotyl expanding horizontally over the soil surface. Common bean genotypes vary considerably in adventitious rooting allocation, and this trait is controlled by phosphorus uptake (Miller et al., 2003; Ochoa et al., 2006). In the tropics, under P soil deficiency, a field study demonstrated that bean cultivars with superior growth and high phosphorus uptake allocated relatively more adventitious roots in relation to basal root when compared to the phosphorus inefficient cultivars (Miller et al., 2003). Adventitious roots are found to have numerous advantages for topsoil exploration, since their horizontal growth tends to maintain foraging in the top horizon. Other benefits are related to the anatomical and morphological variations between adventitious and basal roots, where adventitious roots have higher root length per unit of root mass (specific root length) compared to other root classes, allowing the plant to explore a greater bulk of soil per unit of metabolic investment in root tissue (Lynch & Brown, 2008).

The development of cultivars with improved productivity that are able to thrive where water and nutrient availability is limited, is a fundamental strategy in addressing challenges in marginal lands with low use of input (Lynch, 2019; Lynch & Brown, 2008). Since roots are strictly responsible for resource acquisition and transport (water and nutrients), drought as well as nutrient deficiencies are difficult to sustain and mitigate. Therefore, understanding the fitness landscape of root phenotypes is fundamental for the development of crop cultivars with improved productivity under limited soil resources.

1.2 Research problem

Drought and low soil phosphorus availability are major constraints in common bean production and productivity in developing countries. Most agricultural systems rely on P fertilizers derived from rock phosphate to overcome soil P deficiency for food production and therefore food security (Bouwman et al., 2009; Lynch & Brown, 2012). Since rock phosphate reserves are not renewable resources, the utilization of P fertilizers in sustainable ways in order to improve its efficiency is a major concern. Moreover, the sustainability of using P fertilizer is questionable in the long-term due the depletion of natural resources and the implications for biodiversity. Therefore, the development of common bean varieties, which are less dependent on phosphorus fertilizers, is an important impetus of a new sustainable green revolution. This is especially true in developing countries where agricultural production is characterized by low inputs and very low yields. Under such situations, root architectural traits that improve water and phosphorus acquisition efficiency are key tools in developing crop plants that can achieve higher yields in stressful soils.

Adaptation to low fertilization regimes can also be achieved by other alternatives such as plant inoculation with bacteria in a mutual beneficial relationship or the use of genetically modified organisms. Neither alternative is a viable solution for agricultural systems in developing countries, as they increase the overall production cost. Therefore, the hope of increasing productivity in most developing nations lies in crop adaptation to low soil resources under breeding by improving root traits that increase nutrient and water acquisition efficiency.

The identification of traits which improve the utilization of environmental resources, would be beneficial in selecting tolerant genotypes, deciding on an appropriate common bean roots improvement strategy and selecting desirable parents for breeders to develop genotypes that can achieve greater yields with the use of minimum resources. This study aimed at identifying a particular trait (phene) that would improve crop growth under limited water and phosphorus availability, with high yielding common bean varieties preferred by the farmers.

1.3 Objectives

1.3.1 Main Objective

The overall objective of this study was to assess the physiological responses of traits associated with enhanced drought stress and low phosphorus adaptation in common bean in order to establish a selection criterion that would be used in a bean breeding program.

1.3.2 Specific Objectives

1.3.2.1 To evaluate the relationship between shoot biomass and water use efficiency by leaf water potential to identify the best genotypes with desirable traits for drought tolerance and high yield;

1.3.2.2 To determine the effect of two varying phosphorus levels on vegetative plant growth and yield of different common bean genotypes;

1.3.2.3 To assess the contributions of root systems (lateral root branching) to low phosphorus and drought tolerance in genotypes under suboptimal water and phosphorus supply.

1.4 Research hypotheses

1.4.1 Phenotypes with few taproot lateral branches will perform better in environments where water and phosphorus availability are concentrated in the topsoil;

1.4.2 Phenotypes with deep roots and many taproot lateral branches will perform better in drought environments; and

1.4.3 Phenotypes that are adapted to both environments (low P and drought stress) will have dimorphic characteristics.

1.5 Outline of the Thesis

The specific objectives listed are addressed in the chapters that comprise this thesis. The chapters have been written as independent manuscripts and therefore, overlaps are most likely to occur in terms of content and references among the different chapters. The thesis is organized as follows:

Chapter 1: General Introduction

Chapter 2: General Literature Review

Chapter 3: Shoot traits associated with drought stress tolerance in common beans (*Phaseolus vulgaris* L.*)*

Chapter 4: Evaluation of common bean (*Phaseolus vulgaris* L.) genotypes for phosphorus use efficiency in roots, shoots and yield components

Chapter 5: Root traits related with drought and phosphorus tolerance in common bean (*Phaseolus vulgaris* L)

Chapter 6: General overview and implications of the study

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2. CHAPTER TWO - Literature Review

Taxonomy, history of origin, domestication and common bean (*Phaseolus vulgaris* L.) response to limited water and phosphorus

Abstract

Common bean (*Phaseolus vulgaris* L.) is a diploid species and annual crop that belongs to the legume *Fabaceae* family. The bean crop is cultivated in many parts of the world and is originated from a wide geographic origin in the tropics and sub-tropics of Latin America and comprise of more than 50 species. The major centres of domestication are the Andean and Middle American. The genotypes of Andean and Meso-American gene pools are further categorized into three races based on phenotypic adaptive characteristics. The most widely cultivated species are *Phaseolus vulgaris* L., *Phaseolus lunatus* L., *Phaseolus accutifolius* L.*,* *Phaseolus dumosus* Λ.*,* *P. polyanthus* L. *and Phaseolus coccinus* L. Common bean is a cheap source of proteins, carbohydrates, amino acids and vitamins in diets of people and livestock mostly in developing countries. Fungal, bacterial and viral diseases, and insects are important biotic factors that farmers have to face when growing this crop. Likewise, high seed yield productivity under rain-fed is mostly constrained by drought and low soil fertility in particular low soil phosphorus. The consequences of drought on crop production indicate that it greatly affects over 60% of common bean world production. The effects of drought are mostly in developing countries where resource poor farmers cannot afford irrigation facilities. Low soil phosphorus greatly limits cultivation of common bean mainly in smallholder farming system in the tropics and subtropics with low inputs use, and soil acidity. In developing countries, the majority of smallholder farmers grow beans as a low-input crop, as a consequence, the yields of bean in developed countries are much higher compared to those in developing nations. Therefore, the development of cultivars that can thrive under low use of input, will contribute in increasing yield in the smallholder farmers.

2.1 Introduction

Common bean (*Phaseolus vulgaris* L.) is a dicotyledonous plant of the *Leguminosae (Fabaceae)* family that serves as food for people, livestock feed, and serves as well for non-food uses (Graham & Vance, 2003). Legumes are ranked second after cereals in supplying food, with more than 18,000 existing legume species that are cultivated for food and forage (Broughton et al., 2003). Even though there are more than a thousand edible legume species known, common beans (*Phaseolus vulgaris*), pea (*Pisum sativum*), soybean (*Glycine max*), chickpea (*Cicer arietinum*), and lentil (*Lens culinaris*), are among the most broadly grown species around the world.

Common bean has been reported to be one of the most essential legumes grown worldwide (HarvestPlus, 2016), providing nutrients to more than 300 million smallholder farmers in Africa and Latin America. It accounts for 65% of overall protein, and 32% of energy consumed in Africa and Latin America (Blair et al., 2010; Trott et al., 2016). In addition, it is an important source of micronutrients such as folic acid, iron, zinc, and thiamine (Broughton et al., 2003). There are different types of common beans, which include dry, green, shelling, and popping beans, all which are annual herbaceous plants. Dry-edible beans are essential sources of proteins for plant-based - diets compared to the other classes of beans and includes most species of the genus *Phaseolus* and some species of *Vigna*.

Improving common bean nutritional quality, stress tolerance or resistance to pests and diseases are key objectives for various breeding programmes (Angenon et al., 1999; Suárez et al., 2008). In general, bean production is affected by multiple biotic and abiotic factors, common bean diseases being the main biotic constraint that reduces bean yield. Diseases and insects are important biotic factors that farmers have to face when growing this crop (Broughton et al., 2003). Among the fungal, bacterial and viral diseases that can affect common bean, at least five major ones are widespread: common bacterial blight, bean common mosaic virus, anthracnose, bean golden yellow mosaic virus and angular leaf spot, while several others occur regionally or locally (Broughton et al., 2003). A common bean variety that is resistant to bean golden mosaic virus (BGMV) has recently been developed (Aragão et al., 2013). Most commonly, breeders aim for resistance to one or two diseases and/or pest insects within the same variety. Since wild *Phaseolus* species present traits such as pest and pathogen resistance that are usually not common among cultivated common beans, they may be a potential source of novel alleles (Acosta-Gallegos et al., 2007). Breeding programmes are developing agronomic traits such as nitrogen fixation. Other characteristics are also being explored by common bean breeding programmes, such as the increased content of specific nutrients including protein, minerals and vitamins.

The development of varieties with improved tolerance/resistant to other biotic stressors and to abiotic stressors is another important goal. Regarding the abiotic constraints, drought has been reported as the most limiting factor in yield (Beebe et al., 2014). It has been shown to decrease yields in almost 60% of the bean production systems by 10% to 100% (Beebe et al., 2014; Polania et al., 2016). In addition to drought, smallholder farmers in most of these bean production agro-systems face the challenge of declining soil fertility, which is compounded by limited use of inputs as they cannot afford purchasing those (Douxchamps et al., 2010). In general, phosphorus (P) and nitrogen (N) are viewed as the most limiting nutrients for crop production. Although legumes can acquire part of their needed nitrogen from the atmosphere over the symbiotic nitrogen fixation (SNF), this physiological mechanism is complex and is severely affected by drought stress by impacting the rhizobia and/or the nodulation of legumes (Douxchamps et al., 2010). Biological nitrogen fixation in common bean and other legume species usually reveals low ratio of N fixation even in good-irrigated conditions (Douxchamps et al., 2010). It is, therefore, important to implement technologies that improve the efficiency through breeding efforts that utilize molecular markers and expand the selection methods by targeting morpho-physiological traits related to the plant performance.

The identification and selection of parental lines to use in breeding for superior SNF capability and P efficient varieties under drought stress with other desirable traits, would be helpful in creating common bean varieties suited to drought stress and low soil fertility. This would contribute to solutions being sought after to overcome the recent challenges of climate changes in marginal environments and to ensure food security in developing nations.

The purpose of this review is, thus, to give an insight into aspects that are important to the research focus. It covers the following sections: An overview of origin, history and diversity of common bean; taxonomic group and important species for agricultural production; importance and nutritional benefits of common bean; characterization of common bean; production constraints; plant responses to limited water and phosphorus and plant attributes important in the evaluation of adaptation to drought.

2.1.1 Origin, history and diversity

Common beans were domesticated more than 7000 years ago in tropical and subtropical regions of South and Central America (Kaplan & Lynch, 1999; Kaplan, 2003). However, there were suggestions indicating Asia as the origin of common bean 200 years ago although the majority of evidence indicate that *Phaseolus vulgaris* came from the New World (Freyre et al., 1996; Singh et al., 1991). Archaeological data suggests that the species had their origin and was first domesticated as early as 5000 B.C. (Bitocchi et al., 2013; Graham & Ranalli, 1997). In addition, data from multi-locus sequence demonstrated that common bean domestication started 8 000 years ago (Mamidi et al., 2011).

Variation in a specific DNA sequence among cultivated species and molecular markers for traits like differences in seed protein phaseolin and isozymes, seed size and color, attests Central and South America as two independent centres of common bean domestication (Angioi et al., 2010; Bitocchi et al., 2013; Chacón et al., 2005; Kaplan, 2003; Singh et al., 1991), which resulted in the Andean gene pools and the Middle American (Acosta-Diaz et al., 2015; Angioi et al., 2010; Brücher, 1988; Kwak & Gepts, 2009). Other evidence supports that the differences between these two gene pools have been observed even before breeding efforts were initiated (Acosta-Diaz et al., 2015; Brücher, 1988). The two gene pools can be distinguished through seed and leaf size. The South American cultivars are large-seeded with wider leaves compared to the Central American varieties (Wortmann & Kaizzi, 1998).

Additional records also indicate that the cultivated species developed from a wild-growing vine ancestor in the highlands of Middle America and the Andes (Singh et al., 1991). The two gene pools have seven races of common bean at Peru, New Granada and Chile (the Andean gene pool), while Durango, Jalisco, Mesoamerica and Guatemala belongs to the Mesoamerican gene pool (Broughton et al., 2003; Singh et al., 1991). The domestication of cultivated common bean introduced multiple agronomic traits such as growth habit (indeterminate and bush types); improvement in leaves, pod and seed size; and decreased pod dehiscence and seed dormancy. A range of attributes and market class preferences, such as seed color, shape and size, is also an outcome of the domestication process (Broughton et al., 2003; Singh et al., 1991), as well as improvement in crop earliness by selecting for photoperiod insensitivity (Singh & Schwartz, 2010). Though the domestication process of the common bean has contributed significant reduction in genetic variability compared to the wild species (Bitocchi et al., 2013; Chacón et al., 2005), both domesticated and wild types are diploid ($2n = 22$), and hybridize readily (Salinas et al., 1988; Singh et al., 1991).

It is believed that *P. vulgaris* was brought to Europe in the 16th century by Spanish and Portuguese explorers (Graham & Ranalli, 1997), and later reached Africa through Portuguese traders, spreading it from the upland areas of Central Africa to the entire continent (Trott et al., 2016). Worldwide, records for bean production indicate 28.9 million metric tons of annual global production, with 4.15 and 2.3 million metric tons alone produced in Latin America

Caribbean (LAC) and Africa, respectively (FAOSTAT 2021). India, occupies the first position in world ranking as the highest producer, with more than 3.8 million metric tons annually. In the Latina America Caribbean, the countries with the highest production are Brazil and Mexico, while in Africa they are Kenya, Tanzania, Uganda and Ethiopia (FAOSTAT 2021).

2.1.2 Botany

Classification and nomenclature

Phaseolus vulgaris L., is the scientific name for common bean which belongs to the Leguminosae family, and taxonomically classified as:

Order: Fabales
Family: Fabaceae
Genus: *Phaseolus* L. Species:
Phaseolus vulgaris L.

There are several common bean synonyms used worldwide such as, French bean, haricot bean, salad bean, snap bean, string bean, frijoles (Spanish), feijão and feijoeiro (Portuguese for the seed and the plant, respectively), and mharagwe (Swahili) (Acosta-Gallegos et al., 2007; Graham & Ranalli, 1997; Singh et al., 1991). The genus *Phaseolus* is vast and has more than 80 domesticated and wild species, with *Phaseolus vulgaris* being the most broadly planted species (Acosta-Diaz et al., 2015; Porch et al., 2013). Some other related species to *Phaseolus vulgaris* are *Phaseolus albescens, Phaseolus coccineus, Phaseolus costaricensis, Phaseolus dumosus, Phaseolus parvifolius* and *Phaseolus persistentus* (Table 2.1.), (Angioi et al., 2010; Broughton et al., 2003; Chacón et al., 2005). In addition to *Phaseolus vulgaris*, other four *Phaseolus* species are commonly cultivated: *Phaseolus dumosus* (year bean), *Phaseolus coccineus* (scarlet runner), *Phaseolus acutifolius* (tepary bean) and *Phaseolus lunatus* (lima bean) (Bitocchi et al., 2013; Douxchamps et al., 2010).

Table 2.1: Species closely linked to *Phaseolus vulgaris*

Species	Geographic location
Phaseolus acutifolius	Western Mexico
Phaseolus albescens	Mexico, southwestern United States
Phaseolus coccineus	Guatemala, Honduras, Mexico
Phaseolus costaricensis	Eastern Costa Rica, western Panama
Phaseolus dumosus	Western Guatemala, Mexico
Phaseolus persistentus	Guatemala
Phaseolus parvifolius	Southwestern United States, Guatemala, Pacific coast of Mexico and Central America

Source: Porch et al. (2013).

2.1.3 Importance and nutritional benefits of common bean

According to a FAO report, total dry bean production was over 15 million metric tons in 2019 (FAO, 2019), although this figure includes other bean species with minor relevance as food legumes. Common bean is commonly cultivated by smallholder farmers in Latin America and Africa, where it is typically planted in marginal lands with no or minimal use of inputs (Beebe et al., 2008). It is considered an inexpensive source of protein and calories for smallholder farmers in developing nations that experience poverty (Beebe et al., 2014). Because in developing nations of Latin America and Africa most beans are grown by smallholder farmers in subsistence agriculture (Broughton et al., 2003), much of the production statistics cannot be documented officially leading to inaccurate global production estimation. In addition, intercropping contributes to an overestimation of the total bean area cultivated, leading to an underestimation of global production (Maredia, 2011).

Common bean comprises of two types: dry and snap beans. Dry beans are typically ready to be harvested when the grains are all mature and entirely dry, while snap beans are mostly used fresh when the pods are unripe and fleshy. Market classes of dry beans are improved based on color, shape, and size of the seed. Broughton et al. (2003) listed more than 59 improved bean market classes that are most preferred by the consumers. Pinto is one well known market class, with medium seed and cream brown mottled.

Geil & Anderson (1994) reported that the consumption of beans has been ongoing many years, playing an important role in food security and nutrition. For human consumption, dry beans should be first processed by cooking in water, although some cultivars can be consumed roasted or after milling into flour (Beebe et al., 2006; Chai et al., 2016). Other uses include immature seed pods of snap beans, that are consumed as vegetables in some areas and straw is used as animals feed (Broughton et al., 2003). Moreover, in some regions with low food quality and food security, leaves of specific selected varieties are consumed as a vegetable (Broughton et al., 2003).

In terms of nutritional content, dry beans contain 21-25% raw protein and are rich in amino acids like lysine, methionine and tryptophan, but are fairly low in sulfur (Hermida et al., 2006). The dry bean protein has high levels of amino acids double that from cereals. Lysine, an essential amino acid that helps the body to absorb calcium, is found 5-6 times more in dry beans compared to cereal grains. Great Northern beans were found to have greater protein content, with about 28.50%, among different cultivated varieties (Rui et al., 2011). The U.S. dietary guidelines, classify dry beans as unique grains that belongs to both vegetables and proteins, the two types of food at the same time.

Bean is also an essential source of bioactive compounds and recent studies found that they are high in antioxidants tocopherols, flavonoids, polyphenols, and phenolics (Boschin & Arnoldi, 2011). Most of these antioxidants, such as flavonoids, can resist heating during the food processing without losing their nutritional properties (KON, 1979), thus a health advantage of bean consumption is associated with the bioactive compounds (Cardador-Martínez et al., 2002). In isolated compounds from *P. vulgaris*, polyphenols are present mostly as phenolic acids and flavonoids (Díaz-Batella et al., 2006). The highest content of polyphenols is found in the greatly pigmented and dark bean varieties, predominately in the hulls or seed coats (Oomah et al., 2008). The seed coats of *P. vulgaris* are rich in anthocyanins, flavonols, flavonoids, and tannins. The presence of these polyphenols in the seed coat is partly responsible for the hard-to-cook phenomenon and the post-harvest seed darkening in *P. vulgaris* (Campos-Vega et al., 2012; Marles et al., 2008). Phenolic compounds reported to have been discovered are ferulic acid, sinapic acid, vanillic acid, caffeic acid, p-acid, p-hydroxybenzoic acid, syringic acid, chlorogenic acid, gallic acid and vanillin (Xu and Chang, 2009; Espinosa et al., 2006). These compounds exist in various amounts. Kaempferol, often linked with O- and C-glycosidic (the most common flavonol), red beans, and pinto beans are renowned for bearing large amounts of this flavonol (14– 209 and 148 mg/kg, respectively), much more than grey or black beans (20 mg/kg) (Díaz-Batella et al., 2006). Quercetin, is reportedly present is in grey (7.9 mg/ kg), cream-red (6.7– 9.4 mg/kg), and black beans (9.7– 23.5 mg/kg) (Díaz-Batella et al., 2006). Quite a number of phytochemical investigations have been reported on varieties of *Phaseolus vulgaris*, including the black bean variety. Available literature showed that aqueous extract of black bean is rich in phenolic, tryptophan, and omega-3 fatty acids (Deshpande and Cheryan 1986). Some known polyphenols, such as gallic acid, chlorogenic acid, epicatechin, myricetin, formononetin, caffeic acid, and kaempferol have also been discovered (Ombraet et al., 2016). Furthermore, Ombraet et al. (2016), reported the presence of vitamins B1, B2, and C in both fresh and dry seeds of the black turtle bean, with Vitamin A found to be present in only the fresh seeds. Quercetin 3-O-glucoside was identified as the primary flavonoid in seed coats of black bean, while the most abundant saponin was discovered to be soyasaponin A and B (Chavez-Santoscoy et al., 2014). Chavez-Santoscoy et al. (2014) reported the presence of myricetin 3-O-glucoside, kaempferol 3-O-glucoside, and Phaseoside.

Many authors have shown that *Phaseolus vulgaris* contains caffeic acid, gallic acid, ferulic acid, coumestrol daidzein, catechuic acid, equol, delphinine, genistein, robinin, phaseolin, malvidin, petunidin, brassinosteroids, proanthocyanidins, naringenin, Protocatechuic acid, Sinapyl aldaric, galactomannans, hemagglutinins, lectins, phaseolamin, phytic acid, and phytohemagglutinin (Bianco et al., 2015; Curl et al.,1988). Yoshikawa et al. (1997) reportedly isolated some saponnins, i.e. Sandosaponins A and B, soyasaponnins I and V, dehydrosoyasaponnins I, soyasapogenol B and E, and sandosapogenol.

Phaseolus vulgaris, especially black bean seed has been used by some families in western Cameroon in the management of sickle cell disease (Kotue et al., 2016). It has also been documented to have been used in traditional medicine for various ailments. Some of its medicinal properties include anticancer, antidepressant, anti-leukemia, antibacterial, antidiabetic, antiviral, mutagenic, and hypoglycemic characteristics (Duke et al., 2002; González et al., 1990).

The carbohydrate content accounts for 60-65%, which is mainly composed of starch with small quantities of monosaccharides and disaccharides. In cooked bean, carbohydrate ranges from 3 to 7 percent, and are found in the form of fiber and typically composed of cellulose and hemicellulose, the two organic compounds (Geil & Anderson, 1994). In cultivated bean, the levels of fats are very low, being 16 % of saturated and 84% of unsaturated fat, and are cholesterol free. The bean mineral content is very high with a single cup serving of cooked beans giving 29% of needed amount of iron for females and 55% for males, plus 20-25% of phosphorus, magnesium, manganese, potassium, and copper, and 10% of calcium and zinc, which are essential elements for health.

Thus, regular consumption of beans is beneficial for humans, reducing the risk of various diseases such as coronary heart disease, diabetes mellitus, obesity, and cancer (Geil & Anderson, 1994). It has been shown that a diet based on regular bean consumption, significantly lowered the concentration of serum lipid, preventing coronary heart disease in human (Jang et al., 2001). Additionally, beans are digested very slowly because of low glycemic index, the production of blood glucose tends to decrease as result of good insulin responses; therefore, patients suffering from diabetes are advised to include in their daily meals at least a small portion of cooked beans. When beans are consumed regularly in adequate amounts, the risk of developing breast and colon cancer is reduced due to the substantial quantity of antioxidant activity which are found in the phenolic compounds of the seed coatin dry bean (Beninger & Hosfield, 2003). Beans are also a good source of folic acid, an essential element especially for women who are pregnant or attempting to be pregnant, since it can help to prevent some major defects of the baby's brain (neural tube defects) when levels of folic acid are low during pregnancy (Gupta & Gupta, 2004).

Beans have also some properties that reduce the feeling of hunger by increasing the sensations of satiation. Therefore, they can be used as nutritional supplement for weight loss or weight maintenance. The scientific confirmation of bean consumption as a means of weight control (Udani & Singh, 2007), and its potential in preventing diseases, such as decreasing colon carcinogenesis (Hangen & Bennink, 2002), have contributed to the demand of bean utilization in commercial food purposes.

2.1.4 Characterization of common bean (*Phaseolus vulgaris L.*)

Cultivated *Phaseolus vulgaris* has a root system based on the axial root with lateral roots normally located in the first 15 cm of the soil, and are generally colonized by *Rhizobium* bacteria that usually results in uneven root nodules (Graham & Ranalli, 1997; Trott et al., 2016). The stems are typically pubescent and depending on genotype, the length and density of the hairs have great variability, and on the younger section of the stem, the hairs are always short and hooked (Chacón et al., 2005; Singh et al., 1991). The stem hairs are an important trait that contributes to disease and insect control. Studies have shown that the hairs stop the multiplication of fungal spores by decreasing secondary inoculum and physically injure the insects resulting in reduced predation (Araus et al., 2008; Mmbaga, 1992). When the growing environment is warm enough to enable a semi-perennial growth habit, the stems of wild *Phaseolus vulgaris* grow up to 1.5 cm diameter and may built-up a live external layer (Gentry, 1969).

The leaves of cultivated *Phaseolus vulgaris* are trifoliate and alternated on the stems, entirely or less pubescent, with 8-15 cm length by 5-10 cm width and small stipules (Graham & Ranalli, 1997; Trott et al., 2016). Depending on the species, bean leaflet shape differs among genotypes, but typically they have wide bases and pointed tips (Singh et al., 1991). Flowers are loaded on axillary racemes, with a variety of colors depending on the cultivar. The most common color are white, pink or violet. The bisexual flowers are wrecked, and the wrack end up in a whorl, with one to two turns (Graham & Ranalli, 1997). Bean seed pods range from 8 to 20 cm long by 1 to 2 cm width with more than 12 seeds per pod. However, most varieties have 4 to 6 seeds with variable colors depending on the cultivar and seed size varying from 150-900 g per 1,000 seeds (Lichtfouse et al., 2009).

Wild *Phaseolus vulgaris* are different from the domesticated types in various traits. The wild species plants have shorter main stems, and are indeterminate climbers when compared to domesticated cultivars that have abundant main stem branches with fewer nodes (Acosta-Diaz et al., 2015; Brücher, 1988). An interweaved growth habit supports plants to foremost compete for sunlight with forest vegetation than a shrubby determinate habit (Kwak et al., 2012). Wild species also generate abundant flowers, pods and seeds. The pods and seeds are smaller than the domesticated species, and the pods are explosively dehiscent due to the slit near the pedicel (Acosta-Diaz et al., 2015; Brücher, 1988). In addition, the flowering period of wild species is much longer than the cultivated species (Brücher, 1988). Wild species are also physiologically different from the cultivated species as they show higher rates of nitrogen use efficiency and CO_2 exchange than the cultivated landraces (Porch et al., 2013).

2.1.5 Common bean production constraints

Common bean originated in subtropical and temperate climates, with distinct wet and dry seasons, hence are more adapted and perform well in areas with moderate rainfall and cannot tolerate dry soil or areas with excessive rainfall (Beebe et al., 2014). Bean plants do not withstand elevations above 3 000 meters above sea level or frost, but they can be cultivated as annuals plants in temperate climates and as annuals or short-lived perennials in tropical climates (Graham & Ranalli, 1997). Heat stress contributes to flower abscission, while low temperatures delay pod formation resulting in empty pods (Broughton et al., 2003).

Regarding to soil proprieties, common bean have preferences to sandy clay or sandy loam soils with a good drainage, equilibrated fertility and mild acidity with pH between 5.8 and 6.5 (Broughton et al., 2003). The use of inputs for bean production also varies and depends on agroecological conditions of the region. However, they can be successfully grown without irrigation in areas with precipitation varying from 250 mm to over 400 mm throughout the growing season (Broughton et al., 2003). Commercial farms in developed nations and in arid subtropical use irrigation to supplement natural rainfall (Trott et al., 2016). In resource-poor countries, the beans are commonly cultivated without application of mineral fertilizers or manure, while in developed nations fertilizers are applied routinely for soil amendment.

In the most developing countries, bean yields are severely affected by biotic and abiotic stress such as diseases, insect pests, low soil fertility and drought, causing serious losses (Broughton et al., 2003). Common bean is affected by multiple insect pests depending on the geographic location, however, a greater variety of arthropods such aphids, beetles, caterpillars, leafhoppers, whiteflies, mites and thrips has been reported to cause economic damage worldwide (Trott et al., 2016). Beans are damaged less after-harvest as the raw dry beans are toxic to rodents and other mammals and chemical pesticides are mostly recommended in commercial production than in smallholder farming system (Singh & Schwartz, 2010).

Low soil phosphorus is a key limitation to common bean cultivation mainly in smallholder farming system in the tropics and subtropics with low inputs use, and soil acidity (Beebe et al., 2006, 2014; Graham & Vance, 2003; Porch et al., 2013). Furthermore, in developing countries the majority of smallholder farmers grow beans as a low-input crop, directing all their scarce resources to other crops, such as cereals (Maredia, 2011). Consequently, the yields of bean in developed countries are much higher compared to those in developing nations (Porch et al., 2013).

Improvement of traits for heat stress and drought resistance contributed significantly to increased bean yields in most of the areas where beans are cultivated (Porch et al., 2013). However, development of bean varieties that can cope with abiotic and biotic constraints is affected by the unavailability of genes for stress resistance. Identification of new cultivars appears to be even more challenging for the breeders as they have to satisfy consumer desires for specific bean size, color, taste, and other relevant traits (Singh & Schwartz, 2010). To overcome these constraints, tepary bean, *Phaseolus acutifolius*, has been reported to be a promising source of genes to improve tolerance to abiotic stresses, including heat and drought stresses and high soil salinity (Porch et al., 2013).

2.2 Plant responses to limited water and phosphorus

The relevant concepts related to plant response to limited water and phosphorus is crucial to the understanding of plant mechanisms and responses to adaptations for double stress (moisture and P limitations). These concepts will help either the breeder or physiologist to seek for traits that preserve productivity and predict better plant growth in non-compromised ecosystems. The simultaneous effect of limited moisture and phosphorus for regular plant function, have permitted some plant species to develop a series of adaptation mechanisms that allow better phosphorus uptake, enough to keep imperative functions within a broad range, at least until a certain amount of phosphorus or water stress is exceeded (Suárez et al., 2020; York et al., 2015). Variation in plant growth and the quantity of phosphorus taken up under double stress (water and phosphorus) is highly dependent on the level of soil dryness or field capacity, and the intensity and duration of the drought event, plant uptake ability, root system plasticity, presence and intensity of hydraulic redistribution, phosphorus remobilization ability, and soil characteristics such as water holding capacity, phosphorus diffusion, mineralization and fixation rates, and the intervention of arbuscular mycorrhizal symbioses (Liao et al., 2001). While soil is drying, both phosphorus fertilization and association with arbuscular mycorrhizal fungi may accelerate performance under drought, until a critical level of water stress is reached.

2.2.1 Drought reduces phosphorus mobility and uptake by plants

Phosphorus in soil is mostly found in many interactive, dynamic reservoirs and can appear in organic or inorganic form (Beebe et al., 2014; Hinsinger, 2001). Depending on whether it is in organic or inorganic form, phosphorus can appear in soil solutions adsorbed on soil exchange complex or tightly bounded in soil particles. Typically, 20 – 80% of phosphorus in soils is found in organic form, where phytic acid is commonly a major component (Hinsinger, 2001). The rest is found in the inorganic portion that includes several mineral forms of phosphorus. However, when chemical reactions happen in soil, there is an ongoing exchange of phosphorus between organic and inorganic pools, as well as between the soil solution and exchangeable and fixed pools. Plant P uptake is limited by low mobility and availability of phosphorus in the bulk soil. As a result, it is delivered by mass flow in very low amounts between 1 – 5% of demanded phosphorus to the plants, and the proportion that can be used by the growing roots is only half and the rest of the required phosphorus should reach the root system through diffusion (Suriyagoda et al., 2014).

Usually, phosphorus used by plants is retrieved from soil solution which is typically found in the inorganic form. Consequently, this uptake is reduced in dry soils when compared with the moist soil. Therefore, phosphorus uptake in dry soil is very low compared with that in wet soil (Hinsinger, 2001). Another factor that limits the availability of phosphorus in dry soils has to do with its natural occurrence in topsoil zone, which is more affected by drought making plant P uptake limited.

2.2.2 Moisture and phosphorus limitation on shoots

Application of phosphorus in drought conditions increases plant aboveground dry weight and relative growth rate on maize, rice bean (Burman et al., 2009; He et al., 2004; Premachandra et al., 1990), and translocation of phosphorus to the soybean seed (Jin et al., 2006). Wilson et al. (1968) found that using annual legumes, the stimulus of growth given by P under dry conditions does not imply that P improve drought resistance. Phosphorus resulted in rapid growth of root and shoot during the first part of the drying period when moisture was still available (Wilson et al., 1968). However, it still doubtful whether plants with P fertilization continue to grow more in drier conditions than P-deficient plants.

Jupp & Newman (1987) also found that P becomes unavailable and P uptake stops after soil dryness, although recovery was seen 2–3 weeks after rewetting, with 40% reduction in the final amount of P taken compared to the well irrigated. When P treatments were compared, the recovery after re-watering was high in P-fertilized than in non-fertilized plants (Burman et al., 2009; Singh et al., 1997). The observed decrease in P uptake and growth under drying soil is a negative balance between increased capacity of plant P-uptake (Matzner & Richards, 1996) and decreased P uptake due to low P diffusion to the root system (Jupp & Newman, 1987), lack of plant adaptation to enhance phosphorus absorption, and death of the roots responsible for phosphorus uptake at the topsoil (Suriyagoda et al., 2011). Therefore, differences in dry weight and quantity of phosphorus utilized among species under double stress (water and phosphorus), greatly depend on the level of soil drying and wetting, changes in dynamics of plant uptake and the severity and length of drought period, as well as soil properties (Suriyagoda et al., 2011).

There is limited information on whether plants with adequate phosphorus would continue growing under drought stress for relatively long period compared with that of P-deficient plants. However, some studies have reported that even a mild drought stress reduced phosphorus assimilation in perennial rye grass, due to unavailability of phosphorus once the soil dried (Dipp et al., 2017; Hu et al., 2014). Although some recovery occurred 2–3 weeks after supplementing with irrigation, 40% decrease in the final amount of phosphorus utilized was observed compared with the control treatment (Muñoz-Perea et al., 2007).

2.2.3 Water-use efficiency

In semi- and arid tropics, where water is the most limiting input to crop production, crop water requirement is an important factor. Crops that use less water are becoming increasingly important as one of the strategies to increase food production under conditions of water scarcity.

Water use efficiency (WUE), or "more crop per drop” is the ratio between grain yield and transpired water and it is considered as an important component of drought resistance in different crops (Sinclair, 2012; Vadez et al., 2014). It has been reported that traits related with conserving water at vegetative stage (lower leaf conductance, smaller leaf canopy), would make more water available for reproductive growth and grain filling, resulting in better grain yield under terminal drought stress conditions (Zaman-Allah et al., 2011; Araújo et al., 2015). Increased WUE reduces the rate of transpiration and crop water use, processes that are crucial for carbon assimilation, biomass production and yield (Blum, 2009; Sinclair, 2012). However, the reduction in water use is generally achieved by plant traits and environmental responses that could also reduce yield potential (Blum, 2005).

Phosphorus fertilization under limited soil moisture increased plant water-use efficiency (WUE) in common bean (Polania et al., 2016; Wissuwa et al., 2016). This increased WUE was attributed to the higher rates of photosynthesis than transpiration (Singh & Sale, 2000; Wilson et al., 1968). For this reason, P-fertilized plants can dry under limited soil moisture to a greater extent, but totally recover from the water-stress symptoms after re-watering, than low-P plants (Singh & Sale, 2000; Klaedtke, 2012). This could be due to water stressed plants growing very slowly in clay soils, enabling them to adjust their water use efficiency to limited available soil water and due to the different responses of photosynthesis, respiration, and transpiration to the slower imposition of water stress.

2.2.4 Leaf water status

Highly fertilized phosphorus plants tend to have higher rates of leaf water potentials under low water conditions when compared to low fertilized phosphorus plants (Polania et al., 2016). Typically, the leaf water potential for a given soil water potential is constantly high for plants in high phosphorus environments (Polania et al., 2016). This high leaf water potential is attributed to the extended activity of aquaporins and higher capability of roots to extract water (i.e., higher hydraulic conductivity (York et al., 2013), greater efficiency of water-use due to increased stomatal and mesophyll resistance (Polania et al., 2016), and lower osmotic potential as a result of solute accumulation and greater cell-wall loosening through the activity of expansion increasing root elongation (Hinsinger, 2001; Suriyagoda et al., 2014).

2.2.5 Effect of phosphorus remobilization

Phosphorus (P) deficiency is a major nutritional constraint limiting crop yields in most of arable land across the globe (Vance et al., 2003). Low use efficiency of applied P in both alkaline and acid soils associated with higher P use in the developed nations had increased the concerns on environmental degradation and depletion of non-renewable rock P reserves (Vance et al., 2003). The situation impels the invention and adoption of strategies to enhance P acquisition and use by plants for sustainable P management. Identification of crop genotypes to cope with root environment deficient in bio-available P is one of the most viable strategies (Aziz et al., 2006, 2011) as genetic differences for P acquisition and utilization have been reported amongst cultivars of several crops (Akhtar et al., 2006; Yaseen and Malhi, 2009; Aziz et al., 2011). Phosphorus utilization within the plants is related to the amount of dry matter produced per unit amount of P uptake, and varies substantially amongst crop cultivars (Aziz et al., 2005, 2006). These variations may be due to differential nutrient uptake characteristics or distribution patterns within the plants because plants grown under nutrient deficiency have different nutrient uptake and distribution pattern compared to those grown under adequate nutrient supply (Akhtar et al., 2006). Once P has entered the roots, for its efficient use, it should effectively reach the metabolically active sites. Plants generally translocate more P from roots to shoots under P deficiency than those grown with adequate P supply hence translocation and re-translocation of P can be up-regulated. Nevertheless relatively less proportions of total P was retained by roots and stems than leaves in P efficient crop genotypes (Clarkson and Scattergood, 1982).

Remobilization of phosphorus can be achieved from senesced tissues of plants grown under limited phosphorus and drought, and has a fundamental role during the grain filling of the crops (Rodriguez & Goudriaan, 1995; Wardlaw, 1987) and in keeping the functionality of active plant cells, especially for those that have been accustomed to low phosphorus environments (Denton et al., 2007; He et al., 2011; Ryan & Kirkegaard, 2012). Increased dry matter production under P deficient conditions by plants may be related to increased rate of its translocation to leaves (Marschner, 1995). Hence, genotypes capable of efficiently remobilize P from stem to younger leaves may perform better under P deficient environment (Gerloff, 1976). Remobilization of P, stored in older leaves, to metabolically active organs may supplement its restricted supply to shoot under P deficient conditions. Genetic diversity in P mobilization may exist among cultivars nonetheless it is rarely explored. Phosphorus remobilization in plants also induced under low nutrient status.

Reports from experiments that were conducted in forest, showed a decrease in soil moisture content to 15%, six years after drought stress imposition, and increased P concentration in leaves by 18%, but stems and roots had their P concentration reduced by 30.9 and 39.8%, respectively (Sardans & Peñuelas, 2007). However, in non-stressed plants, remobilization of phosphorus from stem tissues and roots to leaf was not seen (Sardans & Peñuelas, 2007) and phosphorus resorption from senesced leaves under drought stress was lesser than that in non-stressed treatment (Sardans & Peñuelas, 2007, 2004). Low phosphorus concentration in stems coupled with a decrease in biomass growth led to an overall reduction (one-third approximately) of the total phosphorus content in aboveground biomass. This is supposed to be an adaptive response of perennials crops to limited water and phosphorus deficiency.

Another study on wheat concluded that phosphorus remobilization was incited earlier during growth stages, and the control treatment (high P and non-stressed) remobilized greater amount compared to stressed treatment (low-P and water stress), and this was related to an earlier and effective sink formation for high-P and non-stressed plants (Rodriguez & Goudriaan, 1995). While movement of phosphorus in soil and plant uptake are restricted by drought, P remobilization and reabsorption become highly fundamental in determining the quality and quantity of crop yields, and keeping ecosystem equilibrated in limited water and phosphorus environments (Suriyagoda et al., 2014). Gerloff (1987) observed efficient translocation of absorbed P within plant to metabolically active organs under P deficiency. Hence, differences in redistribution and mobilization of nutrients under P deficiency among species may cause variations in nutrient utilization (Marschner, 1995). Such variations may be exploited to develop crop cultivars efficient in internal P utilization.

2.2.6 Effect of drought and low phosphorus on roots performance

Drought is defined as inadequacy of water availability in quantity and distribution in space and period during plant growth, development and production (White & Juan, 1991; Graham and

Ranalli, 1997). According to Ludlow et al. (1990) indicated that there are three types of drought stress; early drought, intermittent drought and terminal or late drought. Terminal drought occurs when rainfall is too minimal to sustain plant growth or stops at the productive stage, and is frequent in areas that are characterised with bi-modal type of rainfall which is common in the tropical regions (Schneider et al., 1997). Intermittent drought is characterised with periodic drought spells and is common in the sub-tropical regions (Urrea et al., 2009). The effect of drought on crop production are severe, and greatly affects over 60 % of common bean world production (White & Juan, 1991), and mostly occur in developing countries where resource poor farmers cannot afford irrigation facilities (Broughton et al., 2003). Climate change has greatly aggravated the impacts of drought on crop productivity, and estimates indicate that an increase in average temperature of about 4 °C or more by end of this century, the decrease in yields for some of the most important crops in sub-Saharan Africa would be 19 % and 47 % for maize and beans respectively (Thornton et al., 2014).

Apart from drought, it is also reported that in Eastern and Southern Africa, 65 % to 80 % of the area under common bean cultivation, affected by low soil phosphorous (Namayanja et al., 2014). Further to that, Henao and Baanante (2006) reported huge losses of 9 kilograms per hectare per year in cultivated soils of East Africa. Soils under bean production are considered deficient if they contain less than 15 mg/kg of available soil phosphorus (Henry et al. 2010), and usually are acidic, characterized by poor soil management practices for soil amendments to replenish soil-P (Henao & Baanante, 2006). Continuous mono-cropping system contribute to low availability of soil-P, because most smallholder farmers have small land and cannot afford to pay for expensive inorganic phosphate fertilizers. Hence, there is need to identify genotypes with tolerance and develop more improved bean varieties that can perform relatively well under drought conditions or less used of inorganic phosphate fertilisers.

Studies on root system for both drought and low phosphorus have received less attention and are very limited when compared to studies of above ground plant traits (Ho et al., 2005; Wasaya et al., 2018). Plants grown in low phosphorus and drought typically have lower shoot: root ratios due to a higher inhibition of shoot growth than root growth compared to those grown in adequate phosphorus and well-watered conditions (Ho et al., 2005; Lynch & Brown, 2012). Also, studies have shown that root architecture, a shape of root crown in space and time contributes to crop adaptation to low phosphorus and limited water environments (Miguel et al., 2015; Burridge et al., 2016). Most of these crop adaptations are related to improvements such as: a greater portion of roots in the topsoil than in deep horizons; root foraging in nutrient-rich patches; greater allocation of fine roots (i.e., roots with a high specific root length (cm g^{-1} root dry weight), and the development of long, thin root hairs (Miller et al., 2003; Molina, 2001; Rangarajan et al., 2018). However, such adaptation traits are at the expense of the acquisition of deep soil resources such as water (Lynch & Brown, 2008; Lynch, 2018). Therefore, when the drought prolongs, roots in the soil surface die, resulting in reduced phosphorus response (Aroca, 2013).

2.3 Summary

In *Phaseolus vulgaris,* root phenes that change soil foraging depth are important for plant adaptation to phosphorus and water limitation. The simultaneous effect of limited moisture and phosphorus for regular plant function have permitted some plant species to develop adaptation mechanisms. Plant growth in drought stress can be improved through application of phosphorus fertilizer in low phosphorus soils. Increase in growth and drought resistance is also obtained through a set of biochemical, physiological, and structural changes in shoot and root tissues during the soil dryness process which result in better plant phosphorus nutrition and better water absorption. The effects of phosphorus fertilization on growth improvement and drought resistance, could alternatively be achieved through the incorporation of the arbuscular mycorrhizal fungus. The domestication of cultivated common bean has introduced several agronomically useful traits that have contributed significantly in reducing genetic diversity compared to the wild species. There is limited information on whether plants with adequate phosphorus would continue growing under drought stress for relatively long period compared with that of P-deficient plants. However, some studies have reported that even a mild drought stress reduced phosphorus assimilation in other crops such as perennial rye grass, due to unavailability of phosphorus once the soil dried. Studies on root system for both drought and low phosphorus have received less attention and are very limited when compared to studies of above ground plant traits. Therefore, the identification of traits that enhance the use of nutrient resources in climate-induced stress conditions would be beneficial in developing common bean genotypes that can achieve greater yields with the use of minimum resources.

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3. CHAPTER THREE

Shoot traits associated with drought stress tolerance in common beans (*Phaseolus vulgar L.)*

Abstract

Common bean (*Phaseolus vulgaris* L.) is widely considered as a staple food in Africa and Latin America where edaphic stress especially low fertility and drought are major constraints causing low yield. Two studies (pot and field experiments) were conducted at Agricultural Research Institute of Mozambique - Chókwè Research Station, to determine the effect of water stress at different growth stages on shoot biomass, grain yield and yield components of bean genotypes. The pot experiment was done using a randomized complete block design in an 8 x 2 factorial arrangement with four replications, consisting of eight common bean cultivars (factor 1) under two water treatment (water stress and non-stressed – factor 2). The field experiment was arranged as a split plot design with four replications, with water levels as the main plot factor and 24 genotypes as the sub-plots. In well-watered treatments soil moisture level was maintained by supplying water at field capacity until the crop had reached flowering stage, while in the drought treatments, soil moisture was maintained at field capacity from planting up to 23 days after emergence and thereafter irrigation was done whenever the soil moisture was depleted to less than 30% field capacity until the crop had reached flowering stage. Intermittent drought conditions were simulated. Water stress reduced leaf relative water content, leaf water potential and all growth parameters in the pot experiment by decreasing shoot biomass (47%), leaflet growth rates (49%) and number of leaflets (53%). In spite of significant reduction, these variables showed significant, positive correlation with final grain yield, and can be recommended for early selection of the genotypes grown under limited water. In the field experiment, drought stress had a significant effect ($p<0.05$) on yield components. Genotypes SEF 16, SX 14825-7-1, TARS MST-1, SEN 52, BRT103-182, FBN1211-66, IBC 301-204, SER 125 and MHR 311-17 were the most adapted and showed the best yield performances under drought stress, therefore, can be used in breeding programs for improvement of new cultivars suited for drought prone areas.

Key words: *drought stress, common bean, growth stage, phenology, yield component.*

3.1 Introduction

Common bean (*Phaseolus vulgaris* L.) is a cheap source of proteins, carbohydrates, amino acids and vitamins in diets of people and livestock mostly in developing countries (Hummel et al., 2018; Ramirez-vallejo & Kelly, 2014). In this region, it is estimated that bean meets more than 50% of dietary protein required for the households (Broughton et al., 2003). Besides being a cheap source of protein and other nutrients, it is affordable to smallholder farmers in regions with endemic poverty (Arruda et al., 2018). Beans are mostly grown by smallholder farmers in marginal lands, with less inputs (Broughton et al., 2003, Beebe et al., 2013) and where crop yields are mostly affected by series of abiotic factors, especially water deficit (Androcioli et al., 2020; Assefa et al., 2019). The growing period of common bean can go up to 80 days, requiring between 350-500 mm of water depending on the climate, soil depth and genotype (Beebe et al., 2013). It is predicted that water scarcity in the world affects around 60% of cultivated bean areas with prolonged drought periods (Beebe et al., 2013). Drought stress is one of the leading factors that reduces yields, disrupting the security of production systems in developing countries (Asfaw & Blair, 2012).

According to Cortés & Blair (2018) and Hoyos-Villegas et al. (2017), the effects of water deficit on common bean have been widely studied. Severe drought is defined by the stress frequency, duration, and intensity and through the crop development stage where it occurs (Arruda et al., 2018; Chai et al., 2016). Water deficit decreases leaf stomatal conductance and root foraging, reducing water and nutrient assimilation and photosynthetic plant activity. In addition, drought stress can induce flower abortion, pod drop, reduce biological nitrogen fixation efficiency, and consequently decrease seed yield (Asfaw & Blair, 2012; Polania et al., 2016). The effects of drought are mostly in developing countries where resource poor farmers cannot afford installation and operating costs for irrigation facilities (Broughton *et al*., 2003; Androcioli et al., 2020; Hageman et al., 2019). It is a major constraint for bean production especially under dry land conditions and low inputs (Hummel et al., 2018; Manjeru et al., 2007), causing yield reductions of between 10 to 100%.

If the demanding stage of crop growth such as flowering and pod filling occur during the periods of water deficit, the yield reduction can be significant (Kazai et al., 2019). According to Androcioli et al. (2020) and Arruda et al. (2019), drought tolerance is a quantitative trait that is controlled by more than one gene and is highly influenced by environmental conditions. Physiological traits such as stomatal density and conductance, transpiration and photosynthesis rates, and relative leaf water content are well related to drought tolerance (Gonçalves et al., 2019; Rosales et al., 2013).

The availability of water is also influenced by climate change due to alterations in rainfall patterns that reduces the availability and supply of water (Androcioli et al., 2020; Cortés & Blair, 2018). The decrease in rainfall affects available soil moisture and has negative consequences on the roots, the main organs responsible to maintain the crop growth under drought conditions, and consequently crop productivity. Plants that have deeper and vigorous root systems draw water from deeper soil horizons avoiding drought stress (Androcioli et al., 2020; Arruda et al., 2018; Nuñez Barrios et al., 2005). A good plant root system is thus important for adaptation to different drought stress conditions.

Improvement of abiotic stress tolerance in crop plants has been a challenge for plant breeders due to difficulties in simulating correct environmental conditions (Wasaya et al., 2018). However, the development of common bean cultivars adapted to drought stress environments through breeding is a useful strategy to adapt to the challenge of climate change by increasing production and productivity in marginal lands. Implementation of new methods to speed up and increase efficiency in drought tolerance breeding programs, such as the use of biotechnology and the expansion of the selection criteria by recognizing morphological and physiological traits of the plants that are significantly related to performance would be helpful and quick in developing bean cultivars adapted to drought conditions. However, it is expensive for developing nations due to infrastructure investment needed (lab and equipment). A combination of parental lines with complementary traits and additive gene action for resilience traits would increase the potential of selecting for drought tolerance and the germplasm could be assessed more comprehensively than purely testing for yield (Duan et al., 2018; Nuñez Barrios et al., 2005).

Genetic improvement programs to improve adaptation to either drought or heat stress often select the best genotypes based on grain yield (Beebe et al., 2008; Rosales et al., 2012; Soltani et al., 2019). Studies on hybridization of interracial bean cultivars found that they had superior yield, particularly in crosses between Mesoamerican with Durango or Jalisco races (Suárez et al., 2020). Aroca (2013) and Broughton et al. (2003) found that yield can be increased under drought conditions by photosynthate remobilization and biomass translocation, which implies that enhanced yield can be achieved in drought environments. A few bean genotypes that are tolerant to high temperature have been identified (Beebe et al., 2008; Beebe, 2012; Rao et al., 2009; Omae et al., 2012) and these have been developed from interspecific crosses of common bean (*Phaseolus vulgaris*), with other *Phaseolus* species (*P. acutifolius, P. coccineus and P. dumosus*). These species originated from North and Central America are now considered as a valuable genetic resource for improving the yield and stress tolerance of common bean (Bitocchi et al., 2017). Tepary bean originated in the Sonoran Desert (north- western Mexico and the southwestern USA), selected and cultivated in this region for hundreds of years (Traub et al., 2018), showed a greater tolerance to both heat and drought stress. Tepary bean will be a model for drought and heat tolerance, also as a source of genes for tolerance to drought and heat stress (Beebe et al., 2013; Rao et al., 2013; Traub et al., 2018). Drought resistance mechanisms of tepary bean include deep rooting to prevent dehydration by accessing water reserves from deeper soil layers (Blair et al., 2002; Butare et al., 2011), small leaves to reduce water consumption, and stomatal control but not with osmotic adjustment (Mohamed et al., 2005; Beebe et al., 2013). Heat tolerance mechanisms of tepary bean include an advantage in photosynthesis and stomatal control (Porch et al., 2009; Traub et al., 2018, 2017), dehydration avoidance (Mohamed et al., 2002), greater pollen viability (Muñoz et al., 2006) and yield stability (Miklas et al., 1994; Porch, 2006). The adverse effects of heat stress on common bean can be mitigated by developing heat-tolerant genotypes (Wahid et al., 2007), therefore, it is necessary to understand the phenological and agronomic responses of plants to high temperatures for developing possible breeding strategies to improve crop tolerance (Omae et al., 2012; Beebe, 2012).

Furthermore, new breeding methods have been proposed by CIAT and PABRA to breed for seed yield and tolerance to biotic and abiotic stresses, considering grain preference type (market class). This resulted in CIAT and PABRA releasing a vast number of bean cultivars in Africa and Latin America (Buruchara et al., 2011). However, the complexity of breeding for improved drought tolerance involves multiples features of resistance mechanisms and are quantitatively inherited and greatly affected by environments (Eissenstat et al., 2000; Poschenrieder et al., 2017; Rosales et al., 2013). Therefore, the main objectives of this study were to (i) identify specific physiological traits that can be used as selection criteria for drought tolerance improvement in common bean, (ii) to evaluate the relationship between shoot biomass and water use efficiency using leaf water potential and (iii) to identify best genotypes with desirable traits for drought tolerance and good yield.

3.2 Materials and Methods

3.2.1 Experimental site and research approach

Experiments were conducted in two environments, which are; a) pot experiments conducted in 30 cm x 32 cm containers filled with soil media in a stratified water system. These were placed in the shelter at Agriculture Research Institute of Mozambique (IIAM) – Chókwè Research Station, Gaza province in the Republic of Mozambique, and b) a drought study conducted in the field at Chókwè Research Station facilities in Chókwè located at 24° 30'S, 77° latitude, 33° 00'E longitude and an altitude of 33 m above sea level. Details of experiment establishment are given in the following sections.

3.2.2 Pot experiment – Chókwè Research Station

Four pot experiments were conducted during the dry season – April to September in 2017, 2018 and 2019, at Chókwè Research station. A total of eight genotypes characterized as drought-tolerant and drought-sensitive contrasting in root architecture and differing in primary root branching were grown in a system of stratified water (Ho et al., 2005) as shown in (Figure 3.1). Seeds were sterilized in 10% NaOCl for 3 minutes, rinsed twice with distilled water, pre-germinated in rolls of brown germination paper, soaked in 0.5 mM CaSO4, and placed in a dark growth chamber for 72 hours at room temperature of 26°C. After germination, four seedlings were transferred to pots, planted at a depth of 4 cm in pots of 30 cm diameter by 36 cm height, which were filled with sandy soil. The plants were thinned at the stage V3 (when the first fully trifoliate leaves expanded) to leave one plant per pot. The containers were separated into two layers, 0 to 8 cm and 8 to 32 cm depths by a ring. The fertilization in each pot was amended using solid fertilizer and mixed thoroughly with the soil before

planting and it contained 3 g urea, 20 g KNO3, and 8 g micromax granular micronutrients (6.0% Ca, 3.0% Mg, 12.0% S; 0.10% B 1.0% Cu, 17.0% Fe, 2.5% Mn, 0.05% Mo and 1.0% Zn), obtained from Omnia.

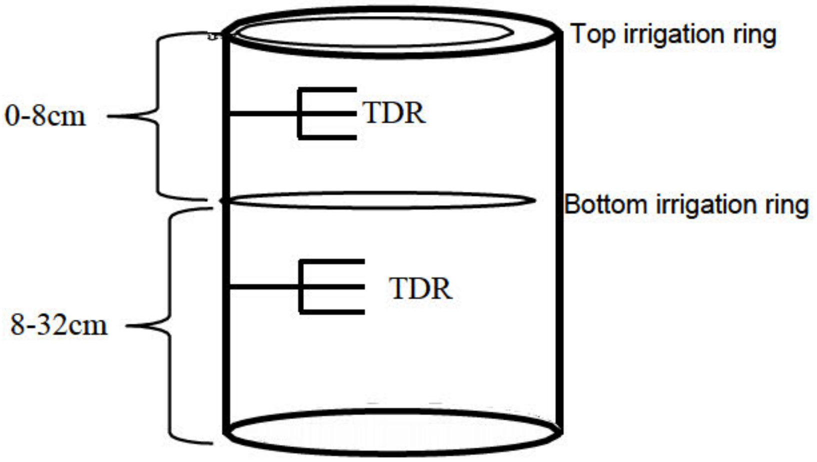

Figure 3.1: Stratified water pot system. Top 0-8 cm and bottom 8-32 cm, horizons separated by an irrigation ring. Figure was recreated from (Ho et al., 2005), and is not drawn to scale.

The water supply in the system was maintained by placing two irrigation rings in each pot, one at 8 cm deep and another on the top of the pot, which permitted a separate irrigation of the two layers using a small water pump. The irrigation frequency depended on the water content in the pots that was monitored by TDR probes. In the bottom layers, the moisture content was maintained at a constant level in all treatments, while in the water stress treatment irrigation was withheld 7 days after planting (DAP). To avoid waterlogging, the top layer was irrigated first followed by watering the bottom layer to the saturation point. The drip ring irrigation used in the experiments (32 cm diameter) supplied 70-80 ml of water in 3 minutes of irrigation, an amount sufficient to maintain adequate growth of the plants.

3.2.3 Field Experiment at IIAM - Chókwè Research Station

Field trials were used to impose drought stress on 24 genotypes by growing the beans in the dry season at IIAM- Chókwè Research Station in the Republic of Mozambique in 2018 and 2019. This site experiences less occurrence of rainfall during the dry season, therefore is considered a potential site for drought studies. During the active crop-growing season the total rainfall was 23.6 mm in 2018 and 14.0 mm in 2019. The maximum and minimum temperature during the crop growth was 31ºC and 22ºC in 2018 and 31ºC and 23ºC in 2019, respectively.

The genotypes were grown under two water regimes (with and without water deficit) to test well-watered (control) and drought stress treatment. After planting, the entire field was furrow irrigated approximately 30 mm of water per time irrigation. Drought stress treatments under rainfed regime in 2018 and 2019 received irrigation 2 days before planting (DAP) and at 6 and 23 DAP. Irrigation to the drought treatments in both years was withheld at 23 DAP to induce terminal drought stress conditions – less water availability at flowering and physiological maturity stages. The irrigated treatment plots continued to receive furrow irrigation, with a total of 6 irrigations to ensure enough soil moisture for optimum growth and crop development.

3.2.4 Plant material

All common bean (*Phaseolus vulgaris L.*) seeds used in these studies were propagated from seeds originally obtained from the International Center for Tropical Agriculture (CIAT, Cali, Colombia). For the pot studies, 16 elite lines (BFS 81, BIOF 2-106, IBC 301-204, INB- 841, MEN 2207-17, MHN 322-49, SB 787, SB-DT1, SEF 16, SEN 52, SEQ 342-87, SER 125, SJC 730-79, SX14825-7-1, TARS LFR-1, TARS MST-1) characterized as drought-tolerant and drought-sensitive and contrasting in root architecture were selected. The criteria for grouping the elite lines were based on phenotypic characterization of tap root lateral branching density from a previous pilot experiment established in May of 2017. Due to limited space in the shelter where the pot experiment was conducted, eight genotypes were tested at each time. For the field experiments at IIAM-Chókwè Research Station a total of 24 lines comprising of 16 elite lines from the pot experiment plus eight lines previously selected from pilot experiment (Amadeus77, Beniquez, BRT103-182, FBN1211-66, MER 2212-28, MHR 311-17, PR 1217-16, USMR 20) were used. Details of seed color, size and growth habit of the lines used are described in the (Table 3.1).

Table 3.1: Characteristics of bean cultivars evaluated under water-stressed and non-stressed conditions.

Cultivar	Seed color	Seed size	Growth habit	Drought reaction	Heat stress reaction
Amadeus 77	Cream	S		Sensitive	
Beniquez	Cream	S		Tolerant	Tolerant
BFS 81	Red	S	II A	Tolerant	
BIOF 2-106	Red	S		Tolerant	Tolerant
BRT 103-182	Red	S		Tolerant	Tolerant
FBN 1211-66	Red	S	II A	Tolerant	Tolerant
IBC 301-204	Red	S		Tolerant	
INB 841	Brown	S	II A	Tolerant	Tolerant
MEN 2207-17	Black	S		Tolerant	
MER 2212-28	Red	S		Tolerant	Tolerant
MHN 322-49	Red	S	II A	Tolerant	
MHR 311-17	Red	S		Sensitive	Tolerant
PR 1217-16	Red	S		Sensitive	Sensitive
SB 787	Black	S		Tolerant	
SB-DT1	Black	S		Tolerant	
SEF 16	Red	S		Tolerant	Tolerant
SEN 52	Black	S	II A	Tolerant	
SEQ 342-87	Red	S		Tolerant	
SER 125	Red	S	II B	Tolerant	
SJC 730-79	Red	S		Sensitive	
SX 14825-7-1	Cream	S		Tolerant	
TARS LFR-1	Red	S		Sensitive	
TARS MST-1	Red	S		Sensitive	
USMR 20	Cream	S		Tolerant	Tolerant

S – small, maximum 25g/100 seeds; M – medium, between 25 – 40g/100seeds; Type IIA – indeterminate, completely upright; Type IIB – indeterminate, inclined upright.

3.2.5 Experimental design

The experimental design used in the pot experiments was a randomized complete block design in an 8 x 2 factorial scheme, consisting of eight common bean cultivars under two water regimes (i) adequate water in both layers: 0-8 cm and 8-32 cm, and (ii) stratified low water: low water in the top 0-8 cm and adequate water in the bottom 8-32 cm, with four replicates. For the field study, a split plot design with four replications was used, where water levels were the main plot factor and genotypes the sub-plots. The experimental unit consisted of 3 rows of 3.0 m length, 0.6 m inter-row and 0.1 m intra-row spacings. There were buffer plots surrounding the entire field as well as 4.0 m buffer zone separating the irrigated and no-irrigated plots. Weed control was managed manually using hoe and insecticides and fungicides were applied as needed.

3.2.6 Plant measurements

Growth and plant biomass measurements

The following traits were measured; leaf appearance rate and leaf size were taken after 14, 21, and 35 DAP to determine differences in length, width, and number of leaves in the control and drought treatments pots. On the day of harvest, which ranged from 35 to 42 DAP, shoot biomass was collected and stored in a paper bag for shoot biomass weight. Shoot tissues were dried in oven at 60ºC until constant mass and then weighed.

The field study at Chókwè research station focused on phenological data and yield parameters recorded in well-watered and drought treatments. Number of pods per plant, number of seeds per pod, seed yield per m^2 and 100 seed weight were measured.

In the pot experiments, measurements were taken in both the control and drought treatments to assess plant water status. The day before the harvest, which ranged between 33-42 DAP, pre-dawn leaf water potential was measured with a Scholander leaf pressure chamber (Wescor Scientific Inc. M 615 Scholander chamber 0-40 bar) in active leaves in all pots. Measurements were made very early in the morning, before sunrise. On the same day after pre-dawn measurements, relative water content (RWC) was determined in both control and drought treatments, by collecting randomly six leaf discs of 2.54 cm diameter from each plant, and immediately sealed in a plastic bag for fresh weight (FW) determination. Then the discs were immersed in water 18-20 h for turgid weight (TW) measurement and finally dried at 60ºC until constant mass for dry weight (DW). Leaf relative water content was determined by ((FW-DW)/(TW-DW)) *100 (Turner, 1991).

3.2.7 Soil moisture measurements

For the pot experiments, volumetric water content was monitored in both layers (as shown in Figure 3.1) with a portable TDR-100 time-domain reflectometry system (Campbell Scientific Inc., Logan, UT, USA). Forty TDR probes were used in all pots; two were placed in each pot at the top and bottom horizon in the control and drought treatments. Measurements of each TDR probe were taken twice per week, and supplementary irrigation was made if the volumetric water content (VWC) in control pots was below 15% and 30% at the top and bottom horizons, respectively. Irrigation in drought treatments was withheld 7 DAP in the top layer, and in the bottom, water content was kept between 10-15% since the preliminary experiments showed that this amount was enough to induce drought stress.

For field studies, volumetric water content was assessed through gravimetric method. Soil cores for soil water content were taken to determine the gravimetric water content and then converted to volumetric water content using bulk density of the soil of 1.5 g/cm^3 at the

Chókwè research station.

3.2.8 Phenological evaluation and yield components

In the field experiments, days to flowering (DF), days to physiological maturity (DPM) were determined for each plot. DF is defined as the number of days after planting until 50% of the plants have at least one open flower, while DPM is the number of days after planting until 50% of plants have at least one pod losing its green pigmentation.

Days to flowering was the number of days after planting and irrigating when 50% of the plants had at least one open flower, while days to physiological maturity was the number of days after planting and irrigating the field until 50% of plants had at least one pod changing its green coloration.

At harvest time, yield components were determined by selecting a 0.5 m long row (0.3 m^2 area) of each plot, and all plants were harvested and put in paper bag for pod number, pod and seed weight, seed number per pod and pod number per area. The remaining rows were harvested to determine grain yield, 100 seed weight and seed moisture content. After recording all data, the following stress indices related to seed yield were quantified according to (Beebe et al., 2013):

- Drought intensity index (DII): $DII = 1 - (X_{ds} / X_{ns})$, where X_{ds} and X_{ns} are the mean yield of all genotypes under drought stress (ds) and no stress (ns) treatments respectively.
- Drought susceptibility index (DSI): for each genotype was determined as $DSI = 1 - (Yds/Yns)/DII$), where Y_{ds} and Y_{ns} are mean yields of given genotype in *ds* and *ns* environments;
- Pod harvest index (PHI%): the PHI for each genotype was calculated by seed biomass dry weight at harvest/pod biomass dry weight at harvest x 100.
- Percentage reduction (PR%): The PR for each genotype was determined as PR = $(Y_{ns} - Y_{ds} / Y_{ns}) *100$, where Y_{ns} and Y_{ds} are mean yields of given genotype in *ns* and *ds* environments.

- φ Geometric mean (GM): the GM was determined for seed yield and 100 seed weight as GM = $(ns \times ds)^{0.5}$ where ns and ds are as defined above.
- φ Grain filling index (GFI): the GFI for each genotype was estimated from 100 seed dry weight under stressed conditions/100 seed dry weight under non-stressed conditions x 100.

3.2.9 Statistical Analysis

The statistical software Minitab Ver. 16 (Minitab Inc. State College, PA, USA) was used for all data analyses. Data from the pot studies were analyzed as a randomized complete-block design, and a fixed model analysis of variance (ANOVA) was used. The linear model $Y_{ijk} = \mu + \tau_i + p_j + (\tau p)_{ij} + \varepsilon_{ijk}$ was applied, where Y_{ijk} = response variable; μ = The general mean; τ_i = The treatment effect; p_j = the block effect; $(\tau p)_{ij}$ = The interaction effect, and ε_{ijk} = experimental error. Analysis of the two water treatments was performed for leaf growth rate, number of leaves, shoot biomass, relative water content and leaf water potential where drought stress/irrigated treatments and replicates were the independent variables. Regression analysis was also performed for growth parameters in the pot experiment. Field studies at Chókwè Research station were analyzed as a split-plot design and Analysis of Variance (ANOVA) was performed. Combined analysis across the two water treatments was performed for phenology (flowering, pod formation, physiological maturity) and yield parameters, where genotypes, water treatments were fixed variables and block was a random variable. Means that were significantly different statistically were separated using Tukey's Honest Significant Difference Test at 5 % probability level. Correlation analysis was performed for all stress indices and shoot traits related to seed yield and other yield traits. The following general linear model was used in this split-plot design experiment: $Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha_i\beta_j) + \gamma k_{(i)} + \varepsilon_{ijk}$, where,

Y_{ijk} = response variable;

μ = Overall mean;

α_i = The whole plot treatment effects;

β_j = The split-plot treatment effects;

$\alpha_i\beta_j$ = The interaction effects;

$\gamma k_{(i)}$ = The whole plot errors;

ε_{ijk} = The experimental error

3.3 Results

3.3.1 Pot study in stratified water system

3.3.1.1 Soil moisture availability

Drought was imposed at seven days after planting (7 DAP), after irrigation of all pots at saturation point. Fourth days after planting, soil moisture content started to be monitored in both depths (0-8 and 8-32 cm), showing small variation in top horizon 0-8 cm, and did not differ in bottom horizon 8-32 cm for both drought and irrigated treatments (Figure 3.2). Moisture content started to decline from 21 DAP until harvest stage, and there was a significant difference between well-watered and drought treatments ($p<0.000$) as well as a difference in the depths in the two sections of the stratified water system ($p<0.000$). Figure 3.3 shows the soil VWC of the drought and control treatments at the end and throughout the experiment. The moisture content of the bottom horizon (8-32 cm) in the drought treatment differed from the moisture content in the well-watered treatment because irrigation in the drought pots at the bottom horizons was completely stopped two weeks before harvest in order to impose a severe drought stress.

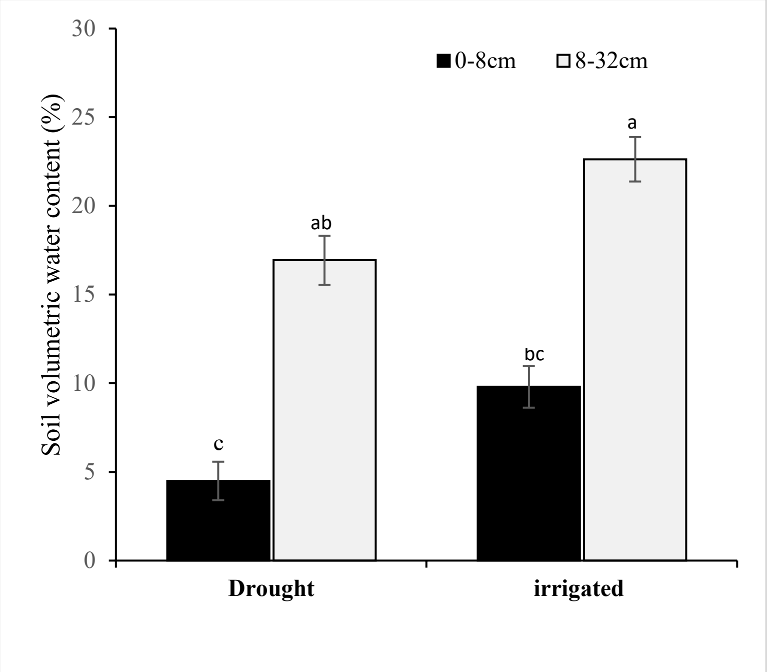

Figure 3.2: Soil volumetric water content, 38 DAP in drought stress and well-watered regimes of the two depths (0-8 and 8-32 cm) in stratified water pot system at Chókwè Research station. VWC was significantly different at the harvest of the experiment. Bar indicate standard error of the mean.

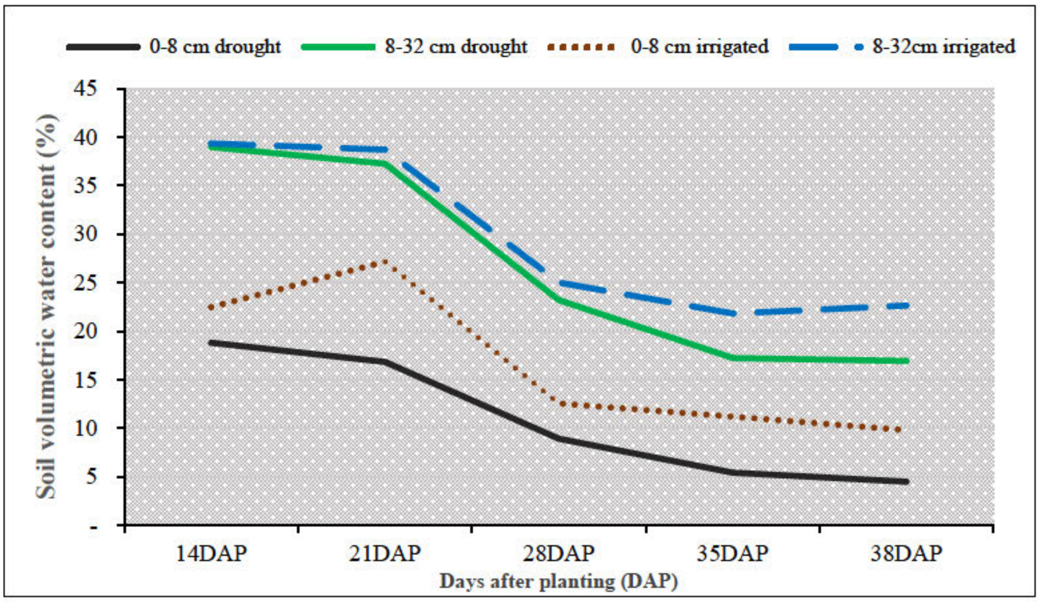

Figure 3.3: Soil volumetric water content of the drought and irrigated treatments of the two depths (0-8 and 8-32 cm) in the stratified water pot system throughout the experiment at Chòkwé Research Station. In the drought treatment, irrigation at bottom horizon was stopped 14 days before harvest to impose strong stress.

3.3.1.2 Leaf water potential

Leaf water potential measured predawn at 38 DAP with pressure bomb showed that plants grown under drought were strongly stressed compared with the well-watered in stratified pot system (Figure 3.4). Measurements were taken before harvest and data were averaged by treatment. Data of all genotypes by treatment, revealed that water potential values were significantly lower in drought stress treatment compared with irrigated, showing that the imposed drought resulted in water deficit compared to irrigated treatment.

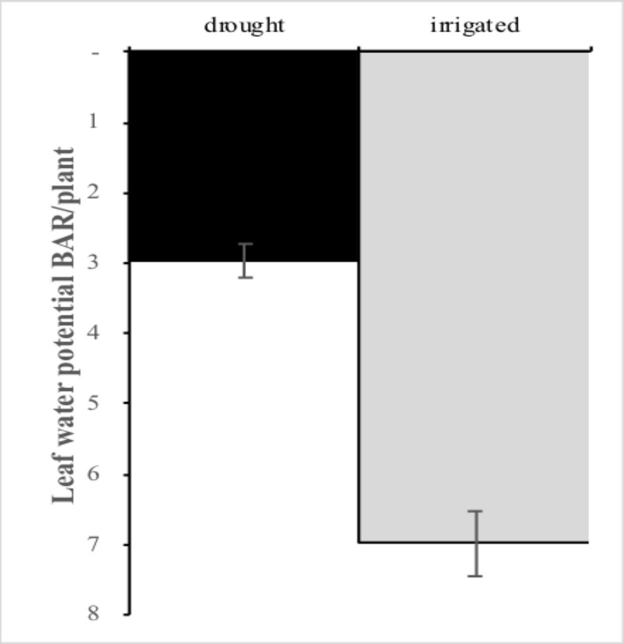

Figure 3.4: Plant water status measured at 38 days after planting with pressure bomb under drought and well-watered treatments in stratified water pot system at Chókwè Research Station. Bars indicate standard error of the mean.

Grouping genotypes by treatment, leaf water potential ranged from 1.75 to 6.5 bar under drought stress, while under irrigated conditions was between 3.5 to 9.0 bar (Figure 3.5). The increase or decrease in leaf water potential in drought stress and irrigated treatments did not correlate with other physiological or agronomic traits such as shoot biomass, leaf growth rate, number of leaves and RWC, meaning that if leaf water potential is maintained at lower values, will not accelerate plant growing resulting in an increase of shoot biomass.

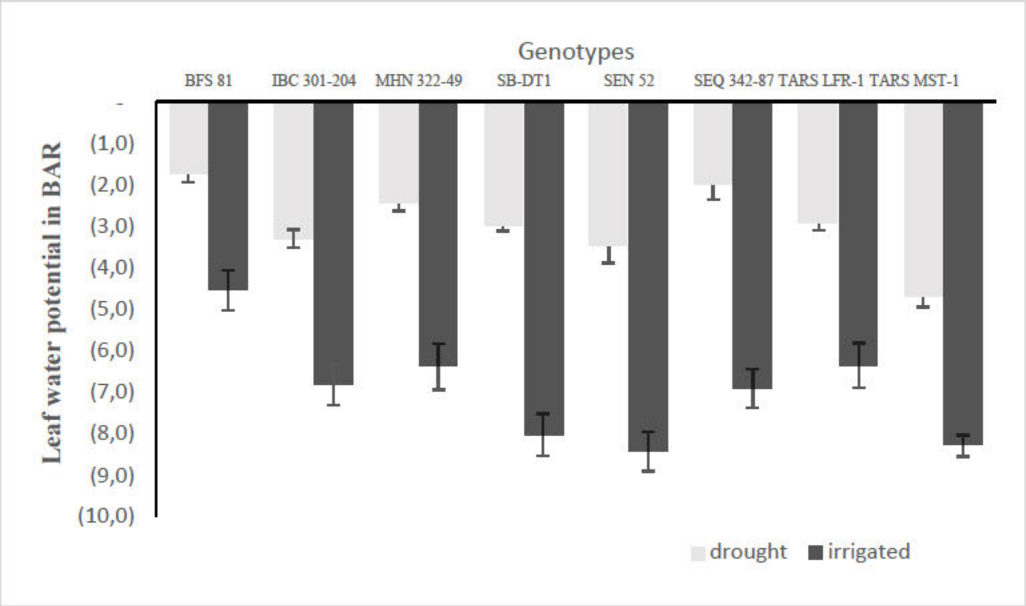

Figure 3.5: Plant water status of the genotypes, measured at 38 days after planting with pressure bomb under drought and well-watered treatments in stratified water pot system at Chókwè Research Station. Standard error bars of the mean are given.

Leaf water relative content revealed that there was strong drought stress imposed on the plants at the harvest period (Figure 3.6). Total shoot biomass was negatively correlated with leaf water relative content. Plants grown under drought with greater total shoot biomass showed higher percentage of relative water content, compared with plants grown under optimum conditions. There was significant effect of water treatment (p $\leq$0.001), which may have influenced an increase of turgid leaf weight, suggesting that genotypes with high RWC, had less water transport and use across the growing season, which resulted in better plant water status.

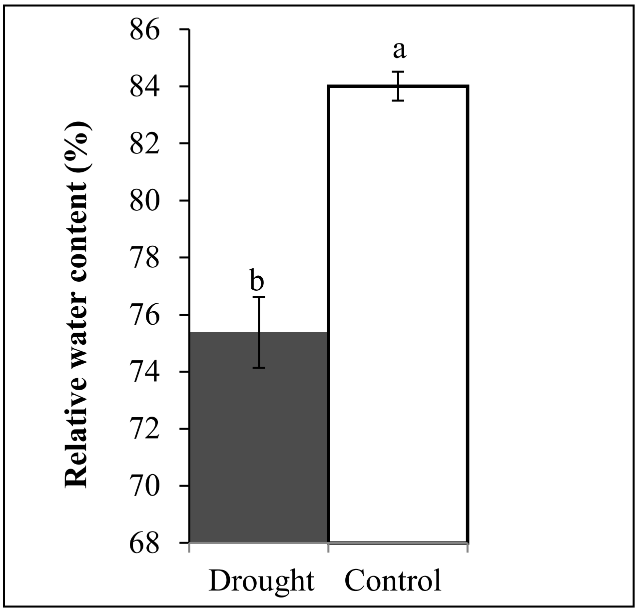

Figure 3.6: Leaf relative water content measured the day before harvest showed difference between the drought and well-watered plants in the stratified water system (pot study) at Chókwè Research Station. Bars indicate standard error of the mean.

3.3.1.3 Leaflet growth rate and shoot biomass

Plant performance assessed by measuring leaflet growth rate and number of leaflets weekly and shoot biomass at harvest were strongly impacted by drought (Figure 3.7). The decrease in leaflet growth rates (LGR) and number of leaves (NL) between drought stress and irrigated conditions was 49 % and 53%, respectively, while for shoot biomass it was 47%. However, when the genotypes were observed individually in each treatment, the percent reductions were significant for LGR and NL ranging from 34% to 70% and 36% to 60%, respectively, while for shoot biomass varied from 45% to 50% and was not significant. Out of the eight genotypes assessed in the pots, two genotypes (MHN 322-49 and IBC 301-204) were the most impacted in all three growth rates (SB, LGR and NL), while for other genotypes the percent decrease was not consistent, since the reduction of LGR and NL, did not necessarily impact the percent decrease of shoot biomass, suggesting that the genotypes are independently affected by these growth parameters under limited water supply. The percent decrease in leaflet growth rate had significant and positive correlation with percent decrease in shoot biomass, and negatively correlated but significant with shoot biomass under drought stress (Figures 3.8a and 3.8d). Yet, weak correlation between decrease in LGR with decrease in leaflet number (Figure 3.8c) was found suggesting that the number of leaflet does not necessarily influence the size of leaflets under drought conditions. Moreover, leaflet growth rate was correlated with number of leaves and shoot biomass under drought stress (Figure 3.9 a and 3.9e) and did not correlate in irrigated conditions (Figure 9 b and d).

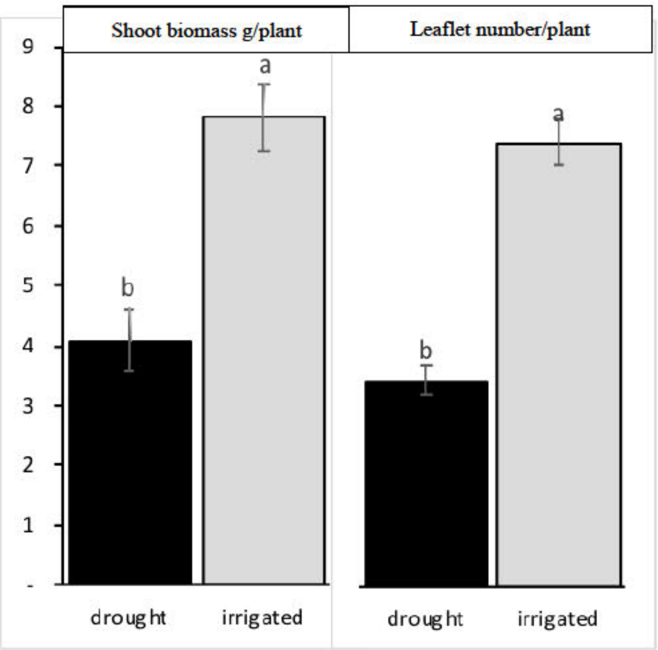

Figure 3.7: Shoot biomass and leaflet number at 36 DAP showed significant differences between the drought stressed and well-watered plants in the stratified water pot system at Chòkwé research station, Mozambique. Bars indicate standard error of the mean.

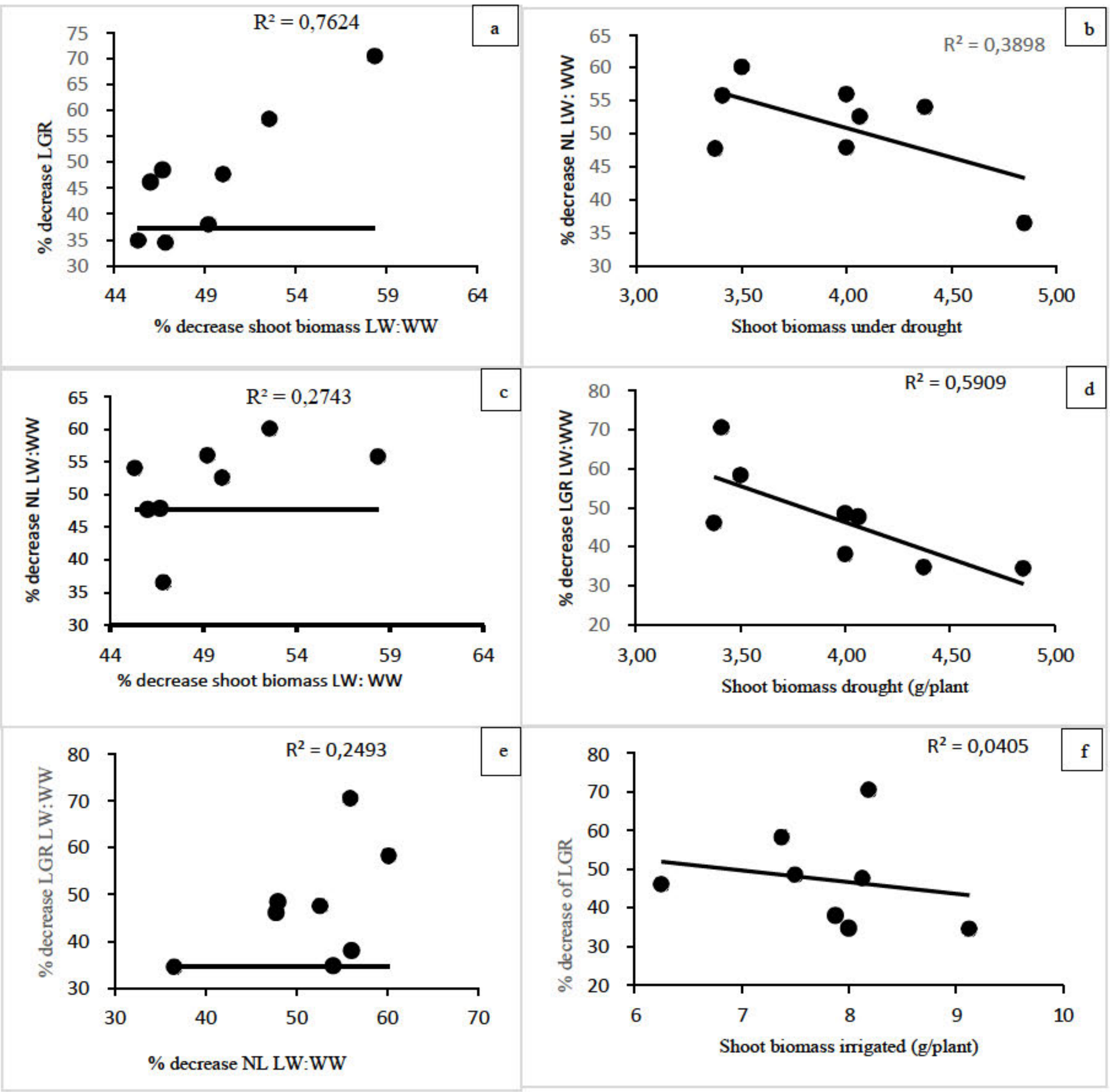

Figure 3.8: The relationship between percent decrease in the genotypes with the growth parameters in the stratified water pot system under drought and well-watered conditions (2019 pot study) at Chókwè Research station, Mozambique.

In the meantime, the influence of drought on leaflet number was positively correlated with shoot biomass and did not correlate under well-watered condition (Figure 3.9 e and f). As was expected, genotypes in which the number of leaflets were strongly impacted by drought had their shoot biomass equally reduced. Similarly, genotypes with high NL in irrigated conditions, also performed better under drought stress.

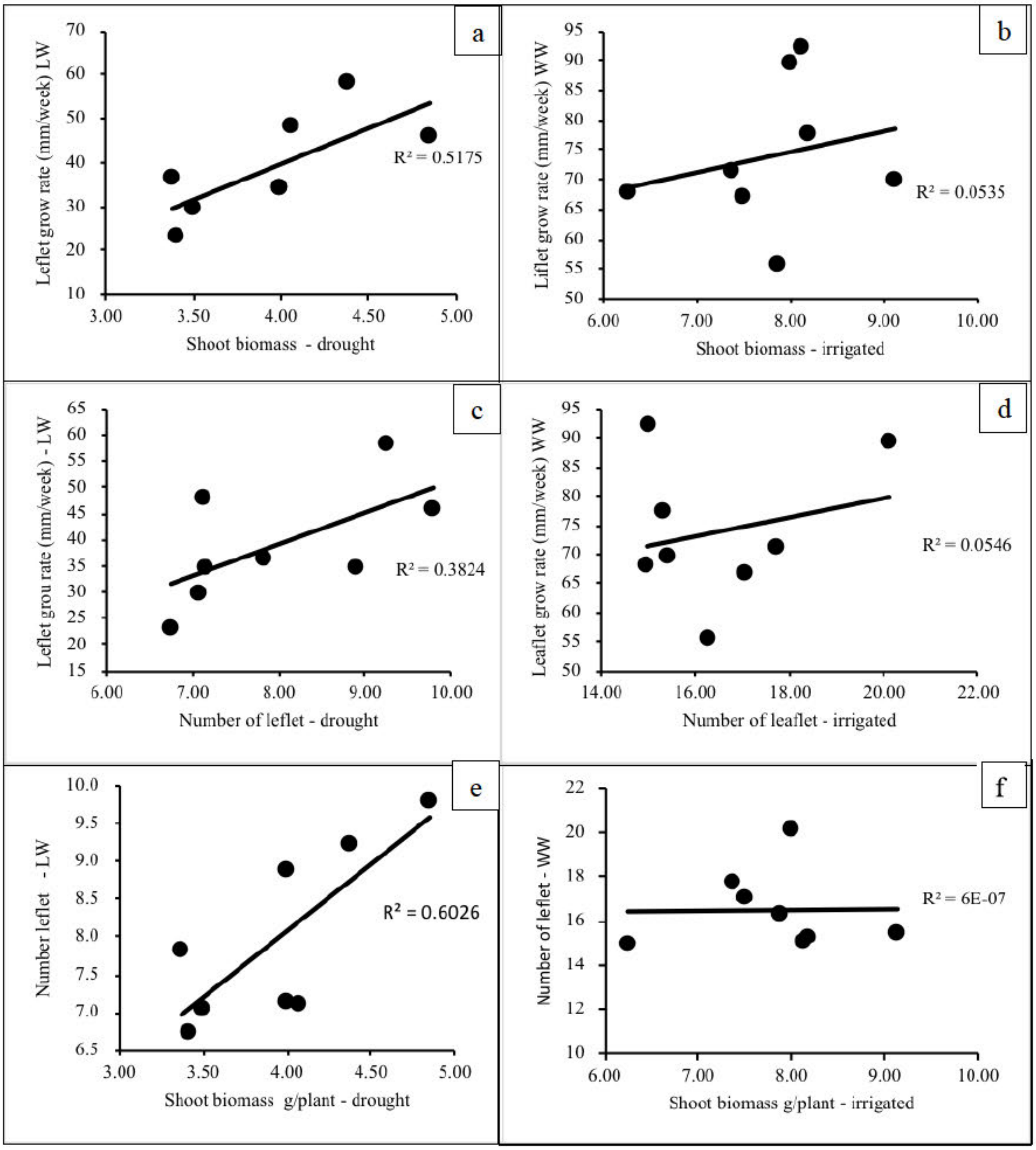

Figure 3.9: The relationship between leaflet growth rate of the genotypes and the shoot biomass and number of leaflets in the stratified water pot system under drought and well-watered conditions (2019 pot study) at Chókwè Research station, Mozambique.

3.3.2 Field Study

3.3.2.1 Meteorological data and soil moisture availability

Local weather station was used to record data on temperature, rainfall and pan evaporation throughout the experiment. The trial received 23.6 mm rainfall, while the recorded evaporation was 39.7 mm, an indication that drought stress impacted the trial during crop development under rainfed conditions (Figure 3.10). Supplementary irrigation in drought treatment was made when the soil volumetric water content was below 10% field capacity.

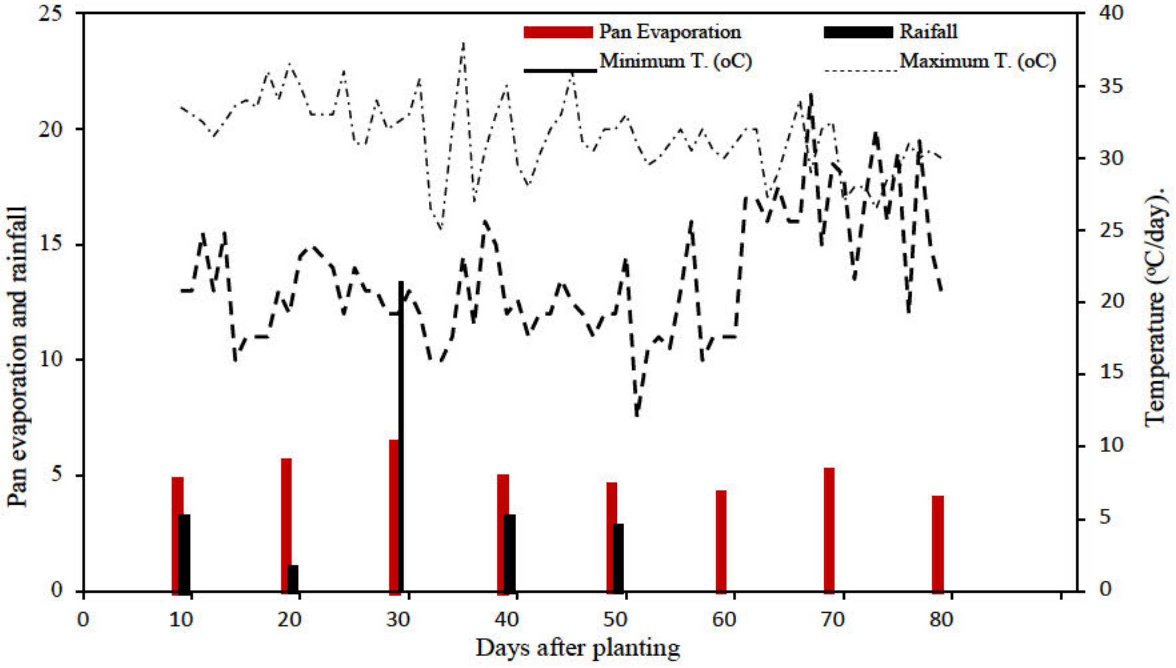

Figure 3.10: Rainfall distribution, pan evaporation, maximum and minimum temperatures during crop growing cycle at Chókwè Research station, Mozambique.

Soil moisture content was monitored through gravimetric water content method. Gravimetric water content in the top layer (15 cm) showed a constant decrease in water content throughout the experiment, and there were no differences at the same horizon at the beginning of drought imposition. In the drought stress trial, soil gravimetric water content was 14% in the top 15 cm and –28% in 40 cm depth, while in well irrigated plots GWC was 24% and – 37.6% in 15 cm and 40 cm depth, respectively (Figure 3.11).

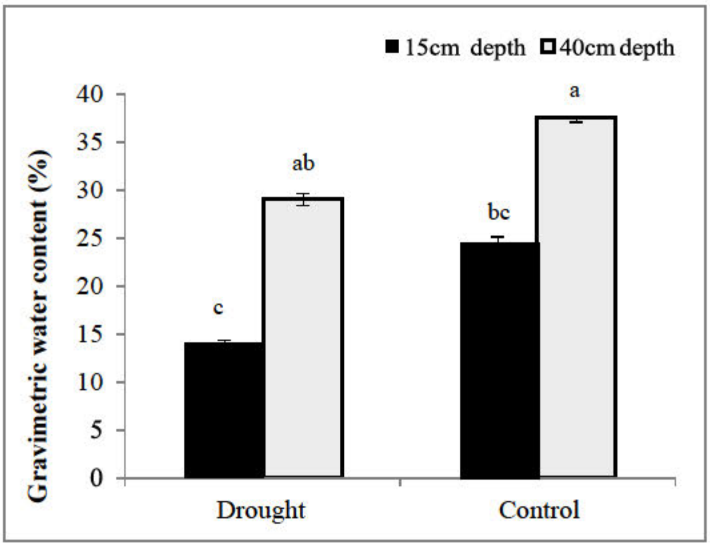

Figure 3.11: Soil gravimetric water content throughout the experiment showed difference in drought plots- and control plots between depths in the field study.

3.3.2.2 Morphological and Phenological assessment

Initial and final plant stand, days to flowering and days to physiological maturity was assessed throughout the growing season. Final plant stand was high and significantly correlated to seed yield both in drought stress and well-watered condition, (r=0.730*** and r=0.814***), respectively (Table 3.2). Under drought stress the final plant stand ranged from 16 to 35 with an average of 27 plants per plot, while in well-watered treatment the final plant stand ranged from 8 to 28 with a mean of 23 plants per plot. The genotypes FBN 1211-66, MEN 2207-17, SB 787, SB- DT1, SEQ342-87, TARS MST-1 and USMR 20 performed the best in terms of plant stand under drought condition.

Table 3.2: Correlation coefficients (r) between final seed yield (kg ha-1) and other shoot traits of 24 genotypes of common bean grown under well-watered and drought stress conditions at Chókwè research station.

PLANT TRAITS	IRRIGATED	DROUGHT
Initial stand	0.010	0.398*
Final stand	0.814***	0.730***
Days to emergency	-0.218	-0.310
Days to flowering	-0.056	-0.290
Days to pod formation	-0.575***	-0.661***
Days to physiological maturity	0.052	-0.408**
Pod number per plant	0.560***	0.552***
Seed number per pod	0.726***	0.694***
Pod weight per area ($g.m^{-2}$)	0.834***	0.830***
Seed weight per area ($g.m^{-2}$)	0.855***	0.842***
100 seed weight (g)	-0.274	-0.333
Pod harvest index (phi %)	0.370*	0.070

*, **, *** significant at 0.05; 0.01 and 0.001 probability levels, respectively

Days to pod formation had strong and negative correlation with seed yield under both drought stress (-0.661***) and well-watered regime (-0.575***; Table 3.2). Under drought conditions, days to pod formation (DPF) ranged from 51 to 60 days, while under irrigation they ranged from 52 to 63 days. Genotypes BFS 81, BRT103-182, INB 841, SEF 16, SER 125 and SX 14825-7-1 had less and similar days to pod formation in well-watered treatment (Table 3.3).

Days to physiological maturity showed negative correlation with seed yield under drought stress (- 0.408**) and was not correlated in well-watered treatment. Days to physiological maturity ranged from 78 to 86 days with an average of 84 days under drought stress. For well-watered treatment, the days to physiological maturity was between 85 to 90 days with mean of 88 days (Table 3.3). The genotypes BFS81, BRT 103-182, INB 841, SEF 16, SEN 52, SER 125 and SX 14825-7-1 matured relatively earlier under drought stress treatment with seed yield values above the average.

Table 3.3: Phenotypic differences of initial and final plant stand, days to flowering, days to pod formation and days to physiological maturity of 24 genotypes of common bean grown under drought stress and irrigated conditions at Chókwè Research Station, Mozambique.

Genotypes	**Initial plant stand**		**Final plant stand**		**Days to flowering**	
	Irrigated	**Drought**	**Irrigated**	**Drought**	**Irrigated**	**Drought**
Amadeus 77	38.00a	36.33abc	23.66abc	26.33abcd	44.33abc	46.33a
Beniquez	35.00abc	33.66cd	20.66bcd	24.00bcdef	40.66bc	46.00ab
BFS 81	37.66ab	33.66cd	26.66ab	24.33bcdef	42.66abc	38.33fg
BIOF 2-106	36.33abc	35.00bcd	24.33abc	28.66abcd	44.33abc	43.66abcde
BRT 103-182	36.33abc	37.66abc	26.33 ab	29.00abcd	42.00abc	47.00a
FBN 1211-66	34.33abc	38.33ab	20.66bcd	34.66a	41.33abc	41.00bcdefg
IBC 301-204	37.66ab	39.66a	27.66a	31.66abc	44.33abc	43.33abcdef
INB 841	36.00abc	36.00abc	24.33abc	25.00bcdef	40.33c	38.00g
MEN 2207-17	33.33bc	38.66ab	20.66bcd	31.66abc	44.66ab	44.33abcde
MER 2212-28	35.66abc	38.66ab	21.00abcd	21.00def	44.33abc	45.33abc
MHN 322-49	34.66abc	31.00d	15.33d	17.00ef	41.66abc	42.66abcdefg
MHR 311-17	36.66abc	35.00bcd	23.33abc	23.33cdef	44.00abc	47.00a
PR 1217-16	37.66ab	38.00ab	21.33abcd	24.00bcdef	45.00a	42.66abcdefg
SB 787	38.33a	36.77abc	22.33abc	29.66abcd	43.33abc	43.66abcde
SB-DT1	38.66a	37.66abc	25.33abc	29.00abcd	43.00abc	47.00a
SEF 16	36.66abc	37.66abc	25.66abc	32.33ab	41.33abc	39.66defg
SEN 52	34.33abc	37.33abc	21.33abcd	30.66abc	41.66abc	40.33cdefg
SEQ 342-87	37.00abc	36.33abc	27.00ab	26.33abcd	44.66ab	44.00abcde
SER 125	37.00abc	39.00ab	25.66abc	32.00abc	41.33abc	40.66cdefg
SJC 730-79	33.00c	37.66abc	19.33cd	25.00bcdef	43.66abc	47.00a
SX 14825-7-1	37.33abc	35.66abc	26.00abc	26.33abcd	42.00abc	39.33efg
TARS LFR-1	37.33abc	37.66abc	24.33abc	25.33bcde	43.33abc	44.66abcd
TARS MST-1	35.66abc	37.33abc	21.66abcd	31.33abc	43.66abc	46.66a
USMR 20	37.66ab	35.66abc	8.33e	16.33f	44.00abc	46.33a
Mean	**36**	**37**	**23**	**27**	**43**	**44**
SE	**2.21**	**2.07**	**3.41**	**4.33**	**2.07**	**2.61**
CV%	**7.45**	**6.92**	**18.48**	**19.74**	**5.91**	**7.35**
LSD	**4.45**	**4.17**	**6.87**	**8.71**	**4.17**	**5.25**

Means followed by the same letter within the same column are not significantly different ($P \leq 0.05$).

Table 3.3 Continued: Phenotypic differences of initial and final plant stand, days to flowering, days to pod formation and days to physiological maturity of 24 genotypes of common bean grown under drought stress and irrigated conditions at Chókwè Research Station, Mozambique.

Genotypes	Days to Pod formation		Days to Physiological Maturity	
	Irrigated	**Drought**	**Irrigated**	**Drought**
Amadeus 77	57.66abc	55.66bcd	89.66ab	86.00ab
Beniquez	57.33bcd	54.33bcd	90.33a	85.33abc
BFS 81	52.33de	51.33d	87.66abcd	81.33defg
BIOF 2-106	53.66bcde	54.00bcd	89.66abc	83.66abcd
BRT 103-182	52.33de	52.66cd	86.00cd	84.33abc
FBN 1211-66	54.00bcde	56.00abcd	88.33abcd	83.33bcde
IBC 301-204	57.6abc	52.33abcd	90.33a	84.00abcd
INB 841	52.00e	52.33cd	86.00cd	78.00h
MEN 2207-17	58.33ab	59.00ab	90.33a	85.00abc
MER 2212-28	55.33bcde	57.66abc	90.00ab	83.33bcde
MHN 322-49	53.33bcde	57.00abcd	87.66bcd	84.00abc
MHR 311-17	54.66bcde	53.66bcd	89.66abc	85.00abc
PR 1217-16	58.33ab	54.33bcd	87.33abcd	84.00abcd
SB 787	56.00bcde	54.66abcd	87.66abcd	83.33bcde
SB-DT1	55.00bcde	53.00bcd	89.33abc	85.33abc
SEF 16	54.33bcde	51.66bc	87.00abcd	80.33fgh
SEN 52	53.33bcde	54.00bcd	87.00abcd	80.66efgh
SEQ 342-87	56.66bcde	56.00abcd	90.00ab	85.00abc
SER 125	52.66cde	52.00cd	86.66bcd	79.33gh
SJC 730-79	56.33bcde	57.33abcd	90.33a	85.00abc
SX 14825-7-1	52.00e	51.33d	87.00abcd	82.66cdef
TARS LFR-1	54.66bcde	55.00abcd	85.00d	84.66abc
TARS MST-1	57.00bcde	54.00bcd	89.33abc	84.00abcd
USMR 20	62.66a	60.66a	87.33abcd	86.00a
Mean	**54.66**	**55.32**	**88**	**84**
SE	**2.49**	**3.07**	**1.75**	**1.44**
CV%	**5.52**	**6.89**	**2.44**	**2.12**
LSD	**5.02**	**6.18**	**3.54**	**2.90**

Means followed by the same letter within the same column are not significantly different ($P \leq 0.05$).

3.3.2.3 *Stress indices and Shoot traits*

Drought intensity index (DII) value of 0.58 confirms the severity of the drought stress of the experiment (Table 3.5). Under drought stress most of the genotypes showed lower values when compared to the well-watered treatment, confirming good imposition of drought stress plots compared to the neighbouring well-watered treatments. (Table 3.5).

The drought susceptibility index (DSI) for seed yield showed high values for all 24 genotypes assessed, with an average of 0.939 (Table 3.5). Genotypes with the lowest drought susceptibility index were TARS MST-1(0.752), FBN 1211-66 (0.759), SB 787 (0.762), PR 1217-16 (0.772) and SEF16 (0.774). Notably, USMR20 showed a negative value of (-0.208).

Imposed drought stress significantly decreased pod number per plant, seed number per pod as well as 100-seed weight for most genotypes (Table 3.4). Pod number per plant ranged between 7-14 under drought stress and 5 - 21 under well-watered treatment. Drought stress caused a reduction in pod number per plant by 26%. However, an increase in pod number per plant under drought stress of 3% and 46% was observed for genotypes BFS 81 and USMR 20, respectively (Table 3.5).

Table 3.4: Variability of bean genotypes with respect to Pod number per plant, seed number per pod, 100-seed weight 24 common bean genotypes evaluated in drought-stressed and irrigated conditions at IIAM - Chókwè Research Station, Gaza – Mozambique in 2019.

	Pod number per plant				**Seed number per pod**			
Genotypes	**DS**	**NS**	**GM**	**% PR**	**DS**	**NS**	**GM**	**% PR**
Amadeus 77	11.13bc	18.23abcd	14	39	5.53ab	6.10a	6	9.3
Beniquez	10.93bc	16.23abcdef	13	33	5.20abc	5.70abcd	5	8.8
BFS 81	11.83bc	11.53f	**12**	**(3)**	5.67ab	5 57abcd	**6**	**(1.8)**
BIOF 2-106	14.06ab	19.00abc	16	26	5.57ab	5.83abc	6	4.6
BRT 103-182	12.03bc	16.17abcdef	14	26	5.80ab	5.67abcd	6	(2.4)
FBN 1211-66	13.46ab	17.33abcde	15	22	5.93a	5.87abc	6	(1.1)
IBC 301-204	11.50bc	15.13bcdef	13	24	5.77ab	5.97ab	6	3.4
INB 841	11.93bc	14.90bcdef	13	20	5.67ab	5.47abcd	6	(3.7)
MEN 2207-17	10.66bc	16.97abcde	13	37	5.57ab	5.97ab	6	6.7
MER 2212-28	10.66bc	17.77abcde	14	40	5.23abc	5.90ab	6	11.3
MHN 322-49	13.46ab	15.17bcdef	14	11	4.17d	5.00cd	5	16.7
MHR 311-17	12.13bc	18.07abcd	15	33	5.60ab	6.13a	6	8.7
PR 1217-16	10.93bc	13.63def	12	20	4.97bcd	5.00cd	5	0.7
SB 787	12.70abc	17.43abcde	15	27	5.20abc	5 30abcd	5	1.9
SB-DT1	12.40abc	17.47abcde	15	29	5.40ab	6.00ab	6	10.0
SEF 16	12.46abc	12.70ef	13	2.0	5.53ab	6.03ab	6	8.3
SEN 52	14.00ab	17.97abcde	16	22	5.27abc	5.90ab	6	10.7
SEQ 342-87	13.73ab	19.83ab	17	31	5.70ab	5 50abcd	6	(3.6)
SER 125	11.36bc	13.03def	12	13	5.90a	4.90d	5	(20.4)
SJC 730-79	9.60bc	14.47cdef	12	34	5.17abc	5.93ab	6	12.9
SX 14825-7-1	16.90a	16.97abcde	17	0.0	4.50cd	5 30abcd	5	15.1
TARS LFR-1	9.86bc	15.80abcdef	12	38	5.07abc	5.20bcd	5	2.6
TARS MST-1	13.20ab	20.80a	**17**	**37**	5.80ab	5.73abcd	**6**	**(1.2)**
USMR 20	7 98c	5.07g	6	(46)	2.37e	2.77e	3	15.1
Mean	**12.0**	**15.9**	**14**	**26**	**5.27**	**5.53**	**5**	**5**
SE	**4.34**	**2.63**			**2.67**	**0.44**		
CV%	**23.44**	**20.30**			**10.28**	**9.83**		
LSD	**2.46**	**5.30**			**2.01**	**0.89**		

Means followed by the same letter within the same column are not significantly different ($P \leq 0.05$). NS = non-stressed, DS = drought stressed, GM (geometric mean) =(ns x ds)$^{0.5}$, PR = percent reduction between the DS and NS conditions.

Table 3.4 continued: Variability of bean genotypes with respect to Pod number per plant, seed number per pod, 100-seed weight of 24 common bean genotypes evaluated in drought-stressed and irrigated conditions at IIAM - Chókwè Research Station, Gaza – Mozambique in 2019.

	Seed weight per (03m^2). g				**100 Seed weight. G**				
Genotypes	**DS**	**NS**	**GM**	**% PR**	**DS**	**NS**	**GM**	**% PR**	**% GFI**
Amadeus 77	106.33abcde	178.0ab	138	40.3	21.67bcd	23.00cde	22.3	5.8	94.2
Beniquez	69.33de	116.0de	90	40.2	17.00ijk	17.00jk	17.0	0.0	100.0
BFS 81	106.00abcde	114.0e	**110**	**7.0**	19.6cdefgh	24.33bc	21.9	9.2	80.8
BIOF 2-106	123.33abcd	172.7abc	146	28.6	19.33defghi	20.00ghi	19.7	3.3	96.7
BRT 103-182	118.67 abcd	155.3abcde	136	23.6	22.00bc	23.00cde	22.5	4.3	95.7
FBN 1211-66	93.30 bcde	157.3abcde	121	40.7	18.00ghij	20.33fghi	19.1	11.5	88.5
IBC 301-204	98.00abcde	154.7abcde	123	36.6	18.67fghij	21.00efgh	19.8	11.1	88.9
INB 841	110.00abcde	147.3abcde	127	25.3	20.67cdef	21.00efgh	20.8	1.6	98.4
MEN 2207-17	73.00bcde	114.0e	91	36.0	14.67k	15.67k	15.2	6.4	93.6
MER 2212-28	71.00cde	172.7abc	111	58.9	22.00bc	21.67defg	21.8	(1.5)	101.5
MHN 322-49	67.33de	125.0cde	92	46.1	17.33hij	18.00ijk	17.7	3.7	96.3
MHR 311-17	129.67ab	165.7abcd	147	21.7	18.33fghij	21.00efgh	19.6	12.7	87.3
PR 1217-16	86.67bcde	112.0e	99	22.6	19.0efghij	20.33fghi	19.7	6.6	93.4
SB 787	82.33bcde	118.7de	99	30.6	19.6cdefgh	19.67ghi	19.7	0.0	100.0
SB-DT1	89.00bcde	138.7bcde	111	35.8	17.00ijk	18.67hij	17.8	8.9	91.1
SEF 16	124.00abcd	171.7abc	146	27.8	20.33cdefg	24.00bcd	22.1	15.3	84.7
SEN 52	127.33abc	196.0a	158	35.0	21.33cde	24.67bc	22.9	13.5	86.5
SEQ 342-87	88.10bcde	134.7bcde	109	34.6	16.67jk	15.67cdef	16.2	(6.4)	106.4
SER 125	121.33abcd	136.7bcde	129	11.2	24.00b	25.00bc	24.5	4.0	96.0
SJC 730-79	95.33abcde	150.0abcde	120	36.4	21.67bcd	22.67cdef	22.2	4.4	95.6
SX 14825-7-1	151.33a	175.3abc	163	13.7	22.00bc	25.67b	23.8	14.3	85.7
TARS LFR-1	76.00bcde	140.0bcde	103	45.7	19.6cdefgh	23.00cde	21.3	4.5	85.5
TARS MST-1	128.67abc	127.3bcde	**128**	**(1.0)**	16.67jk	19.33ghij	18.0	13.8	86.2
USMR 20	48.69e	19.0f	27	(107.9)	33.19a	34.86a	34.0	5.7	94.3
Mean	99.36	141.36	-		20.0	21.64	21.0	7.0	92.8
SE	3.12	25.27			3.05	2.33			
CV%	35.34	21.89			7.25	6.99			
LSD	5.18	50.86			2.3	0.19			

Means followed by the same letter within the same column are not significantly different ($P \leq 0.05$). NS = none stressed, DS = drought stressed, GM (geometric mean) = (ns x ds) $^{\mathbf{0.5}}$, PR = percent reduction in the DS and NS conditions, DSI (drought susceptibility index) = (1 – Yds/Yns)/DII, where Y_{ds} and Y_{ns} are mean yield of a given genotype in DS and NS environments respectively and GFI (grain filling index) = 100 seed weight DS/100 seed weight NS x 100.

Mean 100-seed weight was reduced by 7%, and 100-seed weight ranged from 14.7 to 33 g under drought stress and 15.5 to 34 g under well-watered treatment. USMR 20 showed the highest weight in both conditions drought stress and non-stressed, while MEN 2207-17 had the lowest weight (Table 3.5).

Table 3.5: Variability of bean genotypes with respect to pod harvest index (%) and seed yield of 24 common bean genotypes evaluated in drought-stressed and irrigated conditions at IIAM - Chókwè Research Station, Gaza – Mozambique in 2019.

	Pod harvest index %			**Seed yield kg.ha $^{-1}$**				
Genotypes	**DS**	**NS**	**% PR**	**DS**	**NS**	**GM**	**% PR**	**DSI**
Amadeus 77	65.73d	71.75bc	8.4	947.6abcd	2,391.1abcd	1,505	60	1.045
Beniquez	74.78abcd	68.34bcd	(9.4)	623.1cde	1,309.4fg	903	52	0.908
BFS 81	75.50abcd	70.31bc	(7.4)	822.7bcde	1,965 3abcdef	1,272	58	1.007
BIOF 2-106	70.73abcd	71.47bc	1.0	891.3bcde	2,352.3abcd	1,448	62	1.076
BRT 103-182	79.12abc	72.43bc	(9.2)	1071.4abc	2,629.0a	1,678	59	1.026
FBN 1211-66	72.76abcd	65.25cd	(11.5)	1028.2abcd	1,831.2bcdef	1,372	44	0.759
IBC 301-204	73.71abcd	72.12bc	(2.2)	967.7abcd	2,602.5ab	1,587	63	1.088
INB 841	74.78abcd	74.33abc	(0.6)	770.2bcde	2,293.2abcd	1,329	66	1.150
MEN 2207-17	67.78bcd	74.60abc	9.1	809.3bcde	1,804.8cdefg	1,209	55	0.955
MER 2212-28	68.18bcd	73.56abc	7.3	768.1bcde	2,435.0abcd	1,368	68	1.185
MHN 322-49	67.55cd	71.04bc	4.9	468.5ef	1,250.7fg	765	63	1.083
MHR 311-17	82.49a	77.15ab	(6.9)	942.9abcd	2,265.9abcd	1,462	58	1.011
PR 1217-16	76.95abcd	70.24bc	(9.6)	805.2bcde	1,451.4efg	1,081	45	0.771
SB 787	67.30cd	82.24a	18.2	574.9def	1,026.6g	768	44	0.762
SB-DT1	73.02abcd	70.91bc	(3.0)	848.4bcde	2,206.8abcde	1,368	62	1.066
SEF 16	66.35abcd	72.56bc	8.6	1361.6a	2,460.8abcd	1,830	45	0.774
SEN 52	72.61abcd	74.35abc	2.3	1078.9ab	2,637.1a	1,687	59	1.023
SEQ 342-87	70.65abcd	69.16bcd	(2.2)	747.5bcde	2,537.8abc	1,377	71	1.222
SER 125	76.24abcd	75.22ab	(1.4)	945.6abcd	2,152.1abcde	1,427	56	0.971
SJC 730-79	80.40ab	69.83bcd	(15.1)	725.5bcde	1,736.2defg	1,122	58	1.008
SX 14825-7-1	70.91abcd	73.70abc	3.8	1112.1ab	2,442.4abcd	1,648	54	0.943
TARS LFR-1	81.50a	73.55abc	(10.8)	683.9bcde	2,030.7abcdef	1,178	66	1.148
TARS MST-1	76.81abcd	69.52bcd	(10.5)	1097.9ab	1,940.4abcdef	1,460	43	0.752
USMR 20	71.20abcd	60.59d	(16.2)	136.1f	121.5h	129	(12)	-0.208
Mean	73.2	71.84	__	842.85	1,994.8			
SE	5.56	4.65	__	225.76	391.47			
CV%	10.5	7.93	__	32.8	24.04			
LSD	2.45	9.36	__	454.42	787.99			
DII		0.58						

Means followed by the same letter within the same column are not significantly different ($P \leq 0.05$). NS = none stressed, DS = drought stressed, GM (geometric mean) = (ns x ds) $^{0.5}$, PR = percent reduction in the DS and NS conditions, DSI (drought susceptibility index) = (1 – Yds/Yns)/DII, where Y_{ds} and Y_{ns} are mean yield of a given genotype in DS and NS environments respectively and GFI (grain filling index) = 100 seed weight DS/100 seed weight NS x 100.

Seed number per pod slightly decreased in some genotypes and increased in another with an average of percent decrease of 5% between drought and well-watered treatment. However, drought susceptibility index (DSI), was correlated to seed number per pod and highly significant correlation was found with geometric mean of seed number per pod (Table 3.6). The drought susceptibility index (DSI) for seed yield was high and significantly correlated with pod number per plant with correlation coefficients of (0.464 ** and r = 0.673***) under drought and well-watered conditions, respectively. Equally, DSI was found to be associated with geometric mean yield (r =0.608***) and percent of seed yield reduction (r=0.741***), (Table 3.6). However DSI for seed yield was negative and significantly correlated with 100-seed weight for drought (-0.669***) and well-watered (- 0.674***), as well as negatively associated with geometric mean yield -0.686*** and not with percent of reduction (Table 3.6).

Table 3.6: Correlation coefficients (r) between DSI and other shoot traits of 24 genotypes of common bean grown under well-watered and drought stress conditions at Chókwè research station.

Plant attributes	**Drought**	**Irrigated**	**DSI**
Seed yield subsample (0.3m^{2})	0.328	0.706***	
Geometric mean subsample seed yield (0.3m^{2})			0.581***
PD subsample seed yield (0.3m^{2})			0.880***
100 seed weight	- 0.669***	- 0.674***	
Geometric mean 100 seed yield			-0.686***
Percent reduction 100 seed yield			-0.186
Number of pods per plant	0.464 **	0.673***	
Geometric mean pod number			0.608***
Percent reduction pod number			0.741***
Number of seeds per pod	0.042	0.692**	
Geometric mean seed per pod			0.758***
Percent reduction seed per pod			-0.221
Seed yield per kg ha^{-1}	0.397*	0.731***	
Percent reduction seed yield kg ha^{-1}			0.994***
Geometric mean yield kg ha^{-1}			0.603***

* Significant at *: $P \leq 0.05$; **: $P \leq 0.01$; and ***: $P \leq 0.001$; respectively

The yield per area showed a positive and highly significant correlation with grain yield under both irrigated (0.856***) and drought stress treatments (0.831***) (data not shown). Genotypes SX14825-7-1, MHR 311-17, TARS MST-1, SEN 52, SEF 16, BIOF 2-106 SER 125 BRT 103-182 INB 841 Amadeus 77 and BFS 81 exhibited greater values of yield per area and also performed better with higher yield under drought stress (Figure 3.12). The highest seed yield per area under drought stress was noted for genotype SX14825-7-1, followed by MHR 311-17, TARS MST-1, SEN 52, SEF 16, BIOF 2-106 SER 125 BRT 103-182 INB 841 Amadeus 77 and BFS 81. The line USMR 20 was the most affected with less yield per area under drought stress, followed by MHN 322-49, Beniquez, MER 2212-28, MEN 2207-17 and TARSLFR-1 (Figure 3.12).

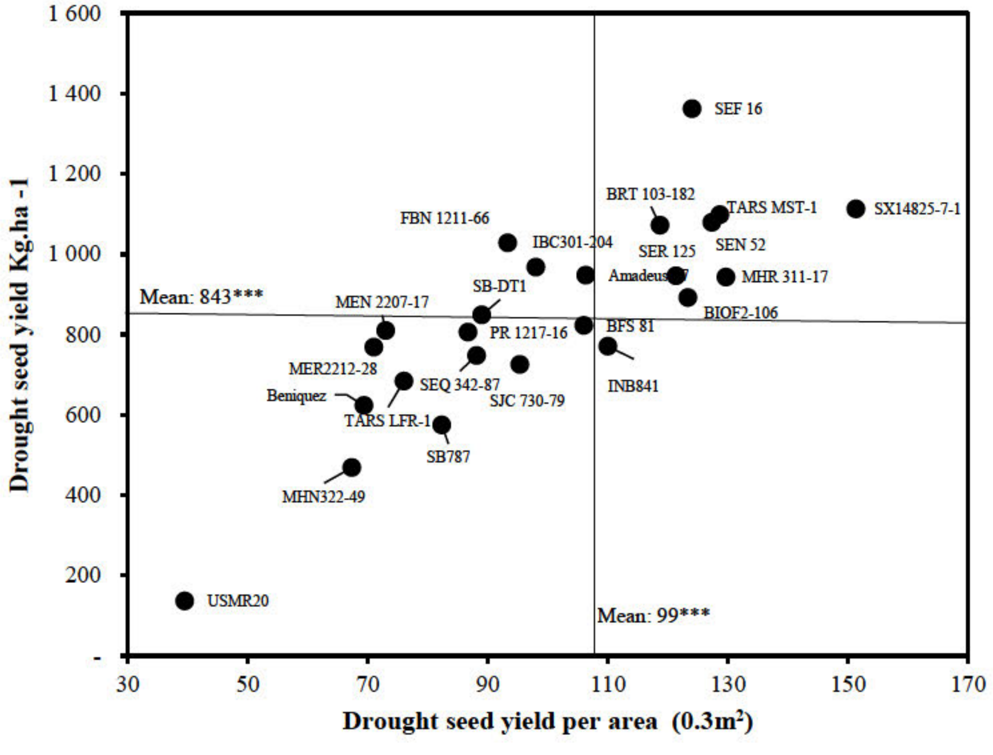

Figure 3.12: Relationship of grain yield kg ha^{-1} and seed number per area showed a strong correlation under drought stress in field experiment at Chókwè Researcher Station. Higher yielding genotypes with greater values of seed number per area are in the upper right quadrant.

3.3.2.4 Seed Yield

Under water stress conditions the mean seed yield (SY) decreased by 58% compared with well-watered conditions (Figure 3.13). All the 24 genotypes assessed under drought stress had seed yield ranging from 136 to 1362 kg ha^{-1}. Among all the genotypes evaluated, SEF 16, SX 14825-7-1, TARS MST-1, SEN 52, BRT103-182, FBN1211-66, IBC 301-204, Amadeus 77, SER 125 and MHR 311-17 were the most adapted to drought stress conditions. A positive relationship between seed yield under drought stress and well-watered treatments (0.792***) showed that SEN 52, BRT 103-182, IBC 301-204, SEF 16, SX14825-7-1, Amadeus 77, BIOF 2-106, MHR311-17, SER125, TARS MST-1 and FBN 1211-66 were not only drought stress tolerant but also responded better under well-watered treatment, with high yield in both environments (Figure 3.13). Among the assessed genotypes, USMR 20 was the most sensitive to drought stress as well as to common bean rust disease, according to field observations made (data not shown).

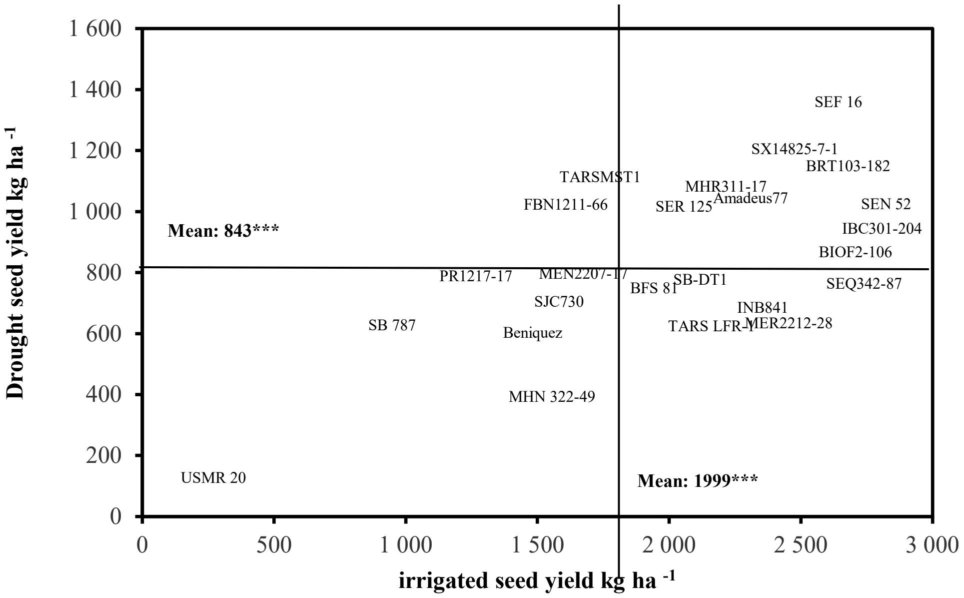

Figure 3.13: Relationship of grain yield kg ha^{-1} under drought stress and irrigated conditions in the field experiment at Chókwè Researcher Station. Adapted genotypes to drought stress and are responsive to irrigation are localized in the upper right quadrant.

Percent decrease in seed yield between irrigated and drought stress treatments was positively associated with number of seeds per pod in non-stressed environment (0.685***), seed yield

(0.394*) and (0.746**), and negatively correlated with 100-seed weight (-0.639***) and (-0.637***) under drought and irrigated treatment, respectively (Figure 3.14). Percent decrease in seed yield showed positive correlation with geometric mean of seed yield (0.610***) and geometric mean of number of seeds per pod (0.738***). Genotypes BRT 103-182 and FBN 1211-66 exhibited the lowest percentage decrease in the drought stress treatment, but had high values of yield (Figure 3.14). It can also be noted that percent decrease (PD) in seed yield due water stress was positively associated with drought susceptibility index (DSI) (Table 3.8).

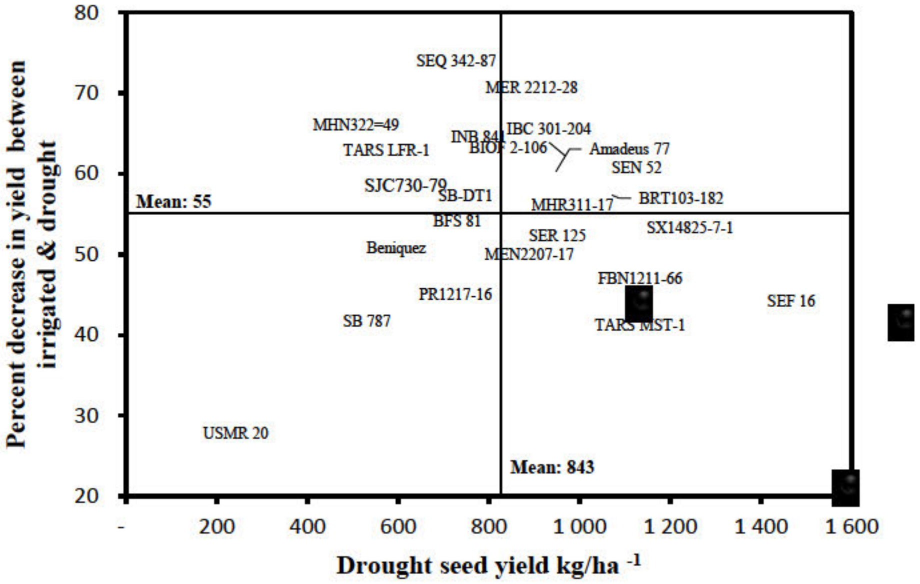

Figure 3.14: Relationship of percent yield decrease between irrigated & drought and grain yield kg.ha^{-1} under drought stress in field experiment at Chókwè Researcher Station. Higher yielding genotypes with lower percent decrease are in the right quadrant.

3.4 Discussion

Observations from both stratified pot system and field experiment highlighted the integration between morphology and physiological traits on water use under drought stress in 24 genotypes evaluated. As expected, leaf water potential showed lower values under drought stress compared to irrigated environment. Traub et al. (2017), who reported that stomatal conductance is an essential parameter to determine the level of drought tolerance among common bean genotypes. Bragg et al. (2004) also mentioned that, measurements taken by the porometer help to analyze the physiological status of the plants.

The stratified pot system showed no significant differences among genotypes in both drought and irrigated treatment. Leaf water potential ranged from 1.75 bar for BFS81 to 4. 5 bar for TARS MST-1 under drought stress and the same were observed for the genotypes under irrigated condition, with values ranging from 3.5 for BFS 8 to 8.4 bar for the genotype SEN52. The lower leaf water potential in the drought stress treatment was caused by low water in the soil due to the imposition of drought. Blum (2015) and Gonçalves et al. (2019) indicated that under dry land environment, the level of abscisic acid can significantly increase in some way, which causes closing of the stomata, resulting in decrease of the gas exchange rate of the leaf. Moreover, higher levels of abscisic acid in the leaves can reduce the rate of cell expansion, and consequently contribute to a decrease in the total shoot biomass of the plant. The reduction of cell expansion has an important function in reducing water loss by transpiration under water stress conditions. In the stratified pot system, leaf water potential showed 57.67% of reduction between irrigated and drought stress treatment.

The significant mean of leaf water potential in the irrigated treatment compared to drought, occurred because stomatal conductance is closely correlated with soil water status (Arruda et al., 2019; Traub et al., 2017). According to Oliveira et al. (2012), stomatal conductance can be considered an important factor that indicates water deficit in common bean. The lower values of leaf water potential recorded under drought stress reveal partial closing of the stomata as a mechanism of avoiding water loss through the leaves in peak periods of stress.

Dipp et al. (2017) and Gonçalves et al. (2019) in their studies using common bean genotypes under drought stress and irrigated at the reproductive stage, observed a decrease of 63.9% in yield and 74.3% in stomatal conductance under water deficit. Those results are closer to the results of this study where reductions of 49% for LGR, 53% for NL, 47% for shoot biomass and 57.67% in leaf water potential were observed. Leaf water potential as a physiological trait indicates that, even under drought, gas exchange continues in plants for photosynthetic processes to continue, and consequently affecting yield capacity. This is a crucial strategy that can be used in breeding programs as a way of increasing yield under drought stress environment, through selection of genotypes that have the best mean values of the trait and its introgression through crosses. As mentioned by Beebe et al. (2013) and Polania et al. (2016), the development of genotypes with maximum potential yield should be a process that involves assessment of adaptive traits since they are closely related with high yield in areas with water restriction.

Shoot biomass showed differences between drought and irrigated treatment, in stratified pot system. The observed mean of 47% reduction between irrigated and drought stress treatment indicate that the genotypes were impacted due to water deficit. The biomass produced is also one of the most useful criteria for the selection of tolerance to drought (Rosales et al., 2013). In stratified pot system TARS-MST-1, FBN1211-66 and MHR311-17, three of the genotypes with the highest biomass production under drought were also the ones with the highest yield under drought stress in the field study, suggesting that shoot biomass production can be one of the attributes for drought tolerance. However, according to Ceccarelli et al. (2007) and Kazai et al. (2019), to achieve maximum yields in terminal drought, a genotype not only requires to produce a large amount of biomass, but must transform it into grain, a condition that was contrary in the case of DOR 390, a genotype that produced abundant biomass at half filling of grain, but its yield was low, therefore, does not present a good accumulation in the reproductive organs, during drought stress.

On seed yield parameters, the drought intensity index (DII) indicates the degree of stress to which the genotypes were subjected and the adequacy of the conditions that were taken in this study to identify common bean lines with tolerance to drought stress. For the field experiment, DII higher than 0.50 were recorded, which is considered high and can cause reductions of 20-100% in biomass production, yield and its different parameters in common beans (Beebe et al., 2006; Muñoz-Perea et al., 2007). The DII values obtained were similar to those found in other studies, from 0.57 to 0.67 (Androcioli et al., 2020; Chai et al., 2016) and in studies conducted in tropical environment such as Puerto Rico, with 0.50 to 0.64 (Polania et al., 2016). The DII value obtained in this study, clearly show optimum conditions of identifying outstanding genotypes that are tolerant to terminal drought stress.

The expressive value of DII of 0.58 obtained in this study, was caused by earlier suppression of irrigation before flowering, low rainfall throughout the growing season and high evapotranspiration. As a result, the reproductive phase that is the most sensitive to drought stress in common beans was affected (Muñoz-Perea et al., 2007) resulting in low yield. Moreover, days with high evapotranspiration were observed throughout the crop cycle, however, this factor only caused incipient wilting of the plants during midday and early afternoon, according to observations made by Campostrini (2018) and Ribeiro et al. (2018).

From the 24 genotypes assessed in the field, the highest yielding under drought stress was SEF 16 followed by SEN 52, BRT 103-182, SX 14825-7-1, IBC 301-204, Amadeus 77, MHR 311-17, TARS MST-1, BIOF 2-106 and SER 125, as shown by the high geometric mean (GM) and relatively low drought susceptibility index (DSI). GM and DSI are considered the best parameters to select drought resistant genotypes (Hummel et al., 2018; Ramirez-vallejo & Kelly, 2014; Ribeiro et al., 2018). The significant correlation that these variables had with yield components and seed yield performance under drought confirms the findings of Hummel et al. (2018); Ramirez-vallejo & Kelly (2014) and Ribeiro et al. (2018). Among the highest yielding genotypes under drought, SEN 52, SEF 16 and SER 125, were consistent in theirperformance as per observations made by CIAT under drought conditions (Beebe et al., 2008, 2014).

In this study drought stress clearly affected 100 seed weight of the genotypes, but it is also a trait closely linked to each genotype, therefore, the best way of interpreting the effect ofstress on this parameter is through yield reduction between non-stressed and drought stressed environment. This reduction is implicit in the grain filling index (GFI), which helps toexplain the response of genotypes to drought stress. In addition to the 100 seed weight, drought decreased the number of seed/m^2. Thus, genotypes that are able to keep a higher number of seed/m^2 under drought stress, such as SX 14825-7-1, MHR 311-17 and SEN 52, good seed filling such as SEN 56 and SER 16, BRT 103-182 and FBN 1211-66, obtain the highest yield under drought stress, inferring that a genotype tolerant to drought must produce a good amount of grains and be able to fill them.

There was no association between days to flowering and yield under both environments, since when the flowering stage started, drought stress was still very incipient. However, days to pod formation had a negative but highly significant correlation with seed yield performance in both drought stress and irrigated treatments, suggesting that early pod formation of a genotype is a trait related to greater tolerance to drought stress. In addition, physiological maturity was negatively associated with yield performance, as most of drought tolerant genotypes reached this stage much earlier. According to Ramirez-vallejo & Kelly (2014) and Ribeiro et al. (2018), one of the responses of the common bean plant to water stress is drought avoidance through shortening of the reproductive stages such as the number of days to reach maturity and grain filling. Beebe et al. (2013) and Rosales et al. (2013) considered early maturity to be an important characteristic for common beans' adaptation to drought, since the filling of the grain can be completed while there is still available water when compared to the later genotypes. The negative and highly significant association between the days to physiological maturity and the yield under drought conditions observed in this study, confirms the findings of Ceccarelli et al. (2007), Ribeiro et al. (2018) and Vincourt & Carolo (2018) in similar studies.

The objectives of most breeding programs that are oriented towards addressing abiotic stress are to develop genotypes that are capable of performing well in both water stressed and non-stressful condition. Considering this approach in our study, genotypes SEN 52, BRT 103-182, IBC 301-204, SEF 16 and SX14825-7-1 were the most outstanding under drought, and outperformed Amadeus, a commercial control.

3.5 Conclusion

Drought stress as an environmental condition, influenced the phenotypic expression of seed yield components (number of pods per plant, number of seeds per pod and 100-seed weight) of common bean. All these parameters were significantly reduced by drought stress, suggesting an instability of genetic variability for drought tolerance in *Phaseolus vulgaris.*

Under drought stress and irrigated conditions, the bean genotypes SEN 52, BRT 103-182, IBC 301-204, SEF 16 and SX14825-7-1 were the highest yielding and superior and can be recommended for stressful environments. Other genotypes that showed good yields under drought and acceptable yield in irrigation environments were TARS MST-1, FBN 1211-66, MHR 311-17 and IBC 301-204. These can be used as parents in breeding efforts, together with SER 125 and INB 841 that are traditionally used and already have traits for tolerance to drought stress.

One of the features related to greater capacity of drought tolerance, is the ability of a genotype to produce biomass and store it in its reproductive organs in order to accelerate maturity and produce a higher amount of seed per unit under drought stress. Since a positive association of percent reduction (PR) with drought susceptibility index (DSI) for seed yield was observed, these traits could be used in combination with the geometric mean yield (GM) to select drought resistant cultivars.

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4. CHAPTER FOUR

Evaluation of the response of common bean (*Phaseolus vulgaris* L.) genotypes for phosphorus use efficiency in root, shoot and yield components

Abstract

In most common bean production systems, yields are decreased by low phosphorus deficiency in the soil. Therefore, two experiments (pot and field) were conducted at Agricultural Research Institute of Mozambique – Chókwè Research Station to evaluate common bean genotypes for phosphorus use efficiency. In the pot study, there were two treatments combining two phosphorus levels x eight genotypes, laid out in a randomized complete block design with four replicates. The genotypes were grown in a system of stratified phosphorus applied as triple super phosphate at the rate of 25 mg P kg^{-1} of soil (low rate) and 200 mg P kg^{-1} of soil (high rate). In the field study, 24 genotypes were assessed in a split plot design with 4 replicates. The main plots were two phosphorus levels; high P applied as triple superphosphate at the rate of 40 kg ha^{-1}, and control level (low P) without phosphorus application; and the genotypes were randomly assigned to the subplots within each block. From the pot study, shoot biomass, root biomass, shoot P concentration, P uptake and P leachate, increased significantly in the high P treatment, whereas the genotypes showed variability in root biomass, shoot biomass and P uptake under low P treatment. Genotypes BFS 81, SEQ342-87 and IBC 301-204 performed better in terms of root and shoot biomass as well as phosphorus uptake under low P and can thus be recommended for low soil fertility environments, especially P deficient soils. Under field conditions, high phosphorus treatment significantly increased all yield components (pods per plant, 100-seed weight, and grain yield), and variability among genotypes was also observed for yield and yield components. Classification of genotypes based on the grain yield efficiency index (GYEI) enabled selection of 10 phosphorus use efficient genotypes that can be deployed for use in low phosphorus conditions as well as in common bean breeding programs.

***Key words**: Phosphorus stress, common bean, P use efficient, grain yield efficiency index.*

4.1 Introduction

Low availability of water and phosphorus contributes to the currently realized low bean yields in Southern Africa that average 600 kg ha^{-1} compared to the attainable yields of more than 1.500 kg ha^{-1} (Chekanai et al., 2018; Lynch & Brown, 2008; Namayanja et al., 2014). Phosphorus (P) deficiency has been reported to be one of the major limiting factors in crop production and productivity, mainly in the tropics and subtropics (Lynch & Brown, 2008; Ramaekers et al., 2010). The situation is worsened by the high cost of fertilizer and lack of capital that prevents many farmers from obtaining commercially available P fertilizer. Another limitation for P fertilization is its economic sustainability (Lynch & Brown, 2008) since periodic applications of fertilizers are needed in order to maintain high yields.

Compared to other macronutrients, P is the least mobile in the soil and therefore the least available to plants (Hinsinger, 2001; Namugwanya et al., 2014). The availability of phosphorus to the plants is determined by chemical and biological reactions, which cause only a small amount of phosphorus in the soil to be present as orthophosphate (Suriyagoda et al., 2014). The reactivity of orthophosphate (Pi) with iron and aluminium oxide in the soil results into compounds becoming unavailable to plants (Lynch & Brown, 2008). In addition, soil chemical and physical properties also affect the phosphorus availability. Sandy soils have low total P content and have limited retention of fertilizer P (Deckers et al., 2001; Namayanja et al., 2014).

An alternative approach to increase productivity in P-deficient soils is to enhance crop adaptation to low phosphorus availability (Fageria et al., 2008; Hoyos-Villegas et al., 2017). Mechanisms for improving P efficiency are associated with soil exploration and phosphate mobilization from unavailable P sources in the rhizosphere (Burridge et al., 2016; Lynch &

Brown, 2012). Traits that enhance topsoil foraging also improve P acquisition, such as shallower root growth angle (Lynch & Brown, 2012; Miguel et al., 2013).

Phosphorus is an essential element, playing important roles in numerous metabolic processes including photosynthesis, respiration, signal transduction and among others. According to Fageria & Baligar (2016) and Lazali et al. (2014), fertilization of common bean with phosphorus consistently showed a positive response on yield, shoot dry matter and number of pods. Chekanai et al. (2018) and Turuko & Mohammed (2014) reported an increased nodule number, weight and volume with the fertilization of phosphorus indicating more effective use of phosphorus.

Genetic and physiological components of plants have strong effects on nutrient use efficiency in plants (Turuko & Mohammed, 2014; Walk et al., 2006). Variation in phosphorus use efficiency is associated with the capability of plants to thrive in soils with phosphorus deficiency and this is attributable to their unique root system morphology, root hair density, and root exudates (Koevoets et al., 2016; Miguel et al., 2013). Fageria & Baligar (2016) also indicated that variations in nutrient use efficiency among genotypes are associated with differences in nutrient assimilation, translocation, shoot demand, dry matter production per unit of nutrient used, and environmental interactions. Variations in inter-specific cultivars for dry bean have been reported for yield components such as number of seeds per pod and weight of 100 grains, and these components have positive and significant associations with grain yield (Fageria & Baligar, 2016). These yield components are also under quantitative genetic control and are thus influenced by environmental elements including abiotic and biotic stresses (Fageria et al., 2012; Namugwanya et al., 2014). Da Silva et al. (2014) and Suriyagoda et al. (2014) indicated that grain yield harvest index (GHI) and grain yield efficiency index (GYEI) in crops are affected by the nature of genotype and environmental factors including phosphorus deficiency. Therefore, these indexes can be useful in separating common bean genotype selection for high grain yield and phosphorus use efficiency under differing soil phosphorus levels (da Silva et al., 2016; Mourice & Tryphone, 2012).

Mourice & Tryphone (2012), reported that beans in one growing season, remove approximately 12.5 kg P/ha from soil, which is higher compared to what is added in terms of phosphorus fertilization by resource-poor farmers. The average yield of the landraces as mentioned by Namugwanya et al. (2014), ranges from 0.3 to 0.5 tons per hectare, which is very low compared to the improved varieties that yield above 1.5 tons per hectare. The main causes for decreased yield include the intrinsically low yielding potential of landraces, biotic and abiotic stresses. Abiotic stresses comprise drought and low soil fertility and among the soil fertility factors, inadequate phosphorus (P) is one of the top abiotic factors negatively affecting bean production in the tropics (Namugwanya et al., 2014, 2018). Soils are considered deficient in phosphorus when the soil plant-availability of phosphorus contents is below 40 mg kg^{-1} P determined by Bray-1 method (Mourice & Tryphone, 2012). However, deficiency in phosphorus can be solved by corrective soil fertility amendment strategies such as applying fertilizers and liming acidic soils.

Use of phosphorus efficient genotypes which can utilize available phosphorous from limiting soil environments helps to increase productivity and decrease cost of fertilizer. Identification of dry bean genotypes that are capable of utilizing phosphorus efficiently will contribute to breeding efforts to develop cultivars for low P environments. The development of new cultivars with greater P use efficiency, integrated with best management practices will contribute to sustainable agricultural systems for the resource poor farmers in the tropical regions. The main objective of the study was to evaluate inter-specific differences on promising common bean genotypes used in a breeding effort for growth parameters, yield components, grain harvest index and P-use efficiency under low and high soil phosphorus levels. The specific objectives were to: (i) determine the effect of P on productivity of selected common bean lines at two different phosphorus levels (low and high rate of phosphorus); (ii) determine the effect of phosphorus levels on vegetative plant growth and yield, and (iii) assess variability of common bean genotypes in relation to phosphorus uptake under P-deficient soils.

4.2 Material and methods

4.2.1 Experimental Site and Research approach

The research consisted of evaluating common bean genotypes (*Phaseolus vulgaris* L.), from the Mesoamerican gene pool in two different environments. These genotypes have been characterized as drought-tolerant and drought-sensitive with some degree of tolerance to low P concentration and associated with root architecture (shallow and deep root system). Experiments were conducted at two locations: a) pots experiments were conducted in 20-liter containers filled with soil media in a stratified phosphorus system in the shelter at Agriculture Research Institute of Mozambique (IIAM) – Chókwè Research Station, Gaza province in the Republic of Mozambique, and b) a phosphorus study was conducted in the field site at Chókwè Research Station facilities in Chókwè located at 24° 30'S, 77° latitude, 33° 00'E longitude and an altitude of 33 m above sea level, Gaza province in the Republic of Mozambique.

4.2.2 Pot experiment – Chókwè Research Station

Pot experiments were conducted during the dry season. Seeds of eight genotypes comprising two phenotypes that differed in primary root branching were grown in a system of stratified phosphorus (Ho et al., 2005) as shown in Figure 4.1. Seeds were sterilized in 10% NaOCl for 3 min, rinsed twice with distilled water, pre-germinated in rolls of brown germination paper soaked in 0.5 mM CaSO4, and placed in a dark growth chamber for 48 h at 28ºC. The seedlings were then planted at a depth of 4 cm in pots of 30 cm diameter x 32 cm height which were filled with a mixture of sandy soil with physical and chemical properties shown in Table 4.1. The containers were separated into two layers, 0 to 8 cm and 8 to 32 cm depths by an irrigation ring (Ho et al., 2005). All trials were laid out as randomized complete block design with four replicates, two treatments combining 2 phosphorus levels (i) control: high phosphorus in both layers; (ii) stratified low phosphorus: high phosphorus in the top 0-8 cm, low phosphorus in the bottom 8-32 cm; x 8 genotypes with time of planting as the block.

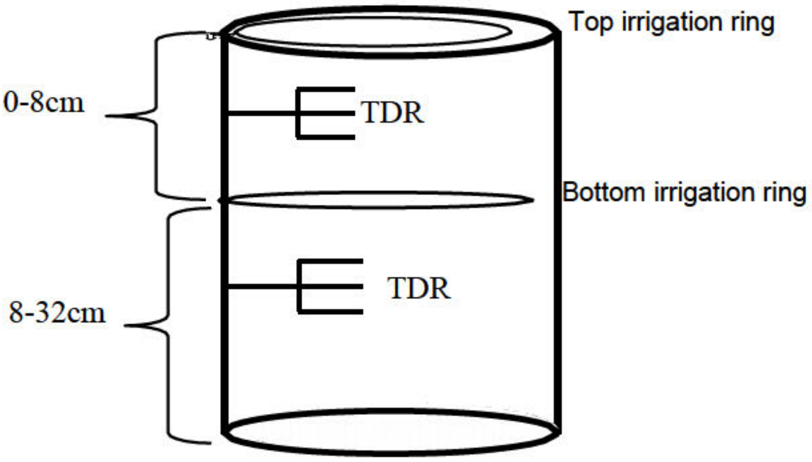

Figure 4.1: Stratified phosphorus pot system. Top 0-8 cm and bottom 8-32 cm, horizons separated by an irrigation ring. Figure was recreated from (Ho et al., 2005), and is not drawn to scale.

The fertilization in each pot was maintained using solid fertilizer that was mixed thoroughly with the media using a mixer and which contained (in grams per pot): 3 g urea, 20 g KNO3, and 8 g micromax granular micronutrients (6.0% Ca, 3.0% Mg, 12.0% S; 0.10% B 1.0% Cu, 17.0% Fe, 2.5% Mn, 0.05% Mo and 1.0% Zn), obtained from omnia. The P treatments were applied as triple super phosphate at the rate of 25 mg P kg^{-1} of soil as low level and 200 mg kg^{-1} of soil as high level. The low rate was determined to give a minimum of phosphorus enough to induce P stress, and based on soil chemical analysis that indicated very low phosphorus content about 3 ppm, which is below the threshold (Table 4.1). According to Henry et al. (2010) soils under bean cultivation are considered deficient if they contain less than 15 mg/kg of available phosphorus. The water supply in the system was maintained by placing two irrigation rings in each pot, one in the middle of the pot at 8 cm and another on the top of the pot, which permitted separate irrigation of the two layers. The irrigation was kept at field capacity and to avoid leaching, the top layer was irrigated first followed by watering the bottom layer to the saturation point. After germination, one plant was maintained in each pot. Plants were grown at field capacity moisture and every day desired amount of water was added to keep the moisture level.

Table 4.1: Chemical analysis of the soil used in pot experiment.

EC 1: 2.5	pH	pH	OM	C/N	Macronutrients					Al	H	Na	CEC
					N	P	K	Ca	Mg				
	(H2O)	(KCl)											
mS/cm			%		%	ppm	cmol$^{(+)}$/kg				cmol$^{(+)}$/kg		
0.06	6.3	5.15	0.49	14.21	0.02	3.3	0.34	0.66	0.32	0.0	0.0	0.26	1.58

Soil Texture							Ca/Mg	Mg/K	Ca+Mg/K
Sand	Silt	clay	Grade	Silt/clay	Coarse sand	Fine sand			
	%				%				
90.7	5.2	4.1	A	1.27	70.8	20	2.06	0.94	2.88

4.2.3 Field experiments at low and high phosphorus

A field site at IIAM- Chókwè Research Station in the Republic of Mozambique, was used to impose phosphorous stress treatments during the dry season of 2018. This site has defined screening plots for low soil fertility studies, particularly for P screening, therefore the P level is maintained depleted over time. Results from chemical analysis of soil sample taken before planting, indicated that the soil P availability was moderately low to induce P stress of the genotypes (Table 4.2). Twenty-four common bean genotypes that differ in root branching density were grown in phosphorus environment with irrigation. The experiment was set in a soil, whose 0-20 cm layer soil chemical and physical properties are shown in the table 4.2. A split plot design with 4 replicates was used, and main plots comprised of two phosphorus levels; high phosphorus (HP), where phosphorus level was maintained by applying mineral fertilizer-triple superphosphate (TSP 46%) with an application rate of 40 kg ha^{-1} applied as basal fertilization 10 days before planting, and control-low phosphorus (LP) without phosphorus fertilization, intended to simulate the real farmer's context in most bean production area. The sub-plot entries (twenty-four genotypes) were randomly assigned to the main-plots. Each genotype was planted in three 3.0 m rows spaced by 0.60 m inter-row and 0.10 m within-row plant spacing. There were buffer plots surrounding the entire field as well as 6.0 m buffer zone separating the high and low phosphorus. After planting the field was farrow irrigated to field capacity to ensure uniform germination.

Table 4.2: Chemical analysis of the soil taken in the Field at Chókwè Research Station between 0-20 cm depth.

EC 1: 2.5	pH (H_2O)	pH (KCl)	OM	C/N	Macronutrients N	 P	 K	 Ca	 Mg	Al	H	Na	CEC
mS/cm			%		%	ppm	cmol$^{(+)}$/kg				cmol$^{(+)}$/kg		
0.31	7.26	6.04	2.5	10.47	0.14	38.1	1.44	20.30	8.34	0.00	0.00	1.18	21.96

Soil Texture Sand	 Silt	 clay	 Grade	 Silt/clay	 Coarse sand	 Fine sand	Ca/Mg	Mg/K	Ca+Mg/K
	%				%				
16.9	50.2	32.4	FGL	1.54	8.7	8.2	2.43	5.59	19.89

4.2.4 Plant material and growing conditions

In the pot studies, eight elite lines developed for multiple abiotic stress (drought-tolerant and low soil fertility) and contrasting in root architecture were selected and used (Table 4.3). The criteria of grouping the elite lines were based on phenotypic characteristics of the root architecture, from a previous pilot study. All common bean (*Phaseolus vulgaris L.*) seeds used in the studies were propagated from seeds originally obtained from the International Center for Tropical Agriculture CIAT, Cali, Colombia. Due to limited space in the shelter where the pot experiment was conducted, eight genotypes were tested for shoot biomass, root biomass, shoot P concentration and P uptake assessment. For the field experiments at IIAM- Chókwè Research Station, a total of 24 lines were used to assess phenology, yield components and total yield per unit. Details of seed color, size and growth habit are described in the (Table 4.3).

Table 4.3: Characteristics of common bean cultivars evaluated under high and low phosphorus conditions.

Cultivar	Seed color	Seed size	Growth habit	Drought reaction	P stress reaction
Amadeus 77	Cream	S		Sensitive	
Beniquez	Cream	S		Tolerant	Tolerant
BFS 81	Red	S	II A	Tolerant	
BIOF 2-106	Red	S		Tolerant	Tolerant
BRT 103-182	Red	S		Tolerant	Tolerant
FBN 1211-66	Red	S	II A	Tolerant	Tolerant
IBC 301-204	Red	S		Tolerant	
INB 841	Brown	S	II A	Tolerant	Tolerant
MEN 2207-17	Black	S		Tolerant	
MER 2212-28	Red	S		Tolerant	Tolerant
MHN 322-49	Red	S	II A	Tolerant	
MHR 311-17	Red	S		Sensitive	Tolerant
PR 1217-16	Red	S		Sensitive	Sensitive
SB 787	Black	S		Tolerant	
SB-DT1	Black	S		Tolerant	
SEF 16	Red	S		Tolerant	Tolerant
SEN 52	Black	S	II A	Tolerant	
SEQ 342-87	Red	S		Tolerant	
SER 125	Red	S	II B	Tolerant	
SJC 730-79	Red	S		Sensitive	
SX 14825-7-1	Cream	S		Tolerant	
TARS LFR-1	Red	S		Sensitive	
TARS MST-1	Red	S		Sensitive	
USMR 20	Cream	S		Tolerant	Tolerant

S – small, maximum 25g/100 seeds; M – medium, between 25 – 40g/100seeds; Type IIA – indeterminate, completely upright; Type IIB – indeterminate, inclined upright.

4.2.5 Growth and phenological measurements

In the pot experiment, plants were harvested after 35 days after planting (DAP) and root and shoot biomass were harvested from each pot for total root biomass dry weight. Shoot biomass material was thoroughly washed in distilled water several times and was dried in an oven at 60 ◦C to a constant weight to determine shoot dry weight accumulation and tissue analysis. Dry tissue was ground and ashen at 490 ºC for 10 -12 h in a muffle furnace and analyzed for phosphorus content using spectrophotometry (Murphy & Riley, 1962). Phosphorous content of media in pot studies was determined by collecting leachate samples at 35 DAP from each pot and analyzed for its concentration (Murphy & Riley, 1962).

In the field experiment, phenology data as flowering and physiological maturity were recorded. At harvest time, yield components were determined by selecting a 0.5 m long row (0.3 m^2 area) of each plot, and five plants were harvested and put in paper bag for pod and seed weight, seed number and pod number per area. The rest of the two rows were harvested to determine total grain yield, 100 seed weight and seed moisture content. Grain yield efficiency index (GYEI = (grain yield at low P level/average grain yield of 24 genotypes at low P level) x (grain yield at high P level/average grain yield of 24 genotypes at high P level)) was assessed to classify genotypes for their P-use efficiency (Beebe et al., 2013; Fageria et al., 2010). Genotypes having GYEI values >1 were classified as efficient (E) P user, genotypes having GYEI values between 0.5 and 1 were classified as moderately efficient P user (ME) and those with GYEI values < 0.5 were classified as inefficient (IE) in P user.

4.2.6 Statistical Analysis.

The statistical software Minitab Ver. 16 (Minitab Inc. State College, PA, USA) was used for all data analyses. Data from the pot studies were analyzed as a randomized complete- block design, and a fixed model analysis of variance (ANOVA) was used for root and shoot biomass where P treatments, and replicates were the independent variables. Field studies were analyzed as a split-plot design and ANOVA, was performed for yield component, where genotypes and phosphorus levels are fixed variables and the block is a random variable. Correlation analysis was also performed for grain yield and yield components. In all cases, means that were significantly different statistically were separated using Tukey's Honest Significant Difference Test at 5 % probability level and all data were tested for normality using the Shapiro-Wilk test before ANOVA.

4.3 Results

4.3.1 Grain Yield and Growth parameters

Analysis of variance indicated that there was a significant effect of phosphorus and interaction (Genotype x P) on the plant's variables grown under the two levels of phosphorus (high and low P) in stratified pot system. The genotypes grown under high phosphorus performed better compared to those under low phosphorus (Figure 4.4).

Table 4.4: Mean squares for the variables of eight common bean genotypes evaluated under P stress

Source	DF	RB	SB	LGR	LC	SPc	Pup	[P] L
Rep/Block	3	4.850	4.266	1.028	5.345	1193.4	23.07	17.416
Genotype	7	3.301^{ns}	1.960^{ns}	5.956**	6.00*	0.5177*	491.7*	4.694**
P	1	1.078***	10.125**	2.759**	16.01***	2.3350***	4831.3***	72.152***
Genotype x P	7	1.810**	3.875^{ns}	7.893***	10.24*	0.5074**	131.9**	4.570**
Error	111	5.139	4.583	8.959	20.03	19.4160	533.8	5.800

* Significant at *: $P \leq 0.05$; **: $P \leq 0.01$; and ***: $P \leq 0.001$; respectively; NS: not significant; DF- Degrees of Freedom; RB – Root biomass; SB - Shoot biomass; LGR- leaf growth rate; LC – Leaf count of trifoliate; SPc Shoot P concentration (%); Pup – Phosphorus uptake; [P]L – [P] leachate.

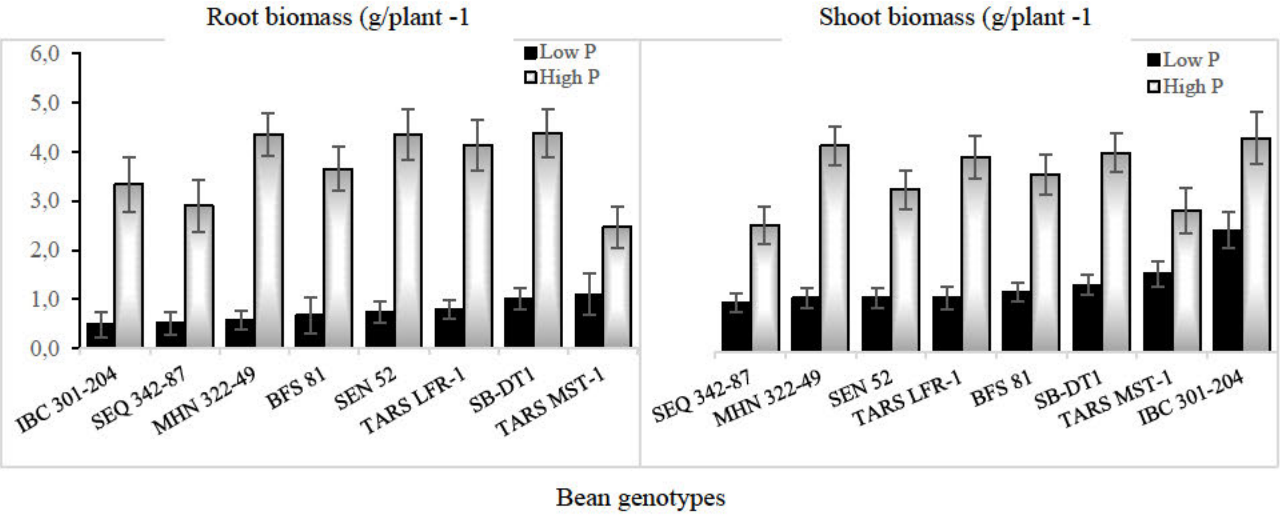

Figure 4.2: Effects of phosphorus and genotype interactions on root and shoot biomass at 32 DAP.

There was a highly significant effect ($p < 0.05$) of phosphorus levels on all variables tested (root biomass, shoot biomass, leaf growth rate, number of trifoliate, shoot P concentration, P uptake and [P] in the leachate samples) in the stratified pot system experiment (Table 4.4).

Total shoot and root biomass assessed 35 DAP increased significantly under high phosphorus level and decreased in low P treatment. The effect of phosphorus levels on root and shoot biomass was highly significant ($p < 0.05$), which resulted in shoot and root biomass reduction with P levels, being lower and higher under low and high P levels, respectively. Likewise, leaf growth rate and leaf count recorded throughout the experiment, also showed highly and significant lowering effects of phosphorus ($p<0.05$) reduction, which reduced the total number of leaves and leaf growth rate under low P treatments. The effect of genotypes and phosphorus by genotypes interaction was also significant for all with exception of one variable tested (Table 4.4). The general trend indicated that root and shoot biomass for all genotypes increased as P increased. The response to high P treatment was high for genotypes SB-DT1, MHN-322-49 and TARS LFR-1. Genotypes TARS MST-42 and SEQ 342-47 showed low increase in root and shoot biomass at high P treatment (Figure 4.2).

Phosphorus analysis from leachate samples also taken at 32 DAP showed higher concentration in high P than in low P treatments in stratified phosphorus pot system ($p<0.05$). In addition, analysis of plant tissues of the stratified phosphorus system revealed highly significant effect of P ($p<0.05$) in percentage shoot P concentration and total plant P uptake between high and low P treatments (Table 4.5).

Table 4.5: Effect of P levels on root biomass, shoot biomass, shoot P concentration, and P uptake of eight genotypes.

P level	RB (g/plant)	SB (g/plant)	LGR (mm)	LC	SPconc. (%)	Pup (mg P/plant)	[P]L
HP	3.75 a	4.57 a	10.54 a	10.15 a	1.37b	39.19a	1.52 a
LP	0.72 b	1.72 b	5.25 b	2.87 b	1.39b	29.14b	1.22 b
Mean	2.24	3.14	7.89	6.51	1.38	34.16	1.38
CV%	63.9	70.0	16.9	35.6	30.2	55.3	65.0
LSD	0.50	0.775	0.46	0.81	0.146	6.18	0.456

Means followed by the same letter within the same column are not significantly different ($P \leq 0.05$). RB – Root biomass; SB - Shoot biomass; LGR- leaf growth rate; LC – Leaf count of trifoliate; SPc Shoot P concentration (%); Pup – Phosphorus uptake; [P]L – [P] leachate

Genotypes effect on assessed growth parameters were not significant at ($p< 0.05$). In general, all genotypes increased root biomass when P was added as treatment. However, genotypes SB-DT1 and TARS MST-1 were the ones that most increased root biomass with values of 1.2 and 1.01 grams per plant. Values for root biomass ranged from 2.47 g/plant $^{-1}$ for TARS MST-1 to 4.38 g/plant $^{-1}$ for SB-DT-1 under high P, and from 0.48 g/plant $^{-1}$ for IBC301-204 to 1.09 grams/plant $^{-1}$ for SB-DT-1 under low P treatment. As observed, genotype SB-DT-1 had the highest value of root biomass (1.01 and 4.38 g/plant^{-1}) in both low and high P treatment, while genotype TARS MST-1 compared to the other genotypes had less roots in the high P treatment and more roots in low P (Figure 4.2).

All genotypes had more shoot biomass under high levels of P. Shoot biomass varied from 3.63 g/plant^{-1} for TARS MST-1 to 5.50 g/plant^{-1} for IBC 301-204 in high P treatment and from 1.25 g/plant^{-1} for SEQ342-87 to 3.12 g/plant^{-1} for IBC 301-204. Although genotype IBC 301-204 had less root biomass among all genotypes under Low P, it showed superiority in producing more shoot biomass in both treatments (low and high P) (Figure 4.2).

Table 4.6: Variability of bean genotypes with respect to root biomass, shoot biomass, shoot P concentration, and P uptake**.**

Genotype	Root biomass	Shoot biomass/plant	LGR (mm)	Leaf count	Shoot P (%)	P uptake (mg P/plant)	[P] leachate
BFS 81	2.35 ab	3.04 ab	8.72 a	6.30 ab	1.43ab	37.90 a	1.31b
IBC 301-204	1.91ab	4.31a	7.06 b	7.06 ab	1.50ab	41.80 a	1.46a
MHN 322-49	2.46 ab	3.34 ab	8.11ab	6.19 ab	1.56b	37.90 ab	1.46a
SB-DT1	2.70 a	3.42 ab	7.20 b	7.41 a	1.57a	38.25 a	1.32b
SEN 52	2.54ab	2.78 ab	8.40 b	5.54 b	1.25ab	32.00 ab	1.34ab
SEQ 342-87	1.71 b	2.25 b	7.74 ab	6.29 ab	1.27ab	28.20 b	1.34ab
TARS LFR-1	2.47 ab	3.19 ab	8.08 ab	7.05 ab	1.22b	29.15 b	1.35ab
TARS MST-1	1.78 ab	2.81ab	7.77 ab	6.24 ab	1.24	28.10 b	1.47a
Mean	2.24	3.14	7.89	6.51	1.38	34.16	1.38
CV%	63.9	70.0	16.9	35.6	30.2	55.33	65.0
LSD	1.002	1.55	0.93	1.62	0.29	12.35	0.91

Means followed by the same letter within the same column are not significantly different ($P \leq 0.05$).

Under low P, the genotypes SB-DT1 and TARS MST-1 performed better by increasing both root and shoot biomass, and at the same time the genotype TARS MST-1 had less root and shoot biomass under high P level when compared to other genotypes (Figure 4.3). Equally, the ratio root/shoot biomass, indicated that genotypes BFS 81, SEN 52 and TARS LFR-1 allocated relatively more biomass when compared to the other genotypes.

Statistically, there was no significant difference ($p \leq 0.\ 05$) in shoot P concentration, between low and high P treatments among genotypes. Shoot P concentration ranged from 1.19 for genotype MHN-322-49 to 1.35 for SB-DT-1 under low P and from 1.42 for genotype SEQ342-87 to 1.70 for SEN52 in high P treatment. Equally, a negligible change in P concentration was observed for genotypes BFS 81, SEQ342-87, IBC 301-204 and TARS LRF-1 under high P, when compared with low P treatment (Figure 4.3). Under low P, all genotypes had low shoot P concentration, whereas genotype SEN 52 and TARS MST-1 had relatively high shoot P concentration under high P.

Overall, P uptake did not increase significantly between low and high treatments among genotypes. However, the genotype IBC 301-204 had the highest P uptake among all genotypes at both low and high P treatment, followed by BFS 81 and SEQ342-87, whereas genotypes TARS LRF-1 had the lowest (Figure 4.3).

P concentration in leachate sample did not show significant differences ($p \leq 0.\ 05$) between low and high P treatments among genotypes. P concentration in leachate sample, ranged from 2.11 for BFS 81 to 3.40 for SB-DT1. Genotypes SEN 52, TARS MST-1 and SEQ342-87 had the lowest concentration under low P treatment compared to the rest of the genotypes (Figure 4.3).

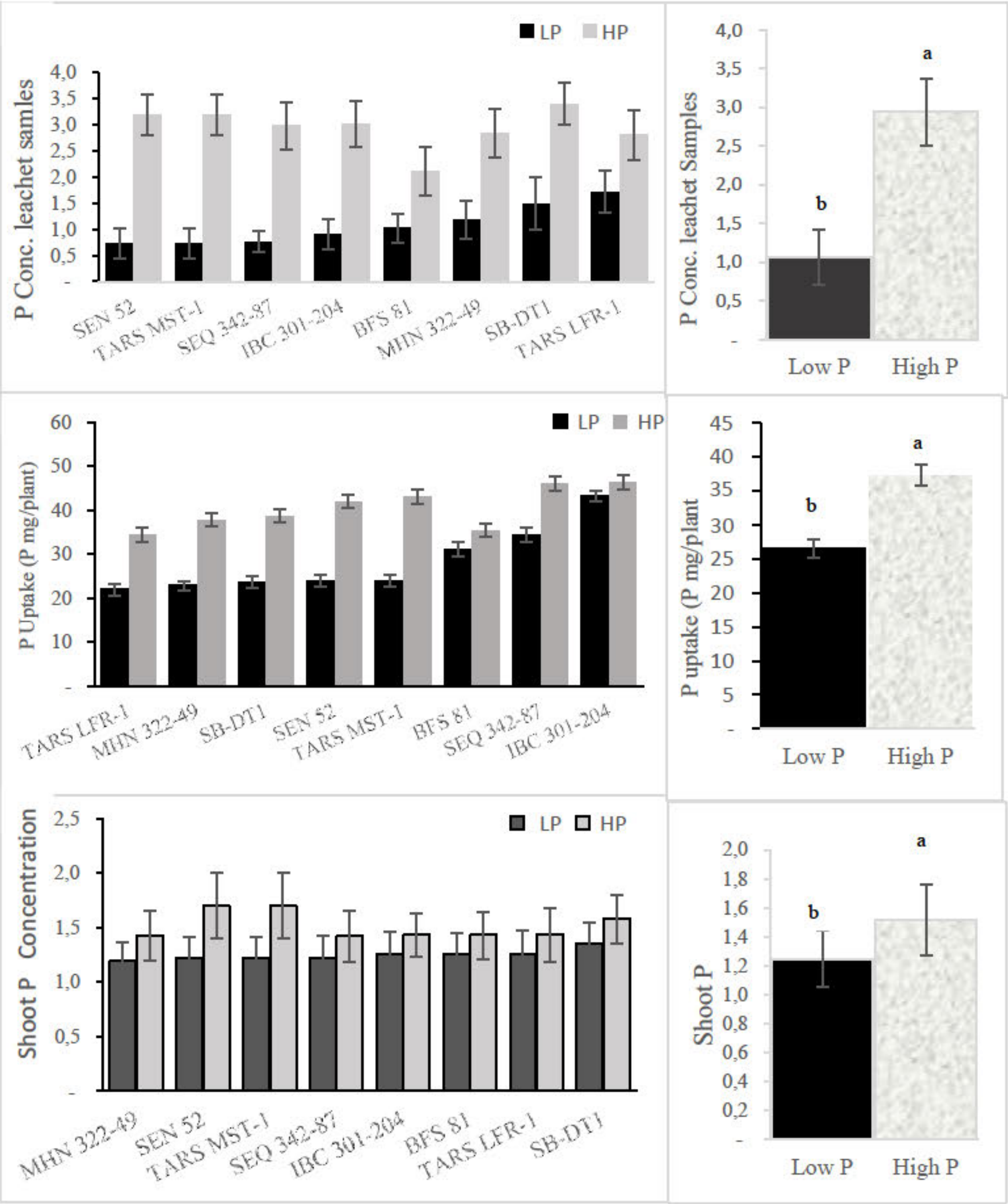

Figure 4.3: Shoot P Concentration, plant P uptake and P concentration in the leachates of the assessed genotypes. Bars indicate standard error of the mean.

4.3.2 Yield components and yield associated plant parameters

Analysis of variance indicated that there was highly significant effect of phosphorus levels on all tested variables under field study at Chòkwé research station. Phosphorus treatment showed significant ($p < 0.05$) effects on the number of pods/plant, seeds/pod, 100-seed weight, pod harvest index and grain yields. With the exception of number of seed/pod, which decreased under high P, all parameters increased under high P treatment (Table 4.7).

Table 4.7: Effect of P levels on number of pods, seed/pod, 100 seed weight, PHI and seed weight of 24 genotypes.

P level	Pod.plant $^{-1}$	Seed.pod $^{-1}$	100 Seed weight (g)	Seed weight g.m $^{-2}$	PHI %	yield Kgha^{-1} x1000
Low P	15.90a	5.73a	23.10b	371.79b	72.78b	2.025b
High P	18.66b	5.53b	26.46a	471.92a	75.78a	3.386a
Mean	17.27	5.63	24.78	421.85	74.28	2.706
CV%	50.8	10.8	16.6	41.3	11.5	39.2

Means followed by the same letter within the same column are not significantly different ($P \leq 0.05$).

There was significant ($P \leq 0.05$) variability among genotypes on number of pods/plant, seeds/pod, pod harvest index, grain yields and 100-seed weights. Genotype MEN 2207-17 had the highest number of pods/plant, while genotype USMR 20 had the lowest (Table 4.7). Genotype MHR 311-17 had the highest number of seeds/pod, while USMR 20 had the lowest. Hundred-seed weight was higher for genotype USMR20 and lowest for MEN 2207-17 compared with other genotypes. Genotype SEQ 342-87 had the highest pod harvest index, while TRS LFR-1 had the lowest for this parameter. For seed yield (g/m^{-2}) and grain yield (kg ha $^{-1}$), genotype BFS 81 had both the highest seed yield (g/m^{2}) and grain yield (kg ha^{-1}), while Beniquez had the lowest seed yield and grain yield (Table 4.8).

Table 4.8: Variability of bean genotypes with respect to pods/plant, seeds/pod, PHI, 100-seed weight grain yields.

Genotype	Pod Nr/plant	Seed Nr./pod	PHI %	100 Seed Weight	Seed yield g m-2	yield Kg/ha
Amadeus 77	18.62	6.29	78.66	25.17	375.00	2,853.50
Beniquez	13.38	5.34	64.30	21.34	274.00	1,421.00
BFS 81	15.67	5.69	78.13	27.00	506.50	3,984.50
BIOF 2-106	16.30	5.78	72.17	22.17	305.00	3,289.00
BRT 103-182	15.95	5.80	74.61	24.50	342.50	2,586.00
FBN 1211-66	19.22	5.54	72.53	21.67	505.00	3,039.00
IBC 301-204	16.43	5.99	75.44	26.67	472.00	2,627.00
INB 841	17.55	5.84	76.31	20.84	489.00	3,000.00
MEN 2207-17	34.12	5.85	71.51	20.00	400.00	2,061.50
MER 2212-28	18.32	6.22	76.99	25.50	489.00	2,961.50
MHN 322-49	16.22	5.30	77.82	24.17	388.00	2,500.50
MHR 311-17	17.60	6.43	78.14	24.83	372.00	2,769.00
PR 1217-16	14.48	5.40	75.47	26.67	444.50	2,637.50
SB 787	16.58	5.35	74.71	25.84	401.50	2,907.00
SB-DT1	17.84	5.94	75.32	24.50	420.00	2,459.50
SEF 16	17.22	5.98	72.77	25.67	444.00	2,959.50
SEN 52	18.42	5.89	77.63	23.34	481.50	3,537.50
SEQ 342-87	19.12	5.59	81.74	20.33	447.50	3,107.50
SER 125	16.12	5.09	77.08	27.00	485.50	3,089.50
SJC 730-79	14.47	5.57	70.81	26.50	468.00	2,669.50
SX 14825-7-1	17.55	5.42	72.58	24.84	401.50	2,007.50
TRS LFR-1	13.46	5.30	60.91	27.50	295.00	1,685.00
TRS MST-1	19.47	5.67	73.77	22.00	427.50	2,504.00
USMR 20	9.73	3.88	73.40	36.67	339.50	2,274.50
Mean	17.24	5.63	74.28	24.78	415.58	2,705.46
CV%	61.5	10.2	13.3	14.2	14.2	14.6
F-Test	3.72	4.31	3.8	2.15	15.74	119.89
P level (P)	*	**	*	ns	***	***
Genotype (G)	ns	***	***	**	*	***
P x G	ns	**	ns	*	***	ns

$^{*, **, ***, ns}$ Significant F values at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.01$ respectively; ns: not significant.

Statistically, significant differences were observed for P levels, genotypes and interaction between phosphorus levels x genotype for seed yield (g m^{-2}) and grain yield (kg ha^{-1}), indicating a greater response of genotypes for P levels (Table 4.8). From all 24-genotype assessed, seed yield ranged from 114 g m^{-2} for genotype TARS LFR-1 to 583 g m^{-2} for genotype SER 125, with an average of 372 g m^{-2} under low P treatment. Similarly, seed yield under high P treatment ranged from 317 g m^{-2} for genotype USMR 20 to 673 g m^{-2} for genotype PR 1217-16 with an average of 472 g m^{-2}. Grain yield kg.ha^{-1}, showed highly significant effects of phosphorus ($p<0.05$), and ranged from 984 kg ha^{-1} for genotype Beniquez to 3,111 kg ha^{-1} for genotype BIOF 2-106, with an average of 2,025 kg ha^{-1} of under low P treatment, while under high P treatment, yield kg ha^{-1} ranged from 1,858 kg ha^{-1} for genotype Beniquez to 5,203 kg ha^{-1} for genotype BFS 81, with an average grain yield of 3,386 kg ha^{-2}. Overall, seed yield increased 21.18%, while grain yield was 40.19% more in soil P levels from increased fertilizer application rate of 20 kg P_2O_5 to 40 kg ha^{-1} of P_2O_5.

Table 4.9: Analysis of Variance and means of Grain yield and seed sample dry weight of 24 common bean genotypes at two soil P levels.

Genotype	Seed yield g m-2		Yield Kg/ha	
	Low P	High P	Low P	High P
Amadeus 77	412 abcde	338 a	2,506 bcdef	3,201abcd
Beniquez	139 ab	409 a	984 a	1,858 a
BFS 81	532 de	481 a	2,766 def	5,203 e
BIOF 2-106	272 abcd	338 a	3,111 f	3,467 bcd
BRT 103-182	298 abcde	387 a	1,979 abcdef	3,193 abcd
FBN 1211-66	486 cde	524 a	2,491 bcdef	3,587 bcd
IBC 301-204	426 bcde	518 ab	1,600 abcd	3,654 bcd
INB 841	534 de	444 a	2,599 bcdef	3,401 bcd
MEN 2207-17	406 abcde	394 a	1,963 abcdef	2,160 ab
MER 2212-28	470 cde	508 a	2,280 abcdef	3,643 bcd
MHN 322-49	290 abcde	486 a	1,937 abcdef	3,064 abcd
MHR 311-17	363 abcde	381 a	2,197 abcdef	3,341bcd
PR 1217-16	216 abc	673 b	1,181 ab	4,094 cde
SB 787	378 abcde	425 a	2,011 abcdef	3,803 cde
SB-DT1	383 abcde	457 a	1,671 abcde	3,248 abcd
SEF 16	354 abcde	534 ab	1,591 abcd	4,328 de
SEN 52	537 de	426 a	3,053 ef	4,022 cde
SEQ 342-87	356 abcde	539 ab	2,653 cdef	3,562 bcd
SER 125	583 e	388 a	2,852 def	3,327 bcd
SJC 730-79	384 abcde	552 ab	1,486 abcd	3,853 cde
SX 14825-7-1	333 abcde	470 a	1,261 abc	2,754 abc
TARS LFR-1	114 a	476 a	1,210 ab	2,160 ab
TARS MST-1	296 abcde	559 ab	1,679 abcde	3,329 abcd
USMR 20	362 abcde	317 a	1,543 abcd	3,006 abcd
Mean	372	472	2,025	3,386
CV%	14.2	14.6	35.2	22.4
F-Test	15.74		119.89	
P Level (P)	***		***	
Genotype (G)	*		***	
PxG	***		***	

*,*** Significant F values at $P \leq 0.05$ and $P \leq 0.001$ respectively; Means in the same column followed by the same letter are not significantly different at the 5% probability level by Turkey test.

Phosphorus level and genotype showed significant effect on all yield components and associated plant parameter with exception of pods per plant, and pod harvest index under low P treatment. Interactions of P level with genotypes had a significant effect on number of seed per pod, but did not have significant effects on pods per plant, pod harvest index and 100 seed per plant suggesting that the response of these yield components/variables were not influenced by altering the P levels (Table 4.9).

The number of pods per plant for most genotypes was slightly higher at low P level than at high P level. Under low P treatment, pods per plant ranged from 10.53 for the genotype Beniquez to 51.27 pods for genotype MEN 2207-17, with an average pod per plant of 18.61; while in high P it was 5.07 for genotype USMR20 to 20.8 for genotype TARS MST-1 with an average of 15.9 pods.

Seed per pod which were high under low P level varied from 4.97 for Beniquez to 6.73 for MHR 311-17 genotype with an average 5.72 pods, while in high P level the range was from 2.77 pods for USMR 20 genotype to 6.13 pods for MHR 311-17 with an average of 5.53 pods. MEN 2207-17 was the most outstanding genotype which produced the highest pods per plant, surpassing the commercial varieties (checks) Beniquez and Amadeus 77.

At low P treatment, 100-seed weight ranged from 17 g for Beniquez to 39.33 g for USMR 20 with an average value of 23.10 g, while at high P level 100-seed weight ranged from 20.67g for MEN 2207-17 and MER 2212-28 genotypes to 34 g for USMR 20, with an average of 26.46g. In both low and high phosphorus treatments, genotype USMR 20 had the highest 100-seed weight, in spite of performing poorly in most assessed traits, and this is attributed to its seed size being USMR 20 a large seeded genotype.

Table 4.10: Number of pods, seed per pod and 100 grain weight of 24 common bean genotypes

Genotype	Pod Nr/plant		Seed Nr./pod		PHI %		100 Seed Weight	
	Low P	High P	Low P	High P	Low P	High P	Low P	High P
Amadeus 77	19.00b	18.23cde	6.47bde	6.10a	78.47a	78.85bcd	20.67abcd	29.67bcde
Beniquez	10.53b	16.23bcde	4.97a	5.70bcd	69.27a	59.33b	17.00c	25.67abcd
BFS 81	19.80b	11.53a	5.80abcde	5.57bcd	77.77a	78.48bcd	25.67cd	28.33abcde
BIOF 2-106	13.60b	19.00cde	5.73abcde	5.83bcd	69.44a	74.90bc	19.67abcd	24.67abcd
BRT 103-182	15.73b	16.17bcde	5.93abcde	5.67bcd	72.25a	76.96bcd	22.67abcd	26.33abcd
FBN 1211-66	21.10b	17.33bcde	5.20abc	5.87bcd	70.88a	74.17bc	20.67abcd	22.67abc
IBC 301-204	17.73b	15.13bcde	6.00abcde	5.97cd	79.53a	71.35bc	25.67cd	27.67abcde
INB 841	20.20b	14.90bcde	6.20bcde	5.47bcd	76.72a	75.89bcd	21.00abcd	20.67b
MEN 2207-17	51.27a	16.97bcde	5.73abcde	5.97bcd	75.92a	67.10ab	19.33abc	20.67b
MER 2212-28	18.87b	17.77bcde	6.53de	5.90bcd	78.52a	75.46bc	26.00b	25.00abcd
MHN 322-49	17.27b	15.17bcde	5.60abcde	5.00bc	73.87a	81.76cd	22.33abcd	26.00abcd
MHR 311-17	17.13b	18.07cde	6.73a	6.13a	75.91a	80.36bcd	23.33abcd	26.33abcd
PR 1217-16	15.33b	13.63bcd	5.80abcde	5.00bc	72.12a	78.81bcd	21.67abcd	31.67de
SB 787	15.73b	17.43bcde	5.40abcd	5.30bcd	74.36a	75.05bc	23.67bcd	28.00abcde
SB-DT1	18.20b	17.47bcde	5.87abcde	6.00cd	78.12a	72.51bc	22.00abcd	27.00abcde
SEF 16	21.73b	12.70bc	5.93abcde	6.03cd	71.08a	74.46bc	24.00bcd	27.33abcde
SEN 52	18.87b	17.97bcde	5.87absde	5.90bcd	75.60a	79.66bcd	22.00abcd	24.67abcd
SEQ 342-87	18.40b	19.83de	5.67abcde	5.50bcd	74.85a	88.62d	18.33ab	22.33ab
SER 125	19.20b	13.03bc	5.27abc	4.90b	75.66a	78.50bcd	25.00cd	29.00bcde
SJC 730-79	15.30b	14.47bcde	5.20ab	5.93bcd	64.00ab	77.62bcd	24.67bcd	28.33abcde
SX 14825-7-1	18.13b	16.97bcde	5.53abcd	5.30bcd	68.89a	76.27bcd	25.00cd	24.67abcd
TARS LFR-1	11.12b	15.80bcde	5.40abcd	5.20bcd	50.04b	71.78bc	24.67bcd	30.33cde
TARS MST-1	18.13b	20.80e	5.60abcde	5.73bcd	72.18a	75.36bc	20.00abcd	24.00abcd
USMR 20	14.38b	5.07b	4.98b	2.77c	71.31a	75.49bc	39.33a	34.00a
Mean	18.62	15.90	5.73	5.53	72.78	75.78	23.10	26.46
CV%	61.5	20.5	10.2	9.7	13.3	8.7	14.2	14.6
F-Test	3.72		4.31		3.80		32.85	
P level (P)	*		**		*		***	
Genotype (G)	ns		***		***		***	
P x G	ns		**		ns		ns	

$^{*,\ **,***\ NS}$ Significant F values at P ≤ 0.05, P ≤ 0.01 and P ≤ 0.01 respectively; ns: not significant. Means in the same column followed by the same letter are not significantly different at the 5% probability level by Turkey's test.

Interrelationships between yield components and associated plant parameters with grain yield indicated that the number of pods per plant was negatively and highly correlated with grain yield at high P level (r = - 0.632***) and not significantly correlated (r = - 0.191) with grain yield at low P level (Table 4.12). At low P, the number of seeds/pod was negatively correlated with 100-seed weight ($r = -0.732$***) indicating that genotypes with average number of seeds/pod were high, their 100-seed weight was low at low P level (Table 4.11). Pod harvest index was positively correlated with grain yield in both high and low P treatment (0.453** and 0.531***), respectively.

Table 4.11: Correlation matrix of yield and yield components at low P treatment

Parameter	yield Kg/ha	Pod Nr/plant	Seed Nr./pod	PHI % LP
100 Seed W LP	-0.175 ns	-0.191 ns	-0.732***	-0.069 ns
PHI % LP	0.453**	0.329 ns	0.187 ns	
Seed Nr./pod	0.201ns	0.234 ns		
Pod Nr/plant	0.16 ns			

$^{**,***,\,ns}$ Significant F values at $P \leq 0.01$ and $P \leq 0.001$; ns: not significant.

Table 4.12: Correlation matrix of yield and yield components at high P treatment

Parameter	yield Kg.ha^{-1}	Pod Nr. Plant^{-1}	Seed Nr.pod $^{-1}$	PHI %
100 Seed W HP	0.146 ns	-0.632***	-0.2 ns	0.053 ns
PHI % HP	0.531***	0.063 ns	0.302 ns	
Seed Nr./pod	0.272 ns	0.35 ns		
Pod Nr/plant	-0.164 ns			

$^{**,***,\,ns}$ Significant F values at $P \leq 0.001$; ns: not significant.

4.3.3 Phosphorus Use Efficiency Genotypes

Grain yield efficiency index (GYEI) was used to classify the genotypes for phosphorus use efficiency. Based on GYEI, genotypes were classified as P efficient; moderately P efficient and inefficient P genotypes (Table 4.13). In this study, based on GYEI, ten genotypes (Amadeus 77, BFS 81, BIOF 2-106, FBN 1211-66, INB 84, MHR 311-17, SB 787, SEN 52 and SER 125) were classified as P efficient users, and twelve genotypes (BRT 103-182, IBC 301-204, MEN 2207-17, MHN 322-49 PR 1217-16, SB-DT1, SEF 16, SEQ 342-87, SJC 730-79, SX 14825-7-1, TARS MST-1, USMR 20) as moderately P efficient utilizers, and the remaining Beniquez and TARS LFR-1 were classified as inefficient P utilizers. Based on this grouping, 46% of the genotypes were phosphorus efficient, 46% were classified as moderately P efficient and 8% were classified as P inefficient, suggesting that dry bean genotypes have high variability in P use efficiency.

Table 4.13: Classification of dry bean genotypes to P use efficiency index based on grain yield.

Genotypes	P-Use efficiency index	Classification*
Amadeus 77	1.17ab	E
Beniquez	0.27b	IE
BFS 81	2.10a	E
BIOF 2-106	1.57ab	E
BRT 103-182	0.92ab	ME
FBN 1211-66	1.30ab	E
IBC 301-204	0.85ab	ME
INB 841	1.29ab	E
MEN 2207-17	0.62ab	ME
MER 2212-28	1.21ab	E
MHN 322-49	0.87ab	ME
MHR 311-17	1.07ab	E
PR 1217-16	0.71ab	ME
SB 787	1.12ab	E
SB-DT1	0.79ab	ME
SEF 16	1.00ab	ME
SEN 52	1.79ab	E
SEQ 342-87	1.38ab	E
SER 125	1.38ab	E
SJC 730-79	0.83ab	ME
SX 14825-7-1	0.51b	ME
TARS LFR-1	0.38b	IE
TARS MST-1	0.82ab	ME
USMR 20	0.68b	ME

*E = efficient, ME = moderately efficient, and IE = inefficient. Means in the same column followed by the same letter are not significantly different at the 5% probability level by Duncan's test.

4.4 Discussion

The results from the pot experiment, showed genotypes that allocated a larger fraction of root biomass under low phosphorus treatment and are capable to thrive under limiting P environments. Results from this study agree with the hypothesis that common bean genotypes differ in their ability to adapt when P is limiting (Beebe et al., 2014). The genotypes that had greater shoot biomass at low P treatment suggest an improved strategy in foraging phosphorus under P-limiting conditions, or use of assimilated P more efficiently to produce relatively large quantities of biomass.

Mourice & Tryphone (2012) stated that at low nutrient availability, plants partitioned a large fraction of their resources to the root system, thus leaf growth and expansion is limited causing a decline in above ground biomass and consequently, seed yield. Therefore, this indicates that phosphorus deficiency limits leaf expansion, resulting in less carbon assimilation that have consequence in low shoot biomass production under low P treatment. Lynch (2013) and Miller et al. (2003) also reported that in the low P treatment, plants may respond to limited phosphorus by diverting large portion of their net carbon assimilation to the production of heterotrophic rather than photosynthetic tissues, which at the end results in an increased root: shoot ratio. The genotypes SB-DT1 and TARS MST-1 were among those that produced high shoot biomass under low P treatment, and can thus be classified as P efficient. This implies that the P in the system was somehow adequate for them or they invested a greater portion of the absorbed phosphorus to the roots for improved soil exploration to support shoot biomass production.

Phosphorus analysis in plant tissues indicate that the critical phosphorus concentration for common bean, below which normal plant growth process may not occur, is 0.2% (Namugwanya et al., 2014). The non-significant variation between low and high phosphorus treatments in shoot P concentration could be attributed to the fact that shoot biomass increased under high P treatment for all genotypes, and a dilution effect might have occurred (Mourice

& Tryphone, 2012; Namayanja et al., 2014). This is where phosphorus is distributed within a larger amount of plant biomass as was observed in our study in the high P treatment. Nevertheless, as observed in this study, shoot P concentration was relatively higher at higher P treatment in most of the cases.

According to Namugwanya et al., (2014), plant P uptake not only depends on P available in the soil, but also on plant adaptation and traits such as root architecture, presence of adventitious roots (Lynch, 2015; Miguel et al., 2013) and exudation of anions in the rhizosphere (Arruda et al., 2018). An increase in phosphorus absorption as a result of increase in P availability among genotypes is reported in a study by Fageria & Baligar (2016) where shoot biomass and P uptake were positively associated at both low and high P for bean cultivars. The variation in P uptake among cultivars across P treatments illustrates the diversity in efficiency in which genotypes are able to absorb phosphorus from the soil at different availability levels. Therefore, P uptake can be considered a good indicator of P acquisition since it combines both shoot biomass and shoot P content. Hence, bean genotypes, which perform better or poorly for either of the two variables (i.e., shoot biomass or shoot P concentration) can be easily identified and these variables can be used as selection criteria.

The increment in the number of pods per plant when P levels is high was confirmed by Gonçalves et al. (2019). However, in this study, phosphorus x genotype interaction was not significant for the variable pods per plant suggesting that perhaps P is not the only element required for pod formation, although P deficiency has adverse effects on pod formation and pod filling in legumes as reported by Marschener (1998) and Namayanja et al. (2014).

Though the number of seeds per pod is an important yield component, it was not correlated to the P levels in all the assessed bean genotypes, agreeing with the results reported by Suriyagoda et al. (2014) from a study on Faba beans. Namugwanya et al. (2014) also found small differences between the number of seeds per pod in mung bean (*Vigna radiata* L.) at different rates of phosphorus and potassium, suggesting that this parameter is genetically controlled or is controlled by multiple environmental factors.

Although in this study significant effect of phosphorus was observed on the variable number of seeds per pod, this trait was negatively affected by high P treatment. It was found that the number of seeds per pod, increased in low P treatment, contradicting to what was found by Wasaya et al. (2018), that reported an increased number of seeds per pod when P level was increased.

Fageria et al. (2012) found that seed weight (100 - seed weight) is controlled by a small number of genes, meaning that the variable's response is influenced by environmental factors. Fageria et al. (2010) and Zhu et al. (2010) observed that drought reduced 100- seed weight by 13%. In our study, this trait was negatively affected when P level increased, as it was also negatively correlated with the number of seeds per pod, suggesting that P may not be the only element that influences seed weight.

Phosphorus is a crucial element for increasing bean productivity as shown by the response of bean cultivars to higher P fertilization. Although grain yield increased with an increase in phosphorus application, cultivars varied in the degree of response to high P levels, implying that bean genotypes differ both within and between phosphorus treatments. Under low P availability, photosynthesis rate is decreased in bean cultivars (Amir et al., 2020; Lazali et al., 2014), thus leading to low seed yield, contrary to those at high P levels. In most situations, seed yield is the most important component for the grower; therefore, it is a fundamental selection criterion for genotypes targeted for low soil fertility environments. Grain yield efficiency index is widely reported to be critical in identifying variation in P-use efficiency among crop species and genotypes within species including dry bean genotypes (Fageria et al., 2010, 2012). Fageria & Baligar (2016) reported that plants differ widely in their capability to thrive in soils with low P, and this has been attributed to several plant characteristics, including morphology of root system and root hair density. However, bean genotypes that are able to maintain some degree of adaptation under low P levels, may be considered efficient and thus worthy of further investigation for inclusion in crop improvement programs. In this study, according to GYEI, genotypes TRANS LER 1 and Beniquez were classified as P use inefficient and genotypes BFS 81, BIOF 2-106 and SEN 52 had the highest GYEI and should be considered for inclusion in breeding for low fertility tolerance, especially phosphorus deficiency.

4.5 Conclusion

Common bean genotypes differ significantly in root and shoot biomass, grain yield production, yield components as well as phosphorus uptake and P use efficiency. Although some genotypes exhibited an outstanding performance in terms of shoot biomass P uptake and yield, improvement of soil fertility would still be very important if economical bean production is to be undertaken in agrosystems with soils that are poor in P, as used in this study. The field experiment in this study was conducted in soil deficient in phosphorus and this represents typical soils to which most common bean production systems take place. Genotypes BFS 81, BIOF 2-106 and SEN 52 were outstanding under low P treatment and based on GYEI, they were the most P efficient genotypes, while TRANS LER 1 and Beniquez genotypes were most P inefficient. The GYEI appears to be an effective indexing tool to use in identifying dry bean genotypes that are efficient utilizers of P. They can therefore be considered for incorporation into breeding program for low soil fertility tolerance. In addition, the three P efficient bean genotypes showed good potential for higher economic yield when P fertilizers are used.

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5. CHAPTER FIVE

Root architecture traits related with drought and phosphorus tolerance in common bean (*Phaseolus vulgaris* L)

Abstract

Roots are key organs for water and nutrient acquisition and transport. Therefore, root phenes that are associated with adaptation to low phosphorus environment could enhance top soil exploration, while deeper allocation is important for acquiring water and mobile nutrients. The understanding of interactions among root phenes can help in the development of genotypes adapted to drought and low fertility through genetic improvement. Two experiments (pot and field) were conducted at the Agricultural Research Institute of Mozambique IIAM to assess the contribution of root phenes to shoot biomass and grain yield under combined stress of drought and low phosphorus in common bean genotypes. The pot study assessed eight genotypes in a randomized complete block design with four replicates, four treatments combining two water levels (drought and non-stress) and phosphorus levels (200 mg P kg^{-1} soil, and 25 mg P kg^{-1} soil). Field study, plants were also grown in high and low phosphorus environment (40 kg P ha^{-1} and control - without P application) under irrigation and limited water in a split plot arranged in randomized complete block design where phosphorus and water levels were each main plots and the genotypes were randomly assigned to the main plots. Grain yield from field data under drought and P stress were correlated with the pot data on root traits. Response of root phene to drought and phosphorus stress appeared to be related to the deep and shallow root systems, respectively. Deep rooted genotypes produced more total root biomass, high taproot lateral branching density, which resulted in high total root length under drought and low P stress. On the other hand, shallow rooted genotypes had low total root biomass and less taproot lateral branching. Increase in shoot biomass and grain yield under drought and low P stress was associated with higher mean values of taproot lateral branching density and total taproot length. Genotypes SER 125, BFS 81, FBN12111-66 and MER 22 11-28, showed greater scores of tap root branching density in the pot study with the highest grain yield in the field under low P and drought stress. Therefore, these can be recommended for use in low phosphorus and drought stress environment or serve as parents for improving phosphorus efficiency use and drought tolerance in common bean.

Keywords: *root phenotyping; drought tolerance; root traits; phosphorus stress, common bean*

5.1 Introduction

Common bean is the most consumed grain legume crop compared to other legumes. Despite its importance, yield of common bean in developing nations is currently very low, only one-third of what is achievable in developed nations, where high inputs are used and irrigation is available to reduce abiotic and biotic limiting factors (Beebe et al., 2014; Lynch & Brown, 2008; Porch et al., 2013; Ramaekers et al., 2010). However, more than half of the global production occurs in marginal lands where people have limited access to food (Beebe et al., 2013; Porch et al., 2013). In most of the bean production areas, drought and soil infertilityare major constraints to production since access to irrigation and fertilizer is very limited. Therefore, the development of cultivars with superior adaptation to limited soil resources is an effective method for increasing yields in harsh environments (Hanslin et al., 2019; Lynch, 2018).

While shoots have an important role in utilizing water and nutrients, the root system is strictly responsible for the acquisition of these resources (Strock et al., 2018). The importance of roots for adaptation to nutrient and water limitations is explained by the increases in root growth compared to shoot growth under edaphic stress (Ho et al., 2005; Strock et al., 2018). Moreover, it has been demonstrated that the root system has a significant effect on bean yield under water stress (Adams et al., 2002; Kaeppler et al., 2011).

According to York et al. (2013), root phenotypes are composed of phenes which are the important elements that are under quantitative genetic control and thus influenced by environmental interactions. Root phenotypes play important roles in improving crop yield under edaphic stress by increasing the metabolic efficiency of soil exploration and by allocating roots in soil domains where several limiting resources are mostly available (Jaramillo et al., 2013; Lynch, 2018).

The three most principal plant nutrient resources are phosphorus (P), nitrogen (N), and water that often interfere with plant growth (Lynch, 2018). Phosphorus is typically more available in the top soil due to deposition of plant residues at the soil surface and low mobility in the soil (Lynch, 2011; Richardson & Simpson, 2011). Roots have an important role in water and nutrients absorption in the plants. Assessment of root traits of common bean by phenotypic analysis under drought stress showed the importance of variations in rooting patterns, which also include deep rooting systems that help to provide water from deeper soil layers (Beebe et al., 2013, 2014; Burridge et al., 2016). Different ideotypes of root systems have been identified for better crop adaptation either for individual or combined abiotic stress conditions (Polania et al., 2016; Zheng et al., 2020).

In *Phaseolus vulgaris,* root phenes that influence soil foraging depth comprise mainly of basal root growth angle (Liao et al., 2001; Miguel et al., 2013; Rangarajan et al., 2018; Yan et al., 2004), basal root whorl number (Miguel et al., 2013; Miller et al., 2003; Ochoa et al., 2006; Rangarajan et al., 2018; Walk et al., 2006), adventitious root abundance (Miller et al., 2003; Ochoa et al., 2006; Rangarajan et al., 2018), and lateral root branching density (Rangarajan et al., 2018).

The development of crop cultivars with enhanced productivity in limited water and nutrient availability is a fundamental strategy in addressing the challenges of low productivity (Lynch, 2019; Lynch & Brown, 2008). Since roots are fundamental for water and nutrient acquisition and transportation, drought as well as nutrient deficiencies are difficult to sustain and mitigate, therefore, an understanding of the fitness landscape of root phenotypes is essential for the development of crop cultivars with improved productivity under soil resource acquisition. The objectives of this study were to assess the contribution of root system to tolerance to low P and drought and to identify genotypes with greater response of lateral branching under suboptimal water and phosphorus supply.

5.2 Material and Methods

5.2.1 Experimental conditions

An experiment was conducted at Chókwè Research Station in pots of 30 cm diameter x 32 cm height which were filled with sandy soil. Seeds of eight genotypes contrasting in root branching were grown in a system of stratified water and phosphorus (Ho et al., 2005) as shown in Figure 3.1 in chapter 3. Seeds were sterilized in 10% NaOCl for 3 min, rinsed twice with distilled water, pre-germinated in rolls of brown germination paper soaked in 0.5 mM CaSO4, and placed in a dark growth chamber for 48h at 28ºC. The seedlings were then planted at a depth of 4 cm in pots separated into two layers, 0 to 8 cm and 8 to 32 cm depths by an irrigation ring.

The fertilization in each pot was applied as solid fertilizer that was mixed thoroughly with the media using a mixer and which contained the following in grams per pot: 3 g urea, 20 g KNO3, and 8 g Micromax granular micronutrients (6.0% Ca, 3.0% Mg, 12.0% S; 0.10% B 1.0% Cu, 17.0% Fe, 2.5% Mn, 0.05% Mo and 1.0% Zn), obtained from Omnia company. Phosphorus levels were maintained by using granular triple superphosphate at the rates of 25 mg P kg^{-1} of soil and 200 mg kg^{-1} of soil as low and high levels, respectively and was mixed thoroughly with the media. The water supply in the system was maintained by placing two irrigation rings in each pot, one in the middle of the pot and another on the top, which permitted separate irrigation of the two layers. The irrigation frequency depended on the pot water content in the pots that was monitored by TDR probes. In the bottom layers, the moisture content was maintained at a constant level in all treatments to simulate normal field conditions, while in the water stress treatment irrigation was withheld 7 days (progressive water stress with no watering after 7 days of growth) in order to simulate terminal drought stress conditions. To avoid waterlogging, the top layer was irrigated first followed by watering the bottom layer to the saturation point. The drip ring irrigation used in the experiments (36 cm diameter) supplied 70-80 ml of water in 3 minutes of irrigation, an amount sufficient to maintain adequate growth of the plants.

Field study was conducted at IIAM - Chókwè Research Station in the screening block with high and low soil fertility, in a soil, whose 0-20 cm layer soil chemical and physical properties were pH (H_2O) = 7.26; organic matter = 2.5 %; C/N = 10.14; P = 38.1ppm; K = 1.44 cmol$^{(+)}$/kg; Ca = 20.30 cmol$^{(+)}$/kg; Mg = 8.34 cmol$^{(+)}$/kg ; Al = 0.0 cmol$^{(+)}$/kg; CEC = 21.96 cmol$^{(+)}$/kg; sand = 16.9%; silt = 50.2% ;clay = 32.4%; coarse sand = 8.7%; fine sand = 8.2%; grade = FGL; silt/clay 1.54; Ca/Mg=2.43; Mg/K=5.59; Ca+Mg/K=19.89. The soil P availability at this site, is moderately low and enough to impose P stress for the genotypes, since the soil P concentration of 38.1 ppm, is higher than the threshold value that is 5 ppm for common bean crop. Twenty-four common bean genotypes that differed in root branching density were grown in high and low phosphorus environment under imposed drought and irrigation.

5.2.2 Plant Material

For the pot study, a total eight elite lines developed for multiple abiotic stress (drought-tolerant and low soil fertility) and contrasting in root architecture were selected, categorized into group then planted. The criteria of categorical grouping were based on phenotypic characteristics of the root architecture, from a previous pilot study. Root morphological components and phenes of the common bean are described in the (Figure 5.1). All common bean (*Phaseolus vulgaris L.*) seeds used in the studies were propagated from seeds originally obtained from the International Center for Tropical Agriculture CIAT, Cali, Colombia. Due to limited space in the shelter where the pot experiment was conducted, eight genotypes were tested. For the field experiments at IIAM-Chókwè Research Station, a total of 24 lines were used. Details of seed color, size and growth habit are described in (Table 5.1).

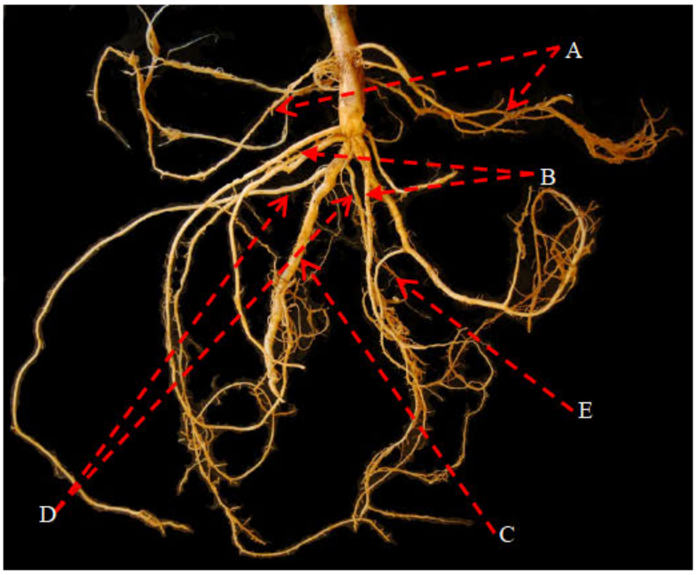

Figure 5.1: Root morphological components of the common bean. a) adventitious root; b) basal root; c) taproot; d) lateral roots 1st order; E) lateral root 2nd order. Root crown was obtained from field experiment at Chókwè Research station, Republic of Mozambique.

Table 5.1: Characteristics of common bean cultivars evaluated under water-stressed and unstressed conditions

Cultivar	Seed color	Seed Size	Growth habit	Drought reaction	P stress reaction
Amadeus 77	Cream	S	IIA	Sensitive	
Beniquez	Cream	S	IIA	Tolerant	Tolerant
BFS 81	Red	S	II A	Tolerant	Tolerant
BIOF 2-106	Red	S		Tolerant	Tolerant
BRT 103-182	Red	S		Tolerant	Tolerant
FBN 1211-66	Red	S	II A	Tolerant	Tolerant
IBC 301-204	Red	S		Tolerant	
INB 841	Brown	S	II A	Tolerant	Tolerant
MEN 2207-17	Black	S		Tolerant	
MER 2212-28	Red	S		Tolerant	Tolerant
MHN 322-49	Red	S	II A	Tolerant	
MHR 311-17	Red	S		Sensitive	Tolerant
PR 1217-16	Red	S		Sensitive	Sensitive
SB 787	Black	S		Tolerant	
SB-DT1	Black	S		Tolerant	
SEF 16	Red	S		Tolerant	Tolerant
SEN 52	Black	S	II A	Tolerant	
SEQ 342-87	Red	S		Tolerant	
SER 125	Red	S	II B	Tolerant	
SJC 730-79	Red	S		Sensitive	
SX 14825-7-1	Cream	S		Tolerant	
TARS LFR-1	Red	S		Sensitive	
TARS MST-1	Red	S		Sensitive	
USMR 20	Cream	S		Tolerant	Tolerant

S – small, maximum 25g/100 seeds; M – medium, between 25 – 40g/100seeds; Type IIA indeterminate, completely upright; Type IIB – indeterminate, inclined upright.

5.2.3 Experimental Design

The pot experiment design was a randomized complete block design (RCBD) with four replicates, four treatments combining two water levels (drought and non-stress) and phosphorus levels (high P:200 mg P kg^{-1} of soil, and low P: 25 mg P kg^{-1} of soil) as bellow described:

(i) Control: high water and high phosphorus in both layers;

(ii) Stratified low phosphorus: high phosphorus in the top 0-8 cm, low phosphorus in the bottom 8-32 cm, and adequate water in both layers;

(iii) Stratified low water: low water in the top 0-8 cm, adequate water in the bottom 8-36 cm, and high phosphorus in both layers and

(iv) Stratified low water and phosphorus: high phosphorus and low water in the top 0-8 cm and low phosphorus and adequate water in the bottom 8-36 cm layers.

For field study, plants were grown in high and low phosphorus environment with full irrigation and limited water. The experiments were laid out in a split plot design and replicated 4 times, where phosphorus and water levels were each main factor. The high phosphorus (HP) level, was maintained by applying 40 kg ha^{-1} of mineral fertilizer- triple superphosphate (TSP 46%) applied as basal fertilization 10 days before planting, and control-low phosphorus (LP) level without phosphorus fertilization, intended to simulate the real farmer's context in most bean production; while irrigation treatment had two soil moisture levels: well-watered (WW), where optimum soil moisture level was maintained by supplying water at field capacity until the crop had reached pod formation stage), and water stressed (WS), where soil moisture was kept at field capacity from planting up to 23 days after emergence and thereafter water was supplemented when necessary until the crop had reached pod formation stage. The sub-plot entries (twenty-four genotypes) were randomly assigned to each main-plot. Each genotype was planted in three rows of 3.0 m length, inter-row spacing of 0.60 m and within-row plant spacing of 0.10 m. There were buffer plots surrounding the entire field as well as 6.0 m buffer zone separating the high and low phosphorus. After planting, the field was furrow irrigated at field capacity to ensure uniform germination. The experimental unit consisted of 3 rows planted in a 3.0 m row length spaced by 0.60 m and within-row plant spacing was 0.10 m.

There were buffer plots surrounding the entire field as well as 4.0 m buffer zone separating the irrigated and no-irrigated plots. Weed control was managed manually using hoe and insecticides and fungicides were applied as needed.

5.2.4 Shoot and root measurements

Shoot and root biomass were harvested between 35 and 42 DAP for the pot trials. Shoot tissue was dried at 60ºC until constant mass.

Roots were harvested by root type and horizon. The entire primary root was sampled for visual measurement of primary root lateral branching. The remaining root biomass from each horizon was stored separately and dried at 60ºC until constant mass and then weighed for root biomass allocation assessment in each treatment.

Total root length (cm) of all genotypes was determined by calculating the specific root length from five root crown randomly selected. The entire tap root was analyzed by imageG for root length, then dried and weight to be used for extrapolation for the rest of the crowns for apparent total root length and tap root form the system.

Roots from field experiment were phenotyped at flowering (approximately 40 days after planting) by excavating and washing root crowns of subsamples of three plants in each plot for visual evaluation. Root crown examination included counting of the number of basal root (BRN), basal root whorl number (BRWN), adventitious root abundance score (1= none; 2 = 2–4 roots; 3 = than 4 roots), number of representative adventitious roots (greater than 1.5 mm diameter) (ARN) and taproot lateral branching and length (Burridge et al., 2020). Other measurements included basal root growth angle (BRGA), stem diameter, and taproot diameter. To relate pot root architecture to field root architecture, comparisons were made in subsets of the genotypes grown in the pot experiment under drought and low fertility conditions.

Root architecture from pot crowns was correlated to seed yield to determine the influence of individual root phenes on seed yield in the field.

Root distribution in the soil profile was determined from soil cores taken 2-3 days before root harvest (at flowering). Three subsamples of soil cores of three representative genotypes of each phenotypic class (dense and sparse lateral branches) were taken. Cores were taken in-row between two neighboring plants at a distance of 5 cm between cores. The cores were divided in depth increments of 10 cm, and depending on the depth of profile reached, 3-6 sections were sampled, washed, dried and weight for specific root length determination.

5.2.5 Statistical Analysis

The statistical software Minitab Ver. 16 (Minitab Inc. State College, PA, USA) was used for all data analyses. Prior to all statistical tests, the normality of the data was determined using the Shapiro-Wilk test, and where data did not meet normal distribution, data transformation was performed. Significant correlations and differences for all data analyses were considered at $p \leq 0.05$ and at $p \leq 0.01$. Pearson's correlation analysis was used for comparisons of phenes measured in pot study with the same phenes measured in the field for each treatment. These correlation analyses were performed using the genotype means for each phene in pot trial and in the field.

5.3 Results

5.3.1 Variation of root traits of common bean genotypes

No significant differences were observed between the soil water treatment regime for all the root variables except for taproot lateral length, taproot lateral number and adventitious root length ($P < 0.05$) (Table 5.2). There were highly significant ($P < 0.01$) differences among the interaction effect between genotypes and water treatment regime for the variables taproot lateral length (TRLL), taproot lateral branching (TRLB) and adventitious root length (ARL).

Table 5.2: Mean squares for root traits of 8 common bean genotypes evaluated under low P and drought stress treatment.

Source	DF	TRL	TRLN	TRLL	TRLB	TRD
Rep/Block	3	111.0	154.29	613.1	15.81	2.6087
Genotype	7	268.5**	37.52^{ns}	229.7^{ns}	18.03**	1.1390^{ns}
P	1	487.6^{ns}	181.13**	37.0^{ns}	10.50*	0.0394^{ns}
H$_2$O	1	90.8^{ns}	41.42**	491.2**	2.80^{ns}	1.2159^{ns}
Genotype x P	7	281.3**	35.88^{ns}	275.6*	9.26^{ns}	0.7062^{ns}
Genotype x H$_2$O	7	1413.0**	98.48^{ns}	133.2^{ns}	14.64**	0.4842^{ns}
P x H$_2$O	1	155.7*	32,83**	2.6^{ns}	6.98**	1.4468^{ns}
Genotype x P x H$_2$O	7	45.1^{ns}	62.64^{ns}	122.5^{ns}	11.22**	0.2652^{ns}
Error	70	179.9	50.78	264.8	10.01	0.8255

*Significant at $P \leq 0.05$; **Significant at $P \leq 0.01$; ^{ns}Non-significant; DF-Degrees of Freedom; TRL – Taproot length; TRLN–Taproot lateral number; TRLL – Taproot lateral length; TRLB – Taproot lateral branching; TD -Taproot Diameter.

Table 5.2 continued: Mean squares for root traits of 8 common bean genotypes evaluated under low P and drought stress treatment

Source	DF	BRN	BRL	BRWN	ARN	ARL
Rep/Block	3	6.446	10.2	0.6352	40.16	608.7
Genotype	7	4.571^{ns}	164.2^{ns}	0.7087^{ns}	18.54^{ns}	215.5^{ns}
P	1	5.745*	90.1^{ns}	0.0362^{ns}	0.43^{ns}	781.1*
H$_2$O	1	2.517^{ns}	93.9^{ns}	1.6333^{ns}	0.07^{ns}	669.6*
Genotype x P	7	2.274*	272.9*	0.1006^{ns}	31.53*	226.9^{ns}
Genotype x H$_2$O	7	2.461^{ns}	196.1^{ns}	0.2491^{ns}	14.67**	296.8*
P x H$_2$O	1	0.768^{ns}	10.0^{ns}	0.6539^{ns}	0.18**	145.0^{ns}
Genotype x P x H$_2$O	7	3.676^{ns}	208.5^{ns}	0.4211^{ns}	24.35^{ns}	109.3^{ns}
Error	70	3.648	191.8	0.3311	17.45	185.0

*Significant at $P \leq 0.05$**Significant at $P \leq 0.01$; ^{ns}Non-significant; DF - Degrees of Freedom; BRN – Basal root number; BRL- Basal root length; BRWN-Basal Root Whorl Number;; ARN – adventitious root number; ARL- adventitious root length.

Interaction effect between genotype and phosphorous had equally significant influence on the total taproot length (TRL), taproot lateral length (TRLL), basal root number (BRN) and adventitious root length (ARL) (Table 5.2). SB-DT1 was the highest performing genotype and SEQ 343-87 the lowest among the eight used genotypes (Table 5.4, Figure 5.2).

Root allocation differs between genotypes and showed a significant interaction effect of phosphorus level and water treatment regime on total taproot length, taproot lateral branching and adventitious root number, though there was no significant interaction effect of genotype, phosphorus levels and water treatment regime, except for taproot lateral branching (p=0.01; Table 5.2). Genotype SB-DT1 always performed better in all taproot variable, regardless of treatment, compared to the other seven genotypes (Table 5.4). There was no statistical difference for total root length (TRL) Taproot lateral length (TRLL) basal root length (BRL), basal root whorls number (BRWN) and adventitious root number (ARN), when phosphorus was limiting. SEN 5 allocated relatively more in total root biomass and total taproot length, after genotype SB-DT1(Tables 5.2 and 5.4).

5.3.2 Pearson correlation coefficients of common bean root variables

Under drought and low phosphorus conditions a positive and significant correlations were observed among root phenes. Likewise, root phenes were also correlated to the shoot biomass and seed yield under combined low phosphorus and drought environment, (Table 5.3).

Shoot biomass production showed a positive correlation with total root length (r =0.782***) and grain yield (r = 0.264***). Likewise, taproot biomass (0.936 ***) was significant and positively correlated to total root biomass (r = 0.801***), taproot length (r = 0.936 ***), basal root number (r = 0.813**), basal root whorl number (r = 0.483*) and grain yield (r= 0.264*). A negative and significant correlation was observed between shoot biomass with taproot biomass (r = -0.063 **), total root biomass (r = -0.402 ***), and total root length (r = 0.402**) (Table 5.3). The related taproot phenes were also significantly correlated among themselves

Taproot lateral branching was negative and significantly correlated with taproot diameter (r = -0.298**) and positively correlated with the grain yield (r = 0.118**); specific root length showed negative correlation with the grain yield (r = -0.66 **), taproot lateral branching (r = 0.739*) and basal root number (r= -0.703**).

Table 5.3: Correlation coefficients (r) among measured traits.

	SB	TRB	tRB	TRL	SRL	TRLB	TD	BRN	BRWN	ARN
TRB	-0.06**									
tRB	-0.40***	0.80***								
TRL	0.78***	0.94***	0.66**							
SRL	-0.40**	0.63	1.00***	0.66						
TRLB	0.46*	0.78*	-0.74	-0.45	-0.74*					
TD	0.45	0.19	0.22	-0.21	0.29	-0.29**				
BRN	0.15	0.81**	0.39	0.55	-0.70**	-0.57	-0.70			
BRWN	0.36	0.48*	0.78**	0.06	0.09	-0.30	- 0.19	0.90***		
ARN	0.45	0.67	0.46	0.15	0.57	0.70	0.81	-0.591	-0.75*	
GY	0.26**	0.46*	-0.45**	0.66	-0.66**	0.12**	0.47	0.148	0.16	0.39

Correlation is significant at 0.05 level; at 0.01 level** and at 0.001 level***; shoot biomass in g.plant^{-1}(SB), taproot biomass (tapbio) in g.plant^{-1}, total root biomass in g.plant^{-1} (tRB), tap root length in cm.plant $^{-1}$(TRL), Specific root length in cm.plant $^{-1}$ (SRL), taproot laterals branching.plant^{-1} (TRLB), tap root diameter in mm.plant $^{-1}$(TD); basal root number.plant^{-1}(BRN), basal root whorls number.plant^{-1}(BRWN), adventitious root number. plant^{-1} (ARN) and grain yield of 24 genotypes under low P and drought stress.

Based on phenotypic categorical grouping criteria, genotypes MER 2212-28, MHN 322-49, SB-DT1, SB 787, SJC730-79, MHR 311-17, PR 1217-16, TARS MST-1, SEQ 342-87, SX 14825-7-1, BFS 81 and SEF 16 were superior in branching density, with a score above 15 in taproot lateral branching under drought and low phosphorus environment. These genotypes were ranked as deep rooted and suited to allocating more roots at deeper horizons (Figure 5.1). The genotypes which scored lower in taproot lateral branching under limited phosphorus and water were SEN 52, USMR 20, BIOF 2-106, TARS LFR-1, BRT 103-182, MEN 2207-17, FBN 1211-66, IBC 301-204, Beniquez, Amadeus 77, INB 841 and SER 125, and were ranked as shallow rooted.

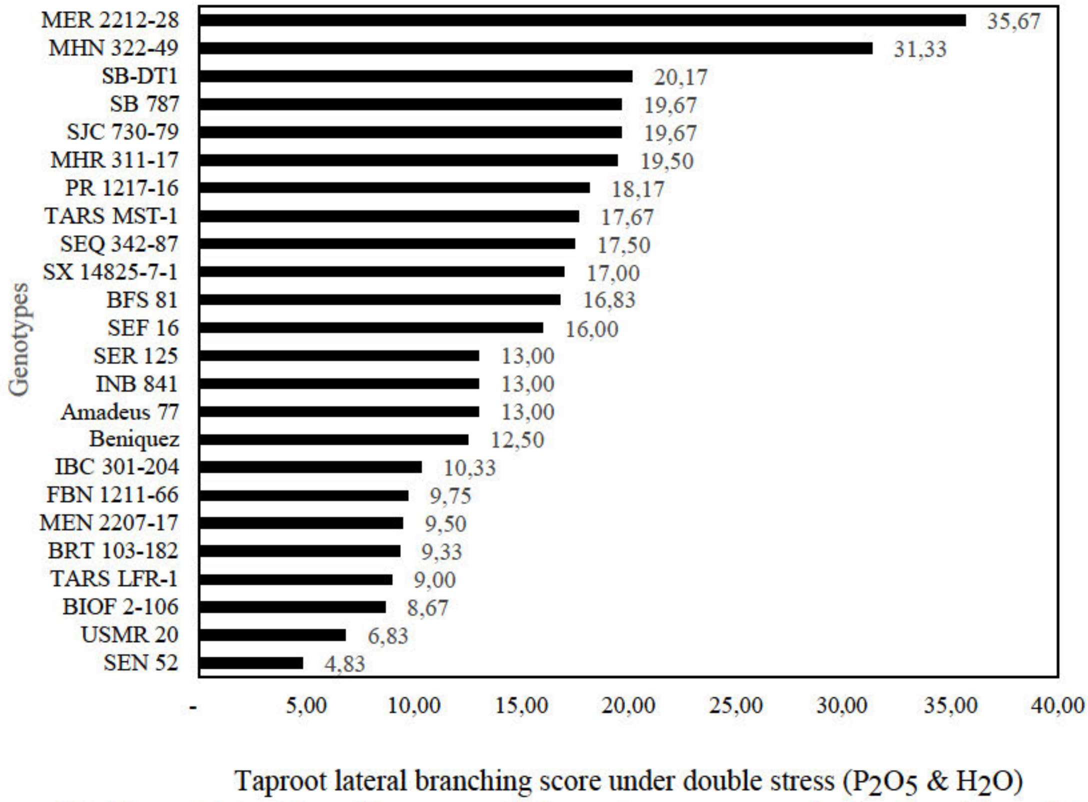

Figure 5.2: Taproot lateral branching score of 24 genotypes grown under low P and drought stress at Chókwè Research Station.

High variability for the root phenes was found among genotypes in response to different environments. In the pot study under double stress (low P and drought stress) genotype SB-DT1 was consistent in allocating high number of roots, but had the lowest value for shoot biomass. In contrast, genotype SEQ 342-87, allocated less roots but showed a higher value of shoot biomass. Genotypes BFS 81 and IBC 301-204 also had poor root systems with superior shoot biomass (Table 5.4).

Table 5.4: Ranking of eight genotypes grown under low P and drought on root and shoot biomass.

Basal Root biomass score (1 -8)	Tap root biomass score (1 - 8)	Total root biomass score (1-8)	Taproot length score (1 - 8)	Total tap root length score (1 - 8)	Shoot biomass score (1 - 8)
1A	1A	1A	1A	1A	8H
2B	2B	4D	3C	4D	6F
3C	4D	3C	4D	3C	5E
4D	7G	6F	7G	6F	4D
5E	5E	5E	5E	5E	7G
6F	3C	2B	2B	2B	2B
7G	8H	8H	8H	8H	3C
8H	6F	7G	6F	7G	1A

1 – 8 represent phene score, where 1 = high presence of root/shoot and 8= low presence/allocation of root/shoot biomass; A to H represents genotypes (A = SB-DT1; B = SEN 5; C = MHN 322-49; D = TARS MST-1; E = TARS LFR-1; F = BFS 81; G = IBC 301-204 H = SEQ 343-87).

SB-DT1 allocate biomass to all roots in equal proportion. Similar variability was observed in the field study under low P & drought stress in relation to taproot branching density. Genotypes SER 125, BFS 81, FBN12111-66 and MER 22 11-28, had a higher score of taproot branching density with the highest grain yield under low P and drought stress, while genotypes TARS LFR-1, IBC301-204, SJC730-79, SB-DT, PR12-7, SEF 16, had lower scores of tap root branching density and also yielded below the average yield under low P and drought stress. Although genotypes INB 814, SEN52, BIOF 2-06 and SEQ342-87 had low scores of taproot branching density, they performed better under low P and drought, as indicated by higher values of yield.

Between the two commercial varieties (Beniquez and Amadeus), both had lower scores for tap root branching, however, Beniquez showed the lowest grain yield among the genotypes, but with a relatively good scoreof taproot branching density, while Amadeus had high grain yield with the lowest score of tap root branching (Figure 5.3).

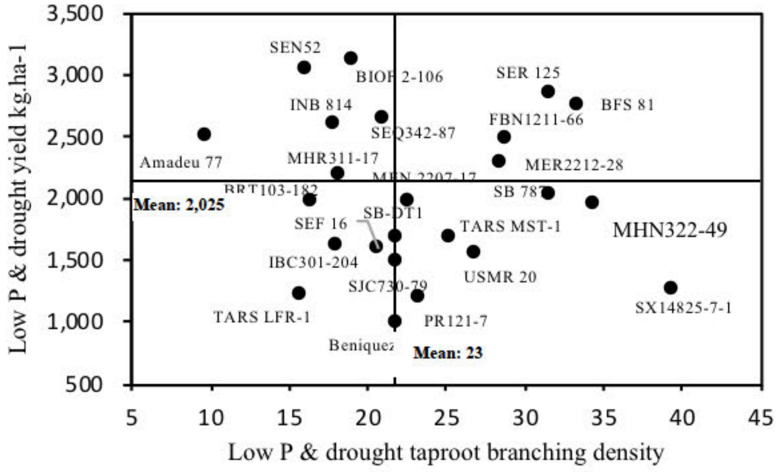

Figure 5.3: Identification of genotypes with superior yield and taproot branching density under low P and drought stress treatment. The outstanding genotypes with higher yield and greater taproot branching are in the upper right quadrant.

Taproot lateral number by order (first and second order) was another phene assessed under low P and drought stress, and the results were quite similar to that of taproot branching density score. Although genotypes GFS 81, MER 2212-28 and MHR311-17 had greater numbers of taproot lateral, the yield was relatively lower compared to the genotypes BIOF 2-106, SEN 52, and SER 125 that showed lower taproot lateral number with greater grain yield under low P and drought (Figure 5.4).

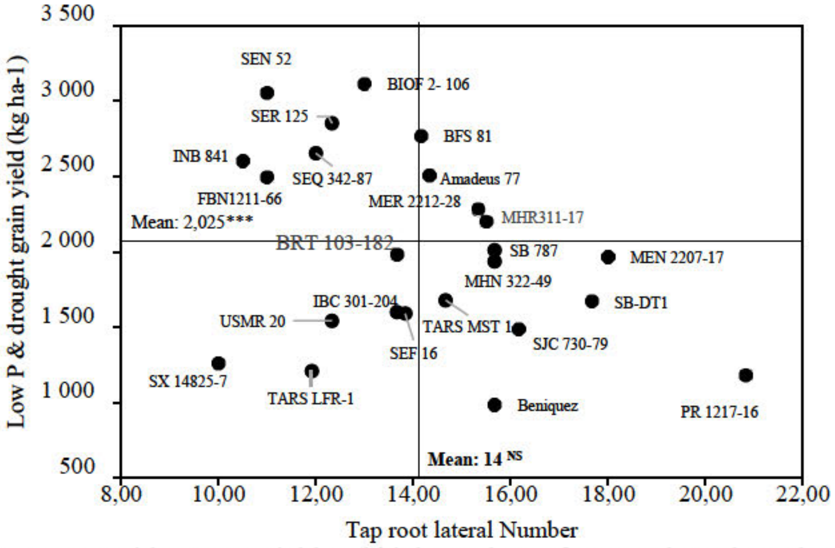

Figure 5.4: Genotypes with greater yield and higher values of taproot lateral number grown under low P and drought treatment. The outstanding genotypes with higher yield and greater taproot branching are in the upper right quadrant.

The sensitive low P and drought genotypes; USMR 20, TARS LFR-1, SEF 16, and IBC 301-

204, had lower taproot numbers with lower grain yield. Even though the genotype PR1217-16 had a high number of the taproot lateral, the phene did not have an effect in increasing grain yield. The commercial variety Beniquez increased the number of taproot lateral, but this did not translate into increased yield (Figure 5.4).

Other root phenes with vital importance to low P and drought environments are basal root whorls number, basal root number and adventitious root. Basal root whorls number was positive and highly significantly correlated with basal root number (r = 0.900***).

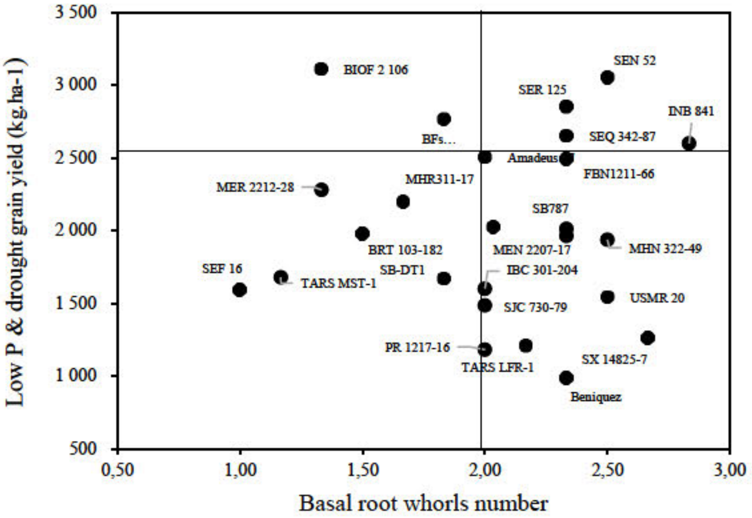

Figure 5.5: Genotypes with higher yield and superior basal root whorls number grown under low P and drought treatment. The outstanding genotypes with superior yield and basal root whorls number are in the upper right quadrant.

Seven genotypes, SEN 52, SER 125, INB 841, SEQ342-87 and FBN1211 had higher numbers of basal whorls with higher grain yield under low P and drought environment. Two genotypes BIOF 2-106 and BFS 81 showed low basal root whorls' number with higher grain yield under low P and drought stress. The two commercial varieties, Amadeus 77 and Beniquez, showed similar number of basal whorls, but Beniquez had the lowest grain yield under low P and drought stress (Figure 5.5).

Results on basal root number under low P and drought stress showed four genotypes SEN 52, SEQ 342 INB 841 and FBN 12 11-46 with high basal root number and also exhibited higher grain yield under low P and drought treatment. For the commercial varieties, Beniquez had relatively higher basal root number and the lowest grain yield, while Amadeus 77, had the lowest basal root number and a higher grain yield under low P and drought stress. The sensitive genotype TARSMST-1 had a lower number of basal root as well as lower grain yield under low P and drought stress. BFS 81, BIOF 2-106, SER 125, MER 2212-28 and MHR311-17 exhibited higher grain yield under low P and drought stress and low basal root number (Figure 5.6).

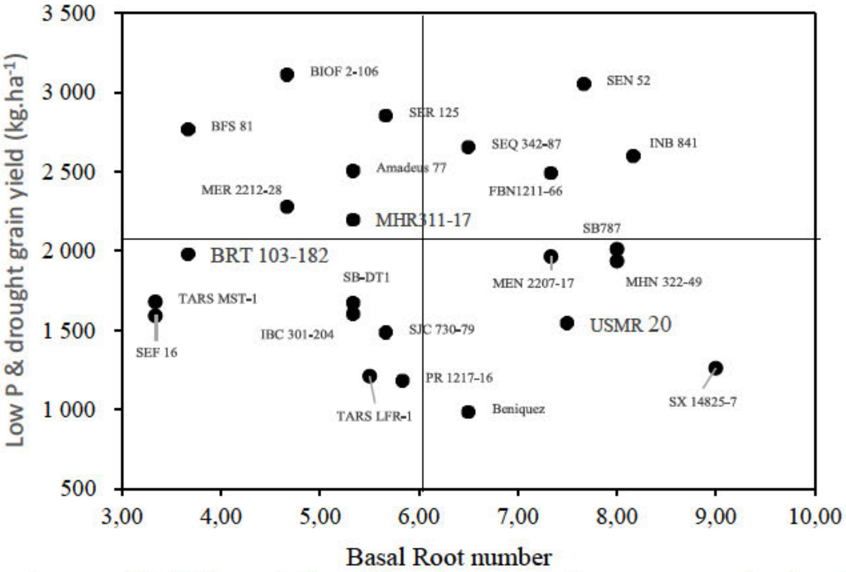

Figure 5.6: Genotypes with higher yield and basal root number grown under low P and drought treatment. The outstanding genotypes with higher yield and greater superior basal root number are in the upper right quadrant.

5.3.3 Root distribution from soil cores

Soil cores showed similar total root length for all phenes at harvest. Overall, the phenotype with dense taproot lateral branches had slightly more total root length in deeper horizons. A higher total root length was observed in the topmost 10 cm and last 10 cm division, which corresponded to 50 cm depth in plants grown under Low P and drought stress as well as high P and irrigated treatments. In general, plants grown under low P and drought showed a larger total root length in all segments of cores compared to high P and well-watered plants (Figure 5.7).

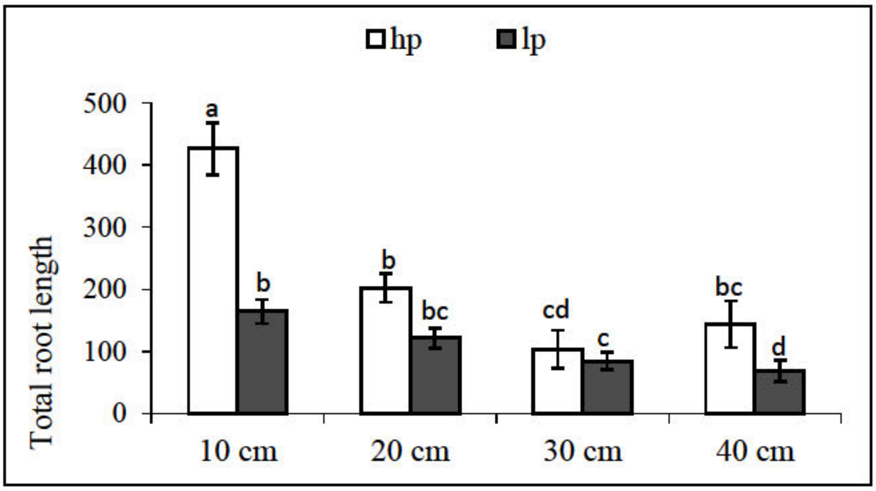

Figure 5.7: Distribution of root within the soil profile grown under combined water/phosphorus treatments at Chókwè research station. Root core samples were taken 5 cm from the plant stem at 42 DAP. Bars indicate standard error of the mean.

5.4 Discussion

In this study was observed that taproot lateral branching and taproot lateral number are phenes under some degree of genetic control and variation exists for taproot phenotype in common bean genotypes. In addition, taproot lateral branching density and taproot lateral number under controlled environment (pot study) had a significant relationship with shoot biomass, total root biomass and taproot diameter under low phosphorus and drought stress in pot, and these were also correlated with yield grain under stressed environments.

The genotype-by-treatment interactions observed suggest that the root phene of the common bean genotypes across to the combined phosphorus levels and water treatment regime were adaptable to specific environment. This findings in this study agree with those of Asfaw and Blair (2014) and Porch et al., (2009) who reported variations in common bean roots response to low and optimum soil phosphorus and drought conditions.

The longest and high taproot number and taproot lateral branching observed under low phosphorus and soil moisture contribute to increased root surface area and plays a very crucial role of enabling the tolerant genotypes explore and access soil moisture resource under limited conditions. Root development and shoot development are interdependent. According to Polania et al. (2017), shoot growth supplies the roots with carbon and certain hormones, in return root growth supplies the shoot with water, nutrients as well as hormones. Increased grain yield through better plant growth in harsh conditions (drought and low fertility) is achieved if the root system is able to supply water and nutrients without absorbing too much photo assimilate from the shoot (Rao et al., 2017; Klaedtke, 2012).

In this current study, a positive correlation between shoot biomass with taproot length, total root length, taproot lateral branching and grain yield was observed, while there was a negative association with taproot biomass and total root biomass. There was also a positive relationship between tap root biomass with total root biomass, tap root length, taproot lateral branching, basal root number, basal root whorls number and grain yield. These positive associations between phenes under pot study and field measurements are of importance in phenotyping process as a tool for screening and selection in breeding programs (Strock et al., 2019).

Asfaw et al. (2017), also reported that interdependency among numerous root phenes are of practical interest in breeding programs where multiple selection of more than one trait would be convenient, since a selection for one trait may influence improvement or deterioration in an associated root phene (Strock et al., 2018). Miguel et al. (2013) and Walk et al. (2006), found that under some environmental conditions, increased adventitious rooting system can decrease allocation of resources to the growth of the taproot and lateral roots emerging from basal roots. This agrees with our pot study, where we observed negative relationship between shoot biomass with taproot biomass and total root biomass under low phosphorus and drought conditions. Negative associations were also observed between total root length with taproot lateral branching and basal root number (Table 5.2). These trade-offs in resources allocation to distinct root classes during early stage of vegetative growth have effects for subsequent vegetative growth and reproduction (Strock et al., 2019).

Although no previous study has explicitly explored the utility of taproot lateral length and lateral branching for plant performance under edaphic stress, results from the present study suggest that an increased taproot lateral branching and length provide benefits under multiple environments. Genotypes that exhibited higher scores of taproot lateral branching in the field such as SER 125, BFS 81, FBN12111- 66 and MER 22 11-28, combining with higher grain yield under low P and drought were ranked as deeper rooted and suited to environments where water is limiting. In contrast, genotypes INB 814, SEN52, BIOF 2-06 and SEQ342-87 had relatively low scores of tap root branching density, but with better yield under low P and drought, and were classified as shallow rooted and suited to environments where P is limiting. The vigor of the taproot associated with many lateral branching of these genotypes, permitted plants to access available water in the deeper horizon and cumulatively foraging for available nutrients through the lateral branching. Although taproot is difficult to evaluate in the field, a longer taproot with higher lateral branching density may be related to greater rooting depth in mature plants (Klein et al., 2020; Strock et al., 2019). Lynch (2013), Lynch & Wojciechowski (2015) and Wasson et al. (2012), reported a positive association between rooting depth and access to water in the deep soil profile under drought.

Previous studies by Miguel et al. (2013) and Rangarajan et al. (2018) on the utility of greater basal root whorls number under P uptake stress have been supported in this study, in addition to showing that more basal whorls number and greater basal root number are associated with increased grain yield in non-stress conditions. The results of this study indicated that genotypes SEN 52, SEQ 342 INB 841 and FBN 12 11-46 produced more basal root whorls number as well as basal root number and higher yield under low P and drought stress. Basal root has been reported to have mixed effects on performance under low P and drought stress, by exploring both shallow and deeper horizons under conditions of high root mortality (Strock et al., 2018). Therefore, basal root whorl number and basal root number are phenes of capital importance for resources acquisition under edaphic stress environment, as it was supported by other studies

(Miguel et al. 2013).

Furthermore, our results supported that plants under water stress increased total taproot length at deeper profiles, as some studies have demonstrated, that the basic difference between shallow and deeper-rooted genotypes is expressed in the respective stress conditions imposed (Nord et al., 2011; Vadez et al., 2008). In addition, some of the reported studies clearly showed that the presumed relation between rooting traits and drought tolerance in some way overlap by escape mechanisms related to the phenology of the genotypes (Beebe et al., 2013; Kashiwagi et al., 2006).

As with the results from field study from soil cores, differences were observed between deeper-rooted phenotypic and depth of rooting. Plants with dense taproot lateral under water deficit localized relatively greater root length below 40 cm compared to the plants with sparse taproot lateral branching. This is in agreement with what some studies have shown supporting that a small volume of roots in deeper layers where water is less limiting, would be enough to fully supply water to the plants when the topsoil is drier (Pierret et al., 2016), thereby improving water uptake. Our interpretation is that the severe the drought the plant faces, the more roots tend to be allocated at deeper profiles as a result of survival mechanism of foraging water.

5.5 Conclusion

Common bean genotypes differed for total root biomass, taproot biomass, taproot length and tap root lateral branching under low phosphorus and drought stress. Phosphorus and drought tolerance in common bean are related to a stronger development of the root system which plays an important role for water and nutrient absorption. Although no previous study has explicitly explored the utility of taproot lateral length and lateral branching for plant performance under edaphic stress, results from the present study suggest that an increased taproot lateral branching and length provide benefits under multiple environments, and this is major contribution in selecting traits associated to drought stress.

Thus, genotypes with greater taproot branching density and length, and high yield were identified as deeper-rooted and suited to water stressed environments, while genotypes with fewer lateral branching density were identified as shallow-rooted and suited to low phosphorus availability environments and genotypes with poor root system (lower taproot branching density and lower yield) were not adapted to either low P or drought stress.

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6. CHAPTER SIX

General Overview

6.1 Introduction

Plant traits that are suited to edaphic stress such as drought and phosphorus tolerance are complex traits and depend on the crop and environment where the plants are grown, the nature of drought and level of phosphorus depletion that is predominant in the target environment. Physiological traits related to adaptation to drought and phosphorus stress in common are numerous, and no single trait bears for its unique and dominant contribution for phosphorus and drought tolerance. The contribution of individual traits such as basal whorls number, basal root number, root hairs and grain yield efficient index for phosphorus uptake and harvest index, pod harvest index, shoot biomass and carbon discrimination for improved stresses in common bean, have been reported in some studies. Therefore, the combination of these traits and how they integrate together to maximize their contribution to better adaptation to phosphorus and drought stresses have still not been explicitly explored.

6.2 Main findings

The results from this work indicate that phosphorus uptake and drought tolerance of the evaluated common bean lines is related to the root system. Shallow rooting system is suited to environments where phosphorus is limiting, as they assist plants to access more topsoil resources, especially phosphorus that is an immobile element; while deep rooting system is suited to the environment where water is limiting, helping the plant to access more water for vegetative growth. In the context of this study, deep rooted genotypes invested in a vigorous taproot with numerous lateral branching, which permitted more effective use of water resulting in good plant performance under phosphorus and drought stress; while shallow rooted genotypes allocated less investment in taproot laterals branching but were able to thrive under low phosphorus and drought for their ability of foraging topsoil resources. Therefore, this performance cannot be attributed solely to the taproot lateral branching, as stated before, no single trait can thrive without contribution of other traits.

6.3 Potential lines for P and drought adaptation

Based on phenotypic categorical grouping, and the differences in the traits assessed in genotypes were targeted for specific agroecological environments. Under drought stress conditions genotypes SEN 52, BRT 103-182, IBC 301-204, SEF 16 and SX14825-7-1 were identified as drought tolerance due to higher yielding; genotypes BFS 81, BIOF 2-106 and SEN 52 were outstanding under low P treatment, and based on grain yield efficiency index, they were the most P efficient genotypes, while genotypes SER 125, BFS 81, FBN12111- 66 and MER 22 11-28 had higher grain yield under combined low P and drought. These genotypes could be grown in areas where rainfall is intermittent as well as low soil fertility, especially low phosphorus.

Phenotypic evaluation either in pot or field conditions demonstrated that more than one plant traits should be considered as useful in bean breeding programs where the aim is to improve phosphorus and drought stress impacts on crop adaptation. Some of these traits are rooting depth and length, basal root whorls number, basal root number adventitious root, taproot lateral branching, pod harvest index, and number of pods and seeds per area, drought intensity index and grain yield efficient index. Since this study aimed to evaluate the physiological responses of lateral branching of the primary root in common bean (*Phaseolus vulgaris l.*), a major contribution of this work is the identification of a common bean genotypes that can thrive in low phosphorus and drought stress environment and could be used as parents for improving common bean adaptation to edaphic stress environment. Besides genotypes, increased taproot lateral branching and length provided benefits under multiple environments, especially in drought stress environment, and will therefore, play a major role in the selection of traits associated to drought stress in plant breeding programs.

6.4 Conclusion

Considerable high root traits and variability was observed among the common bean genotypes assessed after exposure to low phosphorus and drought environment. Low soil phosphorus and moisture increased the average plant performance in shoot biomass and yield through increased taproot lateral branching and length. Out of twenty-four evaluated genotypes, ten outperformed the check genotypes with regard to seed yield. Therefore, the variables evaluated, effectively explained genotypic variability such that genotypes SER 125, BFS 81, FBN12111- 66 and MER 22 11-28 were identified as tolerant to low phosphorus; SEN 52, BRT 103-182, IBC 301-204, SEF 16 and SX14825-7-1 as drought tolerance, while SER 125, BFS 81, FBN12111-66 and MER 22 11-28 are both drought and low phosphorus tolerance.

6.5 Recommendation

Considering that root phenes of the genotypes were evaluated only under semi-controlled environment in pot experiment, the tolerant genotypes that were outstand under low P, low soil moisture and combined low P and soil moisture treatments, should also be evaluated under heat stress in field conditions, since heat is one of abiotic stressor that negatively impact the common bean production and productivity. A couple of genotypes used in this study, can also be selected and evaluated to determine the genetic inheritance of root phenes for tolerance to combined low soil phosphorous and drought stress. Considering that phenes are under some degree of genetic control and variability exists for root traits under either drought or low phosphorus, one strategy that could be recommended to improve common bean seed yield, is selecting parental genotypes with the desired root features from the adapted bean genotypes and also selecting high seed yielding genotypes from the adapted cultivars. Afterward, perform multiple crosses of the selected genotypes with desired root traits and high seed yielding genotypes in all possible combinations in order to produce recombinants with desirable alleles of multiple traits.