EVALUATION FOR DROUGHT TOLERANCE AND ANTHRACNOSE RESISTANCE OF SELECTED PINTO GENOTYPES OF COMMON BEAN (*Phaseolus vulgaris* L.)

BY

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DECLARATION

I, Brian Pule Mwense do hereby declare that this dissertation represents my own work and that, to the best of my knowledge, it has not been previously submitted for the award of degree at any University.

Signed :…………………………………………………………..

Date :…………………………………………………………..

APPROVAL

This dissertation of Brian Pule Mwense, has been approved as partial fulfilment of the requirements for the award of Master of Science in Agronomy by the University of Zambia

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ACRONYMS

CIAT	The International Center for Tropical Agriculture
CTD	Canopy Temperature Depression
DS	Drought Stress
EL	Electrolyte Leakage
GART	Golden valley Agricultural Research Trust
HI	Harvest Index
HSW	Hundred Seed Weight
NS	Non-drought Stress
PHI	Pod Harvest Index
PN	Pod Number
SDW	Shoot Dry weight
UNZA	University of Zambia

ABSTRACT

Common bean (*Phaseolus vulgaris* L.) is an important legume crop grown for household revenue, food and nutrition security in many parts of the world. Common bean is classified into Andean and Middle American gene pools. Most of the beans grown in east and southern Africa countries including Zambia are Andean. In the recent years there has been interest in Zambia in growing pinto beans, which is a major Middle American market class especially in the US, but new to Zambia and most other African countries. The adaptation of pinto beans to Zambia isn't well established especially that the country is prone to drought and anthracnose disease. Anthracnose is the most important disease of common bean in Zambia, which is likely to affect production of this market class in a similar way it has affected other currently grown market classes. Additionally, drought has become an increasingly important production constraint because of frequent drought episodes in bean growing areas caused by climate change. Furthermore, production of common bean has been expanding to geographical areas that are prone to drought. Identification of pinto beans genotypes that are tolerant to drought and resistant to anthracnose could support production and productivity of pinto beans in Zambia and other African countries. The objectives of this study were: (i) Evaluate the agronomic and morpho-physiological response of selected pinto genotypes to terminal drought, and (ii) Determine the response of selected pinto genotypes to seven races of *Colletotrichum lindemuthianum* the causative fungus for anthracnose. A total of 56 pinto bean genotypes and 4 checks were evaluated for tolerance to drought and resistance to seven races of *C. lindemuthianum*. Evaluation for drought tolerance was conducted using one pot experiment and two field trials. For the pot experiment, Electrolyte leakage and canopy temperature depression was measured on the 56 pinto genotypes and 2 checks grown under drought stress (DS) and Non-Drought stress conditions. The two field trials for drought tolerance were conducted at the University of Zambia (UNZA) and Golden Valley Agriculture Research Trust (GART) Research farms under drought stress and non-stress conditions. The primary traits measured included pod number, shoot dry weight, seed weight and seed yield. The traits computed from these primary traits were harvest index (HI), pod harvest index (PHI), yield geometric mean (GM) and yield percentage reduction (YPR). There were significant differences among 56 genotypes in all primary and computed traits indicating significant genetic differences in their response to drought. The average YPR for GART and

UNZA were 43.6% and 58.5%, respectively. The genotypes with highest GM at GART and UNZA were NDZ14048-5 (1,200 kg ha^{-1}) and NDZ14048-2 (1,331 kg ha^{-1}), respectively. Seed yield was significantly correlated to PHI (r=0.55***) and HI (0.68***) under DS suggesting the important role that these two partitioning indices played in the observed drought tolerance for some of the genotypes. Therefore, PHI and HI can be used to indirectly select for drought tolerance. The genotypes NDZ14048-2 and NDZ14048-5 were particularly outstanding in their seed yield performance under both DS and NS conditions. The 56 pinto genotypes and checks were evaluated for resistance to races 51, 65, 73, 247, 253, 263 and 1085. Significant differences were observed among the 56 pinto genotypes in their reaction to seven races of *Colletotrichum lindemuthianum*. In general, the reaction of the 56 genotypes was skewed towards susceptibility except for races 51 and 73. There was no genotype that was resistant to all seven races. In general, the genotypes that showed resistance to most of the races were those that carried Co-4^2, which highlighted the importance of this locus to anthracnose resistance. The three genotypes NDZ14006-4, NDZ14110-4 and NDZ14043 showed superior resistance among the 56 pinto genotypes used in the current study as each was resistant to six of the seven races used in the current study. The genotypes NDZ14048-2 and NDZ14048-5 which showed superior seed yield under both DS and NS, and carry the Co-4^2 anthracnose resistance loci were resistant to five of the seven races used in the current study. The combination of high seed yield (under both DS and NS) and anthracnose resistance exhibited by NDZ14048-2 and NDZ14048-5 make them ideal for use to enhance the adaptation of pinto beans to anthracnose and drought.

CHAPTER ONE

INTRODUCTION

Common bean (*Phaseolus vulgaris L.*) is an important grain legume grown for primarily for direct human consumption (Broughton et al., 2003). Common bean is grown mainly in Latin America, Eastern and Southern Africa where it plays a major role in household food and nutrition security. It is a source of protein and the essential micronutrients iron and zinc, which tend to be deficient in most African diets. Further, common bean is an important source of dietary fiber and carbohydrates (Wortmann, 1998). In Zambia, smallholder farmers mostly cultivate common bean and its nitrogen fixation ability fits well into the low input farming system (Beebe, 2011).

Several biotic and abiotic stress factors limit the production of common bean (Appiah-Kubi et al., 2022). Among the most significant abiotic stress factors include drought, heat stress, aluminum toxicity and poor soil fertility. Drought affects about 60 % of the common bean growing areas worldwide (White and Singh, 1991). Climate change, which is mostly cited in terms of drought risk it poses, affects nearly 73% of common bean producing areas in Africa (Farrow and Muthoni-Andriatsitohaina, 2020). Unfortunately, small-scale farmers cannot not afford irrigation to mitigate drought. Development and use of drought-tolerant common bean varieties is the most cost-effective mitigation strategy for drought.

Significant genetic variation for drought tolerance exists within common bean primary gene pool to support development of drought tolerant varieties (Rao et al., 2013; Dramadri et al., 2021). Most of this variation is found in the Middle American gene pool (Terán and Singh, 2002; Beebe et al., 2013). Beans belonging to the Middle American gene pool are classified into four races (Meso-American, Durango, Jalisco and Guatemala) based on plant morphology, adaptation range and agronomic traits. The Durango race, which includes pinto beans, among other market classes has been widely reported to contain drought tolerance and drought-tolerant genotypes from this race have been used to improve drought tolerance of other market classes (Beebe et al., 2012).

Drought tolerance is genetically complex involving several traits and mechanisms (Schneider et al., 1997; Beebe et al., 2013). These traits are essentially categorized into shoot and root traits. The root traits including root depth maximizes water uptake while the shoot traits optimize the water use during drought stress. Shoot traits include morphological, physiological and agronomic traits. Understanding how these traits relate to seed yield is critical to development of selection indices for drought tolerance of common beans. Shoot traits including pod harvest index, harvest index, which reflects a genotype ability to mobilize assimilates to the pod and seed under drought stress have been reported to be significantly associated with seed yield (Assefa et al., 2013; Polania et al., 2016). These partitioning efficiency indices have been used to enhance adaptation of beans to drought (Assefa et al., 2013). Physiological traits including electrolyte leakage (measure of cell membrane integrity) and photosynthesis have also been reported to be associated with drought tolerance in legumes including common bean (Vasquez-Tello et al., 1990; França et al., 2000).

Anthracnose caused by *Colletotrichum lindemuthianum* is a major disease of common bean and can cause yield losses of up to 100 % depending on varietal susceptibility and environmental conditions (Pastor-Corrales and Tu, 1989). High humidity coupled with cooler ambient temperature enhances spread of anthracnose. Anthracnose affects all areal parts of the plant including leaves, stems, and pods (Schwartz and Pastor-Corrales, 2005). It is a seed borne disease, whose primary mode of transmission is through the planting of infected seed (Ferreira et al., 2013). The informal seed system practice of sharing farm-saved seed, which is prevalent in many African countries makes control and management of anthracnose challenging in most African countries including Zambia. Though anthracnose can be effectively controlled by use of fungicides, small-scale cannot afford them and there are health risks associated with their use. Therefore, development and use of anthracnose resistant varieties is the most cost-effective and environmentally friendly management strategy.

Development of common bean varieties with durable resistance to anthracnose remains challenging because of the extensive genetic variability for *C. lindemuthianum*. To date, over 182 races characterized (Padder et al., 2019) using a set of 12 race differential cultivars (Pastor-Corrales 1991). Extensive genetic variation for *C. lindemuthianum* has also been reported in Zambia (Mungalu et al., 2020). The extensive genetic variability and rapid evolution of new races makes it challenging to develop varieties with durable resistance to anthracnose. *C. lindemuthianum* has co-evolved with the Andean and Middle American gene pools, resulting in Andean races that are virulent mostly on Andean genotypes and Middle American races that are virulent mostly on Middle American genotypes.

Resistance to *C. lindemuthianum* is oligogenically controlled involving mainly major-effect resistance locus Zuiderveen et al., 2016. To-date about 13 major effect loci (*Co-1* to *Co-13*) have been reported. Resistance provided by these major-effect loci follows gene-for-gene action i.e., conferring resistance to specific races (Flor, 1955). These major-effect loci have been classified into Andean and Middle American loci. Clusters of resistance (R) genes underlie some of the resistance loci such as *Co-3* (Oblessuc et al., 2015; Campa et al., 2020). Though there is no single gene that confers resistance to all races, the locus *Co-4*^{2} confers resistance to multiple races of *C. lindemuthianum*.

Pinto bean, which belongs to the Durango race of the Middle American gene pool is a major market class in countries such as the US. Anecdotal evidence suggests that there is a growing interest in pinto beans in some African countries such as Tanzania and Zambia. Despite the growing interest, Anthracnose and drought may hinder pinto bean production. Currently, there is no previous study to determine the response of pinto beans to terminal drought stress and to the predominant races of *C. Lindemuthianum*. Therefore, identification of pinto bean germplasm that is adapted to drought and resistant to *C. Lindemuthianum* is important for meeting the varietal needs of the farmers in Zambia of this new market class. Further, understanding the relationships between agronomic and morpho-physiological traits could support development of selection indices for drought tolerance in pinto beans. This is because identification of pinto bean genotypes tolerant to drought and resistant to anthracnose is important for the genetic improvement of pinto beans and other market classes for drought and anthracnose.

1.1 Study objectives

1. Evaluate the agronomic and morpho-physiological response of selected pinto genotypes to terminal drought stress

2. Evaluate the response of selected pinto genotypes to seven races (51, 65, 73, 247, 253, 263 and 1085) of *Colletotrichum lindemuthianum*

1.2 Hypothesis

- There are some agronomic and morpho-physiological traits play an important role in the response of selected pinto genotypes to terminal drought.
- There are pinto common bean genotypes that are resistant to the seven races of *C. lindemuthianum*

CHAPTER TWO

LITERATURE REVIEW

2.1 Common bean gene pool, evolutionary relationship and systematics

The existence of archaeological, botanical, and historical evidences indicate that the common bean is a New World crop that originated – 7,000 years back in a long arc from the present day northern Mexico through Central America and the Andes mountains to northwest Argentina (Broughton et al., 2003). The two taxonomic subdivisions of Phaseolus have been described as *P.vulgaris var. aborigineu*s and *P.vulgaris* var. mexicanus (Delgado-Salinas et al., 1993) based on morphological and molecular differentiation. The individuals of this important legume are well characterized on the basis of morphological and molecular traits into two gene pools; the Andean originating from the Andes mountains of South America (Southern Peru, Bolivia and northern Argentina) and Mesoamerican from Central America and Mexico with at least seven races (Beebe et al., 2001, Koenig and Gepts. 1989, Valasquez and Gepts., 1994). The morphological and agro-ecological adaptation traits have been further used to distinguish the races among these gene pool (Singh, et al., 1991). Race structure studies demonstrated that members of each race have distinctive and specific physiological, agronomic, biochemical and molecular characteristics and diverse from other races in the allelic frequencies at specific loci (Singh et al., 1991).

2.2 Domestication

Domestication is an outcome of the selection process that leads to increased adaptation of plants to cultivation and utilization by humans. The domesticated common bean originated from wild gene pools through independent domestication events (Kwak and Gepts, 2009) at two centers of domestications i.e., Mesoamerican and Southern Andes of South America. The interspecies diversity in relation to the common bean is organized in Primary, Secondary, Tertiary, and Quaternary gene pools.

The major common bean gene pools have been further divided into races based on plant morphology, adaptation range, and agronomy traits. The Mesoamerican gene pool includes the races; Durango (prostrate bush types with medium sized seed from the dry highlands Mexico), Jalisco (Climbing beans from the moist highlands of central Mexico), and

Mesoamerica (small-seeded types, mostly bush habits , from the lowlands, Central America and Mexico (Singh et al., 1991)

The Andean gene pool includes races Peru (predominantly highland climbing beans with mid-altitude adaptation), Nueva Granada (mostly bush beans with mid-altitude adaptation), and Chile (prostrate bush or weak climbers, with temperate adaptation to higher altitudes). Beebe et al. (2000) suggested existence of a fourth race – Guatemala in the Mesoamerican gene pool (mostly climbing beans from Guatemala and southern Mexico) as well as some systematic variation within the races. Mesoamerican cultivars became predominant in the lowlands of South America and southwestern United State of America, whereas Andean cultivars became predominant in Africa, Europe, and northeastern USA.

2.3 Domesticated gene pool

The interspecies diversity in relation to the common bean is organized in primary, secondary, tertiary and quaternary gene pools. Intraspecies diversity in the common bean is separated into two major gene pools (Andean and Middle American). Cultivars are further divided into races, each with their distinctive characteristics (Pathania et al., 2014). Landraces are distinct but variable populations, which usually have a local name, lack formal crop improvement, are characterized by a specific adaptation to the environmental conditions of the area of cultivation (tolerant to the abiotic and biotic stresses of that area), and are closely associated with the uses, knowledge, habits, dialects, and cerebrations of the people who developed and contribute to grow it. Although landraces are an important component of agrobiodiversity, most of them are at risk of extinction because they are grown by small scale farmers and need to be preserved in situ and ex situ.

Both molecular data and screening of germplasm showed that landraces harboring significant levels of diversity are not found in advanced breeding gene pools. Therefore, bean breeders have actively sought to broaden the domesticated gene pool by crossing with landraces of the two gene pools in the centers of origin. Landraces can provide sources of genetic diversity for disease and pest resistance and tolerance to abiotic stresses (Beebe et al., 1997), Most of the present day bean cultivars have resulted from crosses between closely related cultivars belonging to either of the gene pools. Pedigree and Restricted Fragment Length Polymorphism (RFLP) data revealed that commercial classes of common bean possess low levels of genetic diversity, presumably because of little or no introduction of genetic diversity from outside these classes. Limited diversity in commercial cultivars has serious consequences for bean breeding and emphasizes broadening of the cultivated gene pool through effective breeding programs. (Beebe et al., 1997).

2.4 Diversity in common bean germplasm

Cultivated common bean is a morphologically diverse crop (Geneva, 1928) and wide variations exist for growth habit, pigmentation, pod, seed , phenology, and other characters (Leakey, 1988) (Singh., 1991). There are two major groups with suggested existence of sub-groups within each of the major Andean and Mesoamerican groups, with distinctive morphology, adaptation, and disease resistance. The domestication process has led to a reduction in the genetic diversity within each group of the bean gene pools (Sonnante et al., 1994). Several evolutionary forces played a role in shaping the *P.vulgaris* genetic diversity, which include the introgressive hybridization between *P.vulgaris* and *P.coccineus* in Mesoamerica, gene flow and selection between wild and domesticated populations, evolution of disease resistance (Blair et al., 2012). Inspite of the germplasm resources available in national and international gene banks, only a small proportion of the genetic variability has been utilized by bean breeders for the identification and development of high-yielding bean in national and international bean breeding programs (Singh et al., 2001)**.**

2.5 Global Common bean production

Common bean is cultivated worldwide and accounts for one-third of cultivated legumes (FAOSTAT, 2019). The biggest producers are Brazil, United States of America and Mexico, Africa. Common bean is the third most important legume in the world and shows considerable variation in seed characteristics, maturation and growth habits (De Ron et al.,2015).

Common bean is important source of nutrition, especially to the low-income people, in the developing countries it is often considered as poor man meat (Tharanathan and Mahadevamma.,2003). It’s high levels of protein and carbohydrate make common bean a staple in food, and it is an important source of dietary fiber, vitamins and minerals, including iron, magnesium, potassium, zinc, selenium and calcium (Broughton et al., 2003, de Almeida Costa et al., 2006, Blair et al., 2009, Blair et al., 2016, Ozturk et al., 2009). Common bean consumption reduces the risk of chronic diseases such as cancer, diabetes, cardio vascular diseases and obesity (Hayat et al., 2014). It is usually grown with other crops and it matures quickly, supply food, income and enhances soil fertility through nitrogen fixation its production is constrained by both biotic and abiotic factors.

2.6 State of Common bean production in Zambia

Common bean production is ranked second to groundnuts as major legumes produced in Zambia. Production areas are cooler, higher altitudes and high rainfall areas of Northern Zambia. Most farmers grow landraces for their taste and colour. Although a number of improved varieties have been developed, their adoption rates remain low with most farmers growing low yielding landraces with average yields slightly above 500 kg per hectare. The constraints affecting common bean production in Zambia are well documented (International Center for Tropical Agriculture - CIAT, 1989) and broadly include poor agronomic practices, soil fertility, lack of improved cultivars, moisture stress, weed competition and stress caused by weed competition as well as pests and diseases. The Ministry of Agriculture in collaboration with non-governmental and international organizations (CIAT) and the United Nations (e.g Food and Agriculture Organization –FAO, United Nations Development Programme – UNDP), have devoted efforts to solve these problems with the aim of ensuring house hold food security and increased incomes among small holder farmers. The National Agriculture Research Systems (NARS) in Zambia have released ten varieties to date, seven of them are bush varieties, two are semi-climbers and one is of climbing type. These varieties have high yield potential (1 – 2 tons/hectare) and the bush-type varieties only take two to three months to mature. The earliness of bush varieties has enabled many common bean growers in the high-rainfall northern region of Zambia to grow at least two bean crops in a single season. In terms of management, most of the varieties developed so far are adapted to relatively low fertility conditions. However, additional research is still needed in terms of nutritional content as well as moisture stress, pest and disease resistance (Hamazakaza et al., 2014).

2.7 Ecological and production requirements

The common bean is adapted to temperate and cool tropical climates. In Africa, production is concentrated in the cool highlands of central and tropical eastern Africa where beans are the most important legume crop. Within the highland areas, the production environment is diverse; the altitudes range from 800 to 2,300 m above sea level. Soil type also varies considerably between regions of production. In the major production areas in Ruhengeri district of northern Rwanda and to the west of Arusha in northern Tanzania, the soils are excellent fertile volcanic soils. In other production regions highly acidic soils with a pH as low as 4.2, are found in the bean producing areas of Mbala district of northern Zambia, in the Usambara mountains near Lushoto in Tanzania, and on the Nile Zaire crest of Rwanda. The mean temperatures in the principal areas of bean production ranges from 16 to 24 ^{o}C and annual precipitation in the range 500 – 200 mm., with a bimodal distribution in eastern Africa as a result of movements of the intertropical convergence zone. The average annual rainfall varies substantially with location and, in some places, particularly in the drier regions at the unstable frontiers of rainfall systems, rainfall is markedly variable from year to year (Bunting, 1961). The wide variability of production environments results in a wealth of diversity in cropping systems as well as in agronomic constraints to bean production

2.8 Factors that are limiting common bean production

Constraints to bean productivity vary with region and various other concerns like marginal land of farmers in Asia, Africa, and Latin America and the largescale producers commonly found in the USA and Europe. For most of farmers in Asia, Africa and Latin America, the greatest challenge is to stabilize yield under marginal conditions and using limited technical input. Common bean suffers both from abiotic and biotic production constraints (Pathania et al., 2014).

2.9 Abiotic stress

The principal abiotic stresses that limit common bean grain yield are drought and low soil fertility such as low phosphorus availability and aluminum toxicity in the soil (Marschner, 1995). These problems tend to occur in combination. Drought is estimated to affect 60 % of bean production in the tropics, especially in regions such as Central America and Southern Africa (Thung and Rao, 1999) and approximately 67 % of globally cultivated lands are affected by Phosphorus deficits (Batjes, 2009). It has been estimated that 50 % of common bean production area worldwide suffers moderate to severe Phosphorus limitation (CIAT, 1998). Low Phosphorus is worsened in strongly acidic or alkaline soils, mainly due to formation of phosphates complexes with Aluminum and Iron in acidic soils and Calcium complexes in alkaline soils (Marschner, 1995).

In soils with pH < 5.5 affect more than 50 % of world wide’s potentially arable land (Zhang et al., 2016) and up to 60 % of the acid soils in the world occur in developing countries in South America, Central Africa and South-East Asia (Gupta et al., 2013). High temperature (>30 ºC Day or 20 ºC night) during anthesis or seed setting at lower elevations (<650m) or at higher altitude during summer, especially when relative humidity is low, severely reduces bean production. Low temperature (<10 ºC) and frost during the beginning and end of the growing season at higher elevations (>2,000 m amsl) significantly lower the yield of beans.

2.9.1 Climate Change and Crop Production

The global change in climate will severely affect the United Nations goal to sustainably produce adequate food by the year 2050 to provide sufficient nutrition for the projected global population of 9.1 billion people. The previous progress in addressing global undernourishment with increased food production by agricultural intensification and expansion is under serious threat. The change in global climate has a long term impact in form of several abiotic stresses (Redden, 2013). The impact will lead to large parts of the world to become drier with extended drought periods, more intense heat and erratic rainfall patterns. The anticipated changes will extremely affect agriculture as well as the stability and distribution of food supply chain (Kellogg, 2019). The major driving factor towards temperature increase and altering precipitation patterns is a higher atmospheric carbon dioxide concentration (Kellogg, 2019). The hot and drier climatic conditions, resulting in increased evaporative losses, will drive the increased demand for more water. Th greater part of the already experiencing chronic soil water deficits due to severe drought conditions (Nadeem et al., 2019).

The impacts of drought and heat stress range from critical physiological, biochemical and metabolic pathways severely disrupted (Fahad et al., 2021). Extreme temperatures are strongly associated with significant reduction in crop yield relative to precipitation extremes, and irrigation partly limits it’s adverse effects of high temperatures (Vogel et al., 2019). The combined effect of drought and heat stress has been established to reduce yields of maize, soybean and wheat (Matiu et al., 2017). The physiological characterization of plant responses to either drought or heat stress, or the combination of both stresses, have indicated that the combined effect has numerous distinctive characteristics. The recorded effects include reduced photosynthesis occurring together with increased respiration and closed stomata combined with increased leaf temperature (Maxwell and Johnson, 2000).

2.9.2 Effect of drought stress on common bean production

Drought is a major source of yield losses globally and affects about 60% of the common bean growing area worldwide (White and Singh, 1991). Climate change, which is most frequently cited in term of the drought risk it poses, affects nearly 73 % of common bean producing areas in Africa (Farrow & Muthoni-Adriatsitohaina, 2020). Drought can be defined meteorologically as a period of prolonged and abnormal moisture deficiency (Palmer, 1965) or insufficiently moisture necessary for a plant to grow normally and complete its life cycle (Graham and Ranalli, 1997). Agriculture accounts for 70 % of the fresh water withdrawals worldwide, more than any other use category (FAO, 2019). As the population increases, more of the world fresh water resources will be needed for direct use by humans, reducing the amount that is available for crop production. For optimal growth and high seed yield, long-season dry beans require about 400 – 500 mm of rain, with rainfall well distributed during the first 10 -12 weeks of its growth (Farrow and Muthoni-Adriatsitohaina, 2020).

Drought stress can be classified into three types, early, terminal or intermittent drought (Ludlow and Muchow, 1990). Terminal drought is defined as optimum rainfall until some point in the growing season when rainfall greatly diminishes and does not resume for the remainder of the season usually occurring during the reproductive period of the crop cycle. Terminal drought is becoming an increasingly phenomenon in the major bean growing areas of the world. Intermittent drought occurs when periods of rain are limited and erratic during the growing season and thus cause periods of stress (Schneider et al., 1997). The majority of drought studies use terminal drought as it is much easier to simulate than intermittent drought. Regardless of the type of drought, the effects are devastating in much of the developing world where common bean are a major subsistence crop and are a major source of protein (Broughton et al., 2003). When the common bean experiences intermittent drought at seedling stage overall plant growth is adversely affected, whereas terminal drought stress significantly limits bean seed yield and seed size during the critical reproductive period (Singh, 2007). Terminal drought also negatively affects seed quality when experienced under prolonged periods. The incidence and duration of drought events are expected to increase with climate change, therefore the need for approaches to breeding common bean for drought tolerance (Beebe et al., 2013).

Common bean is an excellent crop for breeding for drought tolerance since it has an average growing season of less than 100 days and thus has much lower water requirements that many other crops (White, 1992). Breeding for drought tolerance can be difficult as the effects are complex and plant response is highly variable and based on many interacting factors (Ramirez-Vallejo and Kelly, 1998). The development of drought resistant crop varieties is a slow and very difficult process (Subbarao et al.,1995) and particularly important for common bean, because 60% of production area worldwide is prone to some level of drought stress (White and Singh, 1991). Genetic diversity for drought tolerance is found in wild, cultivated and related bean species and can be utilized when breeding for drought tolerance (Beebe et al., 2008).

In the last 40 years, significant research efforts have been made to better understand common bean adaptation to drought and enhance this adaptation to achieve sustainable yields in drought years or in bean producing areas prone to drought (Beebe et al., 2013). Phenotyping studies across the world have screened experimental lines for traits such as canopy temperature, root morphology, pollen integrity, pod harvest index and normalized difference vegetative index (NDVI) to learn more about the characteristics that confer drought tolerance. Normalized difference vegetative index quantifies vegetation by measuring the difference between near-infra red (which vegetation strongly reflects) and red light (which vegetation absorbs) and ranges from -1 to +1. In the context of plant physiology, drought tolerance in modern dry bean cultivars is more a function of efficient assimilate partitioning to grain as opposed to improved photosynthetic rate per unit leaf area. The efficiency indexes such as harvest index and pod harvest index were positively associated with drought tolerance (Hageman and Volkenburgh, 2021). The indices significantly reflected the genotypes ability to remobilize photosynthates to the seed during drought stress and are critical traits associated with mechanisms for drought tolerance (Beebe et al., 2013). In view of the high heritability of the identified drought associated traits, the two partitioning indices can be used to indirectly select for drought tolerance.

2.9.3 Drought stress responses

2.9.3.1 Morphological responses to drought

The first effect of drought on the plants is the poor germination and impaired seedling development and establishment. A number of studies have pointed out the numerous adverse impacts of drought stress on germination and seedling development (Farooq et al., 2009). The reduction in germination potential, early seedling growth, root and shoot dry weight, hypocotyl length, and vegetative growth have been reported in important field crops that includes, alfalfa (*Medicago sativa* L.), rice (*Oryza sativa* L.) and pea (*Pisum sativum* L.) under drought stress. Plant growth is mainly accomplished through cell division, enlargement, and differentiation. Drought stress impairs mitosis and cell elongation which results in poor growth (Hussain et al., 2008). Drought constrains the process of cell growth mainly due to the loss of turgor pressure (Lazar, 2003). The turgor pressure determines the expansion of the leaves and the translocation of assimilates. It has been reported that reduced turgor pressure and slow rate of photosynthesis under drought conditions mainly limit the leaf expansion (Rucker et al., 1995). Total shoot fresh and dry weights are also severely reduced under moisture-limiting conditions (Deng et al., 2006).

2.9.3.2 Morpho-physiological mechanisms of adaptation to drought avoid

Plants respond and adapt to and survive under drought stress by the induction of various morphological, biochemical and physiological responses. Drought tolerance is defined as the ability to grow, flower and display economic yield under suboptimal moisture supply (Farooq et al., 2009). Drought stress affects the plant water relations at cellular, tissue and organ levels, causing specific as well as unspecific reactions, damage and adaptation reactions (Beck et al., 2007). To cope with the drought, tolerant plants initiate defense mechanisms against water deficit (Chaves and Oliveira, 2004). Adaptation to drought encompasses a diversity of mechanisms at whole plant, physiological and molecular levels. Manifestation of a single or a combination of inherent changes determines the ability of the plant to sustain itself under limited moisture supply that enable plants to survive and produce in periods of dry weather. The mechanisms of drought resistance are grouped into three categories; drought escape, drought avoidance; and drought tolerance.

2.9.4 Drought stress escape

Drought escape is defined as the ability of the crop to complete its life cycle before serious soil and crop deficits develop, allowing plants to reproduce before the environment becomes too dry. This mechanism involves rapid phenological development (early flowering ad early maturity), developmental plasticity (variation in duration of growth period depending on the extent of water deficit), and remobilization of photosynthates to the grain (Araus et al., 2002). Crop duration is interactively determined by genotype and the environment interaction, that in turn determines the ability of the crop to escape from climatic stresses including drought (Dingkuhn and Asch, 1999). Matching growth duration of plants to soil moisture availability is critical to realize high seed yield (Siddique et al., 2000). Drought escape occurs when phenological development is successfully matched with periods of soil moisture availability, where the growing season is shorter and terminal drought stress predominates (Araus et al., 2002).

The time of flowering is a major trait of a crop adaptation to environment, particularly when the growing season is restricted by terminal drought and high temperatures. Developing short-duration varieties has been an effective strategy for minimizing yield loss from terminal drought, as early maturity helps the crop to avoid the period of stress (Kumar and Abbo, 2001). However, yield is generally correlated with the length of crop duration under favorable growing conditions, and any decline in crop duration below the optimum would tax yield (Turner et al., 2001).

2.9.5 Drought stress avoidance

Drought avoidance consist of mechanism that reduce water loss from plants, due to stomatal control of transpiration, and also maintain water uptake through an extensive and prolific root system (Turner et al., 2001**.** Under drought avoidance the plant enhances the ability of the crop to maintain relatively high-water tissue water potential, despite a shortage of soil moisture. It is achieved through increased rooting depth, an efficient root system and increased hydraulic conductance, and by reduction of water loss through reduced leaf conductance, reduced absorption of radiation by leaf movement/rolling, and reduced evaporation surface.

The root characters such as biomass, length, density and depth are the main drought avoidance traits that contribute to final yield under terminal drought environments (Subbarao et al., 2000). A deep and thick root system is essential for extracting water from considerable depth (Kavar et al., 2008). The maintenance of a high tissue water potential is facilitated by glaucousness or waxy bloom on leaves, and is therefore considered as a desirable trait for drought tolerance (Richards et al., 1986).

2.9.6 Drought stress tolerance

Drought tolerance is defined as the ability of the crop to withstand water deficit with low tissue water potential. It is achieved through maintenance of turgor through osmotic adjustment (a process which induces solute accumulation in the cell), increase in cell elasticity and decrease in cell size, and desiccation tolerance by protoplasmic resistance. Osmotic adjustment, osmo-protection, antioxidation and a scavenging defense system have been the most important bases responsible for drought tolerance. Blum (2005) indicated that an effective drought tolerance mechanism in crop plants is stem reserve utilization for grain filling under drought stress. Plants generally limit the number and area of leaves in response to drought stress just to cut down the water budget at the cost of yield loss (Siddique et al., 2000).

Since roots are the only source to acquire water from soil, the root growth, it's density, proliferation and size are key responses of plants to drought stress (Kavar et al., 2008). It has been well established that plants bearing small leaves are typical of xeric environments. Such plants withstand drought very well, albeit their growth rate and biomass are relatively low (Ball et al., 1994). Leaf pubescence is a xeromorphic trait that helps protect the leaves from excessive heat load. Hairy leaves have reduced leaf temperatures and transpiration (Sandquist and Ehleringer, 2003) whilst inter- and intra-specific variation exist for the presence of this trait. Under high temperature and radiation stress, hairiness increases the light reflectance and minimizes water loss by increasing the boundary layer resistance to water vapor movement away from the leaf surface.

Roots are the key plant organ for adaptation to drought. If tolerance is defined as the ability to maintain leaf area and growth under prolonged vegetative stage stress, the main basis of variation appears to be constitutive root system architecture that permits the maintenance of more favorable plant water status (Nguyen et al., 1997). Evidence suggests that it is quality, i.e., the distribution and structure, and not quantity of roots that determines the most efficient strategy for extracting water during the crop growing season.

Common beans are grown in a wide range of environments where it's exposed to seasonal drought and wide fluctuations in soil moisture availability between years. Hence, the plants have evolved several mechanisms to maintain plant water status within reasonable limits for normal metabolic functioning under drought stress (Beebe, 2012). It has been established from early studies that a set of the same genotypes that were evaluated in several countries indicated that local adaptation is an important component of drought resistance (White, 1988). A number of shoot and root traits contributed to improved drought adaptation. The uptake of water is maximized by optimal root traits whereas, the shoot traits enhance the efficiency use of absorbed water for production of grain during drought stress. Loss of leaf area is the most important morphological adaptation to drought stress. The development emanates from a reduced number of leaves, reduced size of young leaves, inhibited expansion of developing foliage, or leaf loss caused by senescence all of which contribute to reduced seed yield (Acosta-Gallegos, 1988). Through field screening, some relatively drought-tolerant lines of bean germplasm have been identified including BAT 477, A 195, and BAT 1289 (White and Singh, 1991). Drought avoidance through greater root length density and deeper soil moisture extraction are some of the adaptation traits to moisture stress (Sponchaiado et al., 1989).

White and Castillo (1992) grafted diverse shoots genotypes onto selected root genotypes of common beans and evaluated yield under drought. They found variation with shoot genotype, but the effect on growth and yield under drought was found to be small, compared with the effect of root genotype. Sanders and Markhart (1992) also used grafting to examine the importance and mechanisms of the root system's effect on leaf water status in *P. vulgaris* and *P. acutifolius*. They found that the root genotype determined leaf water potential in the most stressed plants, and that roots of tepary bean had greater hydraulic conductivity than those of common beans. Castonguay and Markhart (1991) measured saturated rates of photosynthesis in water-stressed leaves of common and tepary beans, and found that genotypic variance in drought tolerance between the two was not related to differences in mesophyll tolerance of dehydration. Tepary beans relied more on drought avoidance than on drought tolerance. Severe drought impaired nitrogen mobilization, HI and water-use efficiency (WUE) in common bean (Foster et al., 1995). Research work by White (1993) under field conditions indicated that WUE (based on carbon isotope discrimination, CID) that was not a promising indicator of adaptation to drought. Other physiological traits such as shoot dry weight and leaf nitrogen concentration appeared the most promising based on hereditability, strong general combining ability effects, and correlations with seed yield across trials (White et al.,1994a, b).

Phenotypical plasticity is considered to be another mechanism contributing to increased performance under drought stress (Acosta-Gallegos and White, 1995). This particular attribute, accentuated in photoperiod-sensitive cultivars, allows genotypes to shorten their growing cycle dramatically at later planting dates to avoid drought conditions later in the growing season. The breeding programs to improve small seeded cultivars of the race Mesoamerica have utilized genes from the valuable Durango race, that have now been introgressed into this race. In the Central America, North-eastern Brazil, and the Caribbean, lines in the small red, small black, and carioca grains are being developed which present double or more yield under severe stress compared to the respective commercial control (Beebe et al., 2008). The small seeded bean types are often grown in warmer climates where high temperatures exacerbate drought, or under conditions of low fertility or aluminum toxicity that can limit vigor and root development. Therefore. tolerance to low fertility, especially to low soil phosphorous availability, and heat tolerance should be combined with drought resistance. The combination of drought and low fertility tolerance has proven to be useful and practical, since several drought-resistant lines already possesses a relative degree of tolerance to low soil phosphorus availability (Beebe, 2008).

2.9.7 Drought tolerance sources

The attempts to characterize and screen for drought resistance has seen researchers utilize physiological, biomass, and seed yield traits in drought-stressed (DS) and non-stressed (NS) conditions (Pimentel et al., 1999). Genotype that escape terminal drought may mature early enough before onset of severe drought while late maturing genotypes especially indeterminate cultivars that facilitate partial recuperation from a mild drought stress during flowering (Nleya et al., 2001). Cultivars that tolerate higher temperature and low soil fertility and/or that remobilize assimilates and root and shoot reserves to developing pods and seeds during drought stress should give higher yield and improve adaptation in arid environments.

In common bean, the documented highest level of drought resistance is found in Durango common bean followed by races Mesoamerica and Jalisco (Terán et al., 2002). Race Durango common bean traces its origin in the semiarid central and northern highlands of Mexico (Chacón et al., 2005). Breeding for drought resistance has been ongoing in Mexico, Honduras, and Brazil and at CIAT in Colombia for several decades. The greater part of the work has involved evaluation of germplasm with parental materials from CIAT and Mexico (Beebe et al., 2013). There has been germplasm evaluation in the drought prone region of northern Mexico where two sets of germplasm were tested under the typical conditions of insufficient and erratic rainfall with the primary trait measured being seed yield. Drought resistant genotypes were identified mainly in the Durango race (type III growth habit) and Mesoamerica races (type II and III), whereas genotypes from Jalisco race were susceptible. An addition set comprising 800 bush genotypes of the core collection assembled at CIAT were evaluated with the finding that a set of 20 genotypes mostly from the Durango race were classified as drought-resistant (Beebe et al., 2013).

2.9.8 Drought Tolerance Selection indices

The knowledge of plant responses to drought stress has been of great importance for selection of genotypes that are tolerant to adverse environmental conditions (Nouri et al., 2011). Selection for drought tolerance genotypes is not easy, due to the occurrence of strong interactions between genotypes and environment conditions, as well as, the lack of the knowledge in the function and role of different mechanisms of tolerance (Naghavi., 2013). To be useful as a selection criterion, traits should be highly correlated with seed yield, have low genotype by environment interactions (GE) and highly heritable (Mentreddy et al., 2002, Yuan et al., 2002). A comparison of selection indices for dry bean lines grown under drought was conducted when bean were grown under drought stress and non-stress conditions and included arithmetic mean, geometric mean, drought response index, drought susceptibility index, response to drought and percent yield reduction. (Abebe et al., 1998). Abebe et al. (1998) found that the arithmetic mean and geometric mean were the best indicators of high yielding lines under both stressed and non-stressed conditions and that drought response index is a good indicator in stressed environments.

Other alternative indices useful for identification of drought tolerant genotypes to water stresses have been proposed, such as drought susceptibility index (Fischer and Maurer, 1978), Pod harvest index and Harvest index (Klaedtke et al., 2012). Harvest index is the proportion of the whole plant mass that is partitioned to the seed and the index is believed to be a good indicator for how effectively the plant is able to partition resources between vegetative and reproductive growth (Sadok and Sinclair, 2011). When selecting for yield under drought stress, breeders are inadvertently selecting against stay green characteristic and for higher partitioning as measured by harvest index (Beebe et al., 2008). On the list of partitioning influencing drought indices, harvest index was found to be the most reliable (Ramirez-Vallejo and Kelly, 1998). It’s against that background that harvest index is considered a good measure of drought resistance or susceptibility among genotypes. Indices play an important role in identifying overall drought tolerance of genotypes grown under drought stress environments.

2.9.9 Shoot traits for drought tolerance

Drought tolerance indices are an important tool used by breeders to quickly assess the drought tolerance of diverse genotypes. By understanding the specific portions of plant agronomic, morphological and physiological traits, breeders can gain greater insights into specific mechanisms that are critical in drought response. By directly interacting with the environment factors throughout the growing season, plant shoots usually show the first visible symptoms of drought stress and play a very important role in overall plant productivity. The common bean plants have diverse shoots types or growth habits that have been classified into plant types I to IV with type I growth habit being determinate, highly branched and exhibit upright growth (Singh et al., 1991). Type II plants are indeterminate and upright; type III plants are indeterminate, weak stemmed and prostrate and non-climbing; and type IV plants are indeterminate, weak stemmed with a strong ability to climb (Singh et al., 1991). When different common bean shoot genotypes were grafted onto different root genotypes and their performance evaluated under drought stress, the effect of shoot genotype was small in comparison with root genotype, but the shoot genotype still played a critical role in overall drought tolerance (White and Castillo, 1989). The combination of root traits with shoot-based drought tolerance mechanisms in single genotypes should enhance the development of drought resistant common bean. In crop legumes , diverse shoot traits have been used to select drought tolerant genotypes particularly maintenance of green stem was shown to be an important criterion for seedling stage drought tolerance in cowpea (Muchero et al., 2008). The slow wilting trait have been associated with drought tolerance in soy bean (Sadok et al., 2012).

Successful attempts have been made to classify common bean into races based on traits such as leaf shape and size, leaf pubescence, length of internodes, number of nodes to flower, flower and pod types, days to maturity, seed size and shape and geographic distribution (Kelly et al., 1987). Determinacy is an important shoot characteristic and determinate types stop growing when the plant starts flowering. Whereas indeterminate types grow throughout the season and continue to produce leaves and flowers until the sink strength ceases vegetative growth. When determinate plants are stressed during the season, flowers and pods abort resulting in significant yield reduction. Contrary, indeterminate growth habit the plant exhibits continuous production of flowers and pods, the moment favorable growing conditions have resumed and recovery results in less yield loss (Kelly et al., 1987).

Plants with greater transpiration rates yield more because they are utilizing more water. Genotypes that possess the slow wilting trait relies on the interactions between hydraulic conductivity in the leaves, the xylem and guard cells. When transpiration is halted, growth ceases as photosynthesis is stopped. Hence, slow wilting genotypes have an overall lower transpiration rate and therefore a lower yield potential when compared to normal genotypes under favorable conditions whereas genotypes lacking this trait have a higher yield potential when conditions are idea as they are able to transpire and grow faster (Sadok and Sinclair, 2011). In geographical regions where drought stress is frequent, the slow wilting trait is quite valuable because the genotypes are able to slow the transpiration rate and survive during intense drought periods. Conversely, the genotype without this trait tend to completely cease transpiring and photosynthesizing under extreme drought stress conditions compared to those that possess the trait (Ries et al., 2012). Selected target shoot traits that are pertinent for drought resistance breeding in common beans have been clearly defined;

- Seed yield
- Geometric mean
- Harvest index
- Pod harvest index

- Pod wall biomass proportion%
- Pod partitioning Index
- Stem biomass reduction
- Grain filling index
- Seed production efficiency
- Drought Intensity Index
- Drought Susceptibility Index

2.9.1.0 Root traits for drought tolerance

The root architecture is an important component of plant growth and its ability to tolerate stresses that includes drought, soil salinity and acidity among others in common bean. Studies have demonstrated that the root system has a significant effect on common bean yield under drought stress (Trachsel et al., 2011, Adams et al., 2002). Deep rooting has been positively correlated with seed yield, crop growth, cooler canopy temperature, and soil water extraction in common bean (Sponchiado et al., 1989). In another study, drought tolerant bean genotypes were able to extend their roots to a depth of 1.2 m in drought environments whereas sensitive genotypes could not extend beyond 0.8 m. (White et al., 1994). The development of common bean varieties with enhanced productivity under limited nutrient and water resources is the priority strategy in addressing the challenges of low productivity amongst Small scale producers (White and Hammond, 2008), hence root traits could be used for identifying stress tolerant genotypes

2.9.1.1 Leaf cooling trait and drought stress

Regulation of canopy temperature is achieved through maintenance of stomatal conductance (Porch et al., 2013, Prasad et al., 2017). It has been proposed that transpirational cooling could be used by plant breeders to identify bean genotypes with the thermal plasticity to adapt to elevated temperature due to climate change (McClean et al., 2011). The magnitude of transpirational cooling is indicative of plant’s adaptative qualities and has been used by breeders to screen for heat tolerance in spring wheat cultivars (Porch and Hall, 2013). There is evidence that plants that are adapted in extreme environments are able to strongly regulate the temperature of their leaves, decoupling leaf and air temperature. Leaf temperature can exceed air temperature by as high as 20 °C in cooler alpine environments and humid tropics, whereas, in hot and dry desert environment , leaf temperature can be 20 °C cooler than ambient temperature (Blonder and Michaletz, 2018). The relationship between net radiation and evaporative cooling is mediated by leaf thermal traits, including stomatal conductance, size, shape, absorptivity, and emissivity (Michaletz et al., 2016). Stomatal conductance is influenced by internal and external factors that affect the rate of carbon assimilation and transpiration. The simultaneous occurrence of high temperature and drought prompts stomata behavior towards a potential trade-off between leaf cooling and water conservation in extremely hot non-irrigated field conditions. There has been significant progress in understanding the role of transpirational cooling in temperature regulation of rice and wheat plant; and within species variation has been well documented (Weerakoon et al., 2008).

2.9.1.2 Cell membrane thermostability

Cell membrane thermostability (CMT), measured as electrolyte leakage from leaf discs over a range of temperature, is a sensitive and rapid method to evaluate heat tolerance in plants (Wu and Wallner, 1983). A number of studies have confirmed the effectiveness of CMT testing in evaluating genetic variability for heat tolerance among several other agronomic traits in fruit and vegetable crops (Saadalla et al., 1990). Several authors have found that leaf CMT highly correlate with fruit set and yield under high temperature conditions (Fokar et al., 1998, Martneau et al., 1979, Y, 1979).

Cell membrane stability, reciprocal to cell membrane injury, is a physiological index widely used for the evaluation of drought tolerance (Premachandra et al., 1991). This is a genetically related phenomenon since quantitative trait loci for this trait have been mapped in drought stressed rice at different growth stages. The causes of membrane disruption are unknown; notwithstanding, a decrease in cellular volume causes crowding and increase the viscosity of cytoplasmic components (Dhanda et al., 2004). This increases the chances of molecular interactions that can cause protein denaturation and membrane fusion. For model membrane and protein systems, a broad range of compounds have been identified that can prevent such adverse molecular interactions. Some of these are proline, glutamate, glycine-betaine, carnitine, mannitol, sorbitol, fructans, polyols, trehalose, sucrose and oligosaccharides (Hoekstra et al., 2001). Another possibility of leakage from the cell may be due to thermal-induced inhibition of membrane -bound enzymes responsible for maintaining chemical gradients in the cell (Reynolds, 2001).

2.1.0 Biotic stress

The bean crop may be attacked by a wide range of insect pests, diseases, and nematodes. Among diseases, the major constraints in bean production are bean anthracnose (*Colletotrichum lindemuthianum),* angular leaf spot (*Pseudocercospora griseola),* halo bligh*t (Pseudomonas phaseolicola)* rust (*Uromyces phaseoli)* bean mosaic virus, Common bacterial blight (CBB) (Miklas et al., 2006). The seed borne diseases are the major issue with bean production. The extent of damage and loss depends on the type of cultivar, prevailing environments, and above all the production area. The major bean insect pests including leafhoppers, thrips, weevils, white flies, bean flies or bean stem maggot (*Ophiomyia phaseoli* and *O.spencerella*), chrysomelid bettles (*Ootheca bennigseni* and *O.mutabilis*, pod borers (*Maruca vitrata* and *Helicoverpa armigera,* and mites (*Tetranychus cinnabarinus* Boisd, *Polyphago-tarsonemus* latus Banks) attack crops at various stages (Schwartz and Peairs, 1999). Aphids (*Aphis fabae, A. craccivora*) are sometimes a problem on beans during dry spells, especially in the early stages of crop growth. Among nematodes, the root-knot nematodes (*Meloidogyne incognita* and *M. javanica*) can be cause problems in sandy loam soils. Beans are highly susceptible to early weed competition, but their yield is also sensitive to late emerging weeds which are favored by crop foliage loss during reproductive stage.

2.1.1 Common bean production and anthracnose disease

Anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. And Magn.) Bri. And Cavi., is on of the most serious disease that attacks common bean in cool weathers of latin America and Africa. There is documented evidence that yield losses in these regions could occur due to seedling, stem, leaf and pod infestations and have been recorded up-to 90 % in susceptible genotypes under favorable climatic conditions. When the pathogen attacks the common bean leaves, it causes dark brown necrotic lesions and significantly reduces the photosynthetic activity (Bassanezi et al., 2001). The yield loss is attributed to early leaf senescence and plant death, shrunken seed and an increase in the amount of diseased seed that bears lesions on its coat (Schwartz and Corrales, 1989). The infested bean seeds have a repulsive appearance and are not popular among consumers and lowers the marketability and thus the anticipated income from sales. Host resistance, cultural and chemical control methods have been employed to effectively manage and minimize seed borne infection in the field seed production (Yesuf, 2005). The fungus is known to have races that vary from, country, region, location, and variety, to another.

2.1.2 Distribution of common bean anthracnose

The first description of Anthracnose from plant specimens was conducted in Germany in 1875 and ever since, the disease is widely distributed throughout the world and has become one of the most economic important diseases of common bean. There are more than 25 different races of *Colletotrichum lindemuthianum* identified in Brazil (Thomazella et al., 2002). On the Africa continent, the disease is particularly important in Uganda, Kenya, Tanzania, Rwanda, Burundi, Ethiopia and D.R. Congo. Despite plant residues contributing to pathogen survival and dissemination, the infected seed plays an important role in international distribution of pathogen and this is especially so, in African countries where most farmers continuously use infected recycled seed thus contributing to the distribution of pathogen (Schwartz and Corrales, 1989). The documented yield losses in Tanzania are at 40 – 80 % and are estimated to be worth US$ 304 million per annum, whereas in Uganda anthracnose remains an important disease in the high altitude and low temperature areas (Mohammed, 2013). In Zambia, Anthracnose was first recorded in Northern, Copperbelt, Northwestern and Luapula provinces in 1986. The incidence and severity of anthracnose disease in Zambia is highest in region III that exhibit's cool weather, humid and high rainfall followed by regions II and I which experiences low humid and rainfall (Zulu, 2005). The incidence and severity of anthracnose is relatively higher in region III that exhibits a cool , humid and high rainfall rain season (Kachapulula et al., 2010)

2.1.3 Taxonomy of bean anthracnose

Colletotrichum lindemuthianum is considered as hemibiotrophic fungus that belongs to Kingdom Fungi, Phylum Ascomycota, Subphylum Pezizomycotina, Class Sordariomycetes, Subclass Sordariomycetidae, Order Incertae sedis, Family Glomerellaceae, Genus Colletotrichum and Subject Colletotrichum lindemuthianum (Sacc. & Magnus) Lams. -Scrib (CABI, 2023).

2.1.4 Vegetative cycle of bean anthracnose

The fungus displays a complex life cycle which has various development stages and two means of taking food and the distinct development phases enables the fungus to survive. In the imperfect form of *C. lindemuthianum* the reproduction is asexual, and the spores ae produced inside arcevulis and immerse in water soluble mucilage (O'connell et al., 1996). The fungus is both a saprophyte and biotroph; therefore, the fungus is classified as hemibiotrophic. As a saprophyte fungus, the spore germination process begins with the spore adhesion to the plant surface under adequate humidity conditions with the required correct aqueous content in the spore envelope. The spores of fungus round off by water absorption and active growth and is later, the germinating tube is formed, and the hyphae elongates to colonize the substrate. Th aerial mycelia appear; then the fungal reproductive structures are formed where the spores are stored. Finally, their lifecycle is completed and it starts all over again. (Mohammed, 2013).

2.1.5 Plant infection and symptoms of common bean anthracnose

2.1.5.1 Leaf infection and symptoms

The early signs of infection usually appear on the lower leaf surface along the veins, which show a brick red to purplish red discoloration. Subsequently, such discoloration appears on the upper leaf surface and simultaneous brown lesions of various sizes, black, brown, or purplish red margins, develop around small veins (Schwartz et al., 2005). The course of disease development includes vein necrosis appearing first, followed by wilting and bleaching often occurs at the tip of the leaflet before spreading over the margin and finally over the blade (Beshir, 2003). The fungal pathogen produces cell wall degrading enzymes and low molecular weight phytotoxins that may, by killing cells in advance of the invading hyphae, contribute to the necrotrophic growth of the pathogen (Bailey et al., 1992)

2.1.5.2 Pod infection and symptoms

Infection affects the arial parts of the plant that include the leaves, stems and pods. The stem infection is manifested by dark brown eyespots which develop longitudinally along the stems. In the young seedling, if the eyespots enlarge,, the stem may break off, but for older stems the lesion is limited to an approximate length of 5 -7 mm, and the lesion often has a sunken cankerous center (Mohammed, 2013). The infection spreads to the pods with the most striking disease symptoms being small brown specks on rusty brown spots. The spots enlarge with their centers turning brown and many tiny black specks appear randomly on the brown are, replacing the brown specks (Bailey et al., 1992). The lesions on the pod usually reach a diameter of 5-8 mm, are slightly sunken at the center and have a dark brown or purplish brown margin.

2.1.5.3 Seed infection and symptoms

Anthracnose transmission to the next crop generation is facilitated through seed infection and provides means by which the fungus survives unfavorable weather conditions. The pathogen will remain alive on the viable seed, although not all infested and infected seed is capable of transmitting the disease (Mohammed, 2013). The variation in seed transmission relates to the degree of infestation as well as the severity and site of infection in the seed (Tu, 1983). The infection of the seed follows the spread of infection from the pods as such, it has been established that the higher the number of pods infected, the higher is the number of seeds infected. Anthracnose is displayed as brown to light chocolate-colored spots on infected seed coats and in highly infected seed, the lesions may extend into the cotyledons (Mohammed, 2013)

2.1.6 Variability of the anthracnose pathogen

The fungus is known to have many races that vary from country, region and location. Most pathogens exhibit a great variability for pathogenicity that arises from sexual mechanisms of reproduction leading to recombination of nuclear genes, mutation, or by extra chromosomal variation. *Colletotrichum lindemuthianum* has co-evolved with the Andean and Middle American gene pools of Common bean resulting in some races being more virulent on Andean than Middle American and vice-versa (Mahuku and Riascos, 2004). The pathogen has high genetic variability with over 182 races characterized and reported using a set of 12 differential cultivars (Sharma et al., 2019). The high genetic variability of *Colletotrichum lindemuthianum* makes it difficult to develop varieties with durable resistance, and therefore demands continuous breeding programs to help identify new sources of resistance. The high variability of races across regions leads to genotypes being resistant in a particular region and susceptible in other regions (Nalupya et al., 2021). Zulu (2005) characterized 14 races out of 22 isolates from 7 districts, Nalupya et al. (2021) characterized three races from three isolates from three districts, and Kachapulula (2010) characterized 10 races out of 10 isolates from 3 districts. Sansala et al. 2023 characterized 58 physiological races from 103 isolates from 22 major bean growing districts in Zambia

2.1.7 Host plant resistance

Management strategies used to minimize seed-borne infection in the seed production field include host resistance, cultural, chemical and biological control methods. Host resistance is the most effective and efficient method of anthracnose management (Falconí-Castillo et al., 2003), though it has been complicated by the presence of several races of the fungus, and the fact that plants resistant to one race may be susceptible to another. There are nine resistance anthracnose genes that have been reported so far in different parts of the world (Kelly and Vallejo, 2004). Previous studies have established that Cultivar AB 136 and G 2333 could be used as sources of resistance in bean breeding program as they have been found to be highly resistant to different races of *C. lindemuthianum* found in Africa, North and Central America (Mahuku et al., 2002) . In order to determine the effectiveness of resistance to anthracnose, varieties must be tested where they are to be grown to establish their tolerance to the locally prevalent races.

CHAPTER THREE

MATERIALS AND METHODS

Two experiments were conducted. Experiment 1 was conducted to determine the response to terminal drought stress under and green house conditions, while experiment 2 was conducted to assess for *C. Lindemuthianum* in the green house.

3.1 Experiment 1: Evaluation for drought tolerance

3.1.1 Plant materials

A pinto bean collection comprising 56 pinto varieties and breeding lines were used in objective One. These varieties and breeding lines were sourced from public bean breeding programs in the USA. Dr. Phillip Miklas of the USDA-ARS, Prosser, Washington, USA assembled the collection. The names or codes, and the breeding program from where the genotype was sourced is provided in Appendix 10. The genotypes Kabulangeti and SER16 were used as drought-susceptible and drought-resistant checks, respectively. Kabulangeti is an Andean landrace from Zambia while SER16 is a Middle American breeding line developed by the International Center for Tropical Agriculture (CIAT). The 56 pinto genotypes and checks were evaluated for response to terminal drought in two field trials and one green house experiment conducted in Zambia.

3.1.2 Trial design and data collection

The genotypes were evaluated under field and green house conditions. The lay out for each trial is explained in details below;

(a) Field conditions

Trial design

Two field trials were conducted in the year 2022 at two locations namely University of Zambia (UNZA) Research Farm (hereafter referred to as UNZA trial) and Golden Valley Research Trust (GART) (hereafter referred to as GART trial) in Zambia. Both UNZA and GART trials were conducted on soils classified as fine loamy isohyperthermic paleustalf.

Both trials were conducted under irrigation between August and November, and there was no rainfall during this period. Each trial had two moisture regime treatments namely; drought stress treatment (DS) and non-drought stress treatment (NS), separated by a distance of 10 m, which was planted with a buffer crop. A Randomized Complete block design with three replications was used was used for each moisture regime. The experimental unit comprised of two rows that were 4 M long each with an inter-row spacing of 0.5 M. Basal fertilizer (10N: 20P: 10K) was applied at planting at a rate of 250 kg ha^{-1}. Adequate water was applied through overhead surface irrigation (with sprinklers) to both DS and NS from planting until flowering stage. At flowering stage moisture stress was slowly imposed on DS through gradual irrigation withdraw (intermittent irrigation) up to mid-pod fill. At mid-pod fill, irrigation was completely withdrawn from DS (to simulate terminal drought), but normal irrigation continued for NS until physiological maturity.

Data collection

At physiological maturity five plants were randomly selected from each plot, and cut at the ground level. Plant samples were placed in the drier set at 60 ^{O}C for 72 hours. After samples were dried, the primary traits including Shoot Dry Weight (SDW), Pod Number (PN), Hundred Seed Weight (HSW) and seed yield were measured and recorded on the five plants. Average values per plant for these parameters were calculated and reported. Seed yield was measured from the whole plot when crop had reached harvest maturity. The seed yield from each plot was adjusted to yield (kg ha^{-1}) at 10% grain moisture content.

The primary data was used to compute the following secondary traits based on the formulae provided in Beebe 2023:

i. **Harvest index**

   Computed as seed yield divided by total shoot dry weight multiplied by 100

ii. **Pod harvest index**

   Computed as seed dry weight divided by pod weight at harvest multiplied by 100

iii. **Drought intensity index (DII)**

   DII = 1 - (Seed Yield from Drought stressed environment/Seed Yield from Non-Drought stressed environment)

iv. **Geometric mean for seed yield per hectare**

$GM = \sqrt{(GY_{NS} \times GY_{DS})}$, Genotypes with high GM index will be more desirable

v. **Percent Reduction in Yield due to drought stress for each genotype**

Percent Yield Reduction = $\frac{(Y_{NS}-Y_{DS})}{Y_{NS}} * 100$ where Y_{NS} is yield under non-Drought environment and Y_{DS} is yield under drought stress environment

(b) Green house conditions

Trial design

A Completely Randomized Designed with three replications was used in the green house experiment conducted at University of Zambia to evaluate the 56 selected pinto genotypes for electrolyte leakage (EL) and canopy temperature depression (CTD). The experimental unit was a 5 – liter plastic pot that was planted with four seeds. Thinning was conducted at first trifoliate stage to leave two seedlings per pot. The experiment had two moisture regime treatments namely; drought stress treatment (DS) and non-drought stress treatment (NS).

Pots under both DS and NS treatment were watered optimally from emergence up to flowering. Compound D fertilizer was applied one week after emergence to stimulate plant growth vigor. At flowering watering was withdrawn from the DS treatment for 14 days but the NS treatment continued to receive optimum watering (Appiah-kubi et al, 2022).

Data collection

Canopy Temperature Depression (CTD) was measured using the MultispeQ device on two plants in each of the pots just before the leaf tissue samples for electrolyte leakage measurements were collected after 14 days of moisture stress. The CTD measurements were collected during “full sunshine” and “clear sky “(suitable weather condition for CTD (Ayeneh et al, 2002) between 10:00 to 14:30 hours. To take the measurement, the MultispeQ device was placed over a central portion of a fully developed young leaf without altering leaf angle and avoided casting shadow over either the leaf or the PAR sensor of the MultispeQ. Plant leaf samples were collected from both DS and NS treatments after 14 days of imposing moisture stress to analyze for electrolyte leakage. A 10 mm diameter cork borer was used to exercise plan leaf tissues. The plant discs were washed with deionized water to remove surface adhered electrolytes and then plant disc samples were incubated at ambient temperature on a rotary shaker at 100 rpm for 24 hours. The initial electrical conductivity (EL_1) was determined using WTW Cond 3310 SET 1 conductivity meter (Xylem Analytics Germany GmbH). Samples were then autoclaved at 212 ^{o}C for 20 minutes and cooled to room temperature. Following cooling, the final Electrical Conductivity (EL_2) was determined. Electrolyte leakage was expressed as a percentage: (EC_1/EC_2) x 100.

3.1.3 Data Analysis

(a)Field conditions

Data collected was subjected to analysis of variance (ANOVA) per location and also across locations (Combined ANOVA) using R-software. In the individual ANOVA, a mixed model was followed with genotype and moisture regime being fixed and replication random. The model used;

Statistical analyses on all traits measured in the current study were conducted using R software. Normality tests were conducted on residuals for each trait to determine if the data for each trait was normally distributed. Normality test results indicated that all traits were normally distributed. Initial ANOVA was conducted using R following the statistical model:

$Y = \mu + \alpha + k + \beta + l + \alpha * k + \alpha * l + k * l + \alpha * k * l + e$

Where: Y was the response variable e.g., Yield; μ is the population mean; α was the fixed effect of the genotype; k was the fixed effect of water regime (NS or DS); β was the random variable effect of a block; α*l was the random effect of interaction between genotype and location; α*k was the random effect of the interaction between genotype and water regime; α*k*l the random effect of interaction between genotype, location and water regime; k*l was the random effect of interaction between location and water regime; e was the residual (error) associated with replication and was considered as a random variable that was normally distributed with mean =0. The above statistical model showed significant interaction between genotype and location for all primary traits. Therefore, the data for each location was re-analyzed separately using R software following statistical model:

$Y = \mu + \alpha + k + \beta + \alpha * k + e$

Where: Y was the response variable e.g., Yield; μ is the population mean; α was the fixed effect of the genotype; k was the fixed effect of water regime (NS or DS); β was the random variable effect of a block; α*k was the random effect of the interaction between genotype and water regime; e was the residual (error) associated with replication and was considered as a random variable that was normally distributed with mean =0.

(b) Green house conditions

Data for electrolyte leakage and canopy temperature depression was checked for normality, and that check showed that the data was normally distributed. ANOVA was conducted following statistical model below:

$$Y = \mu + \alpha + k + \beta + \alpha * k + e$$

Where: Y was the response variable e.g., Electrolyte leakage; μ is the population mean; α was the fixed effect of the genotype; k was the fixed effect of water regime (NS or DS); α*k was the random effect of the interaction between genotype and water regime; e was the residual (error) associated with replication and was considered as a random variable that was normally distributed with mean =0.

Genetic correlation analysis between traits measured in the field was conducted using multivariate restricted maximum likelihood estimation as described in Holland (2006).

3.2 Experiment 2: Evaluation for resistance to Anthracnose

3.2.1 Plant material

A total of 56 pinto genotypes used in objective One were also used in Objective Two. Additionally, two genotypes Kabulangeti and Lusaka, which are Andean landraces from Zambia were used as susceptible checks. Kabulangeti and Lusaka have previously been reported to be susceptible to anthracnose (Mungalu et al., 2020; Nalupya et al., 2021). The Middle American landrace G2333 was used as a resistant check.

3.2.2 Trial design and data collection

Trial design

The 56 pinto genotypes and checks were inoculated with already characterized races of *C. lindemuthianum* namely races 51, 65, 73, 247, 253, 263 and 1085. These races were selected from a pool that was previously characterized by Sansala *et al* (2023). The races were selected based on their geographical distribution, virulence and frequency of isolation. This allowed for a wide virulence range. Classification of races into either Andean or Middle American was based on the reaction of the 12 differential cultivars as described in Mungalu et al., 2020 and Nalupya et al., 2021. Briefly, a race that was virulent only on Andean host genotypes was classified as Andean while a race virulent only on Middle American host genotypes was classified as Middle American. A race that was virulent on both Andean and Middle American differentials was classified as mixed race.

The 56 genotypes and checks were planted on a Styrofoam tray which had 200 wells with each well measuring 3 cm long, 3 cm wide and 5 cm deep. The experiment was set up in a completely randomized block design with three replications. Each replication had two seedlings, therefore, a total of six seedlings per genotype were evaluated.

Seedlings on Styrofoam trays were grown to the unifoliate (fully expanded primary leaf) stage and then inoculated with races 51, 65, 73, 247, 253, 263 and 1085. Inoculation was conducted for a single race at time. Inoculated seedlings were transferred to the high humidity (> 90 ^{o}C) growth chamber where they were maintained for 72 hours. At the end of 72 hours, the materials were transferred to the green house and where they were maintained at room temperature and humidity for 5 to 7 days to allow for anthracnose disease development.

Data collection

The CIAT scale of 1 to 9 was used to decompose anthracnose severity scores. Plants with no visible symptoms or few small lesions mostly on primary leaf veins were classified as resistant (scale 1-3), seedlings with small lesions mostly on leaves and seedlings stem were considered moderately resistant (scale 4-6) and seedlings with numerous or enlarged lesions, with sunken cankers on leaves and seedling stem, or dead plants were classified as susceptible (scale 7-9).

3.1.3 Data Analysis

Analysis of variance (ANOVA) on Anthracnose reaction severity scores was conducted using R Software based on the statistical model;

$$Y_{ik} = \mu + \alpha_i + \gamma_k + \pounds_{ik}$$

where anthracnose severity score for genotype i in replication k; α_i was the fixed effect of genotype; γ was the random variable effect of a replication; £ was the random error associated with replication k for genotype i.. A test for normality using a Shapiro-wilk normality test revealed that data was not normally distributed, therefore the scores for all seven races were transformed for use in ANOVA. The average severity scores are reported in the untransformed original values.

CHAPTER FOUR

RESULTS

4.1 Evaluation for drought tolerance

The analysis of variance for varying moisture regime revealed that the response of the 56 pinto common bean genotypes and the checks were significantly different ($p \leq 0.05$) for the measured traits at GART and UNZA as indicated in Table 4.1.

4.1.1 Yield, Geometric Yield and Yield Percentage Reduction

Combined analysis of yield from the two locations showed significant ($P<0.05$) differences in seed yield among genotypes. Under DS the average yield ranged from 266 kg ha^{-1} (PT16-20) to 842 kg ha^{-1} (NDZ14048-2) with an average of 544 kg ha^{-1}. The average yields for the drought tolerant SER 16 and susceptible Kabulangeti checks were 681 kg ha^{-1} and 469 kgha^{-1}respectively. Five genotypes (NDZ14048-2, PT16-12-2, PT9-18, PT11-13 and NDZ14006-4) recorded higher yield than the tolerant check. Yields were higher under non drought stressed conditions from 874 kg ha^{-1} (NE2-09-8) to 1,731 kg ha-^{1} (NDZ14048-5), with the average being 1,331 kg ha^{-1}. Genotypic effects on yield were significant for both GART and UNZA. Yield at GART under DS ranged from 262 kg ha^{-1} (PT16-20) to 904 kg ha^{-1} (PT9-18), with the average being 592 kg ha^{-1}. The yield for the tolerant check SER16 and susceptible Kabulangeti were 698 kg ha^{-1} and 423 kg ha^{-1}, respectively.

Yield Percentage Reduction (YPR) from combined analysis of data from two locations ranged from 34.6 % (PT9-18) to 68.4 % (PT16-20), with an average of 48.9 %. The YPR for the tolerant check SER 16 and susceptible check Kabulangeti were 37.6 % and 57.6 %, respectively. At GART, YPR ranged from 18.9 % (PT9-18) to 68.5% (PT16-20), and the average being 43.6%. At UNZA, YPR ranged from 25.6% (PT11-13) to 86.1% (CO-52646), with the average being 58.5%.

Yield geometric mean (GM) from combined data of the two locations ranged from 512 kgha^{-1} (NE2-09-8) to 1,102 kg ha^{-1} (NDZ14048-2). NDZ14048-2 and NDZ14048-5 genotypes had higher GM than that of the tolerant check SER16 (1,016 kg ha^{-1}) and susceptible check Kabulangeti (700 kg ha^{-1}). GM for GART ranged from 595 kg ha^{-1} (PT16-20) and 1,201 kg ha^{-1} (NDZ14048-5), with an average being 891 kg ha^{-1}. At UNZA, GM ranged from 260 kg ha^{-1} (NE2-18-7) to 1,331 kg ha^{-1} (NDZ14048-2) with average being 686 kg ha^{-1}.

Table 4.1. Mean squares from ANOVA for response of the 56 pinto common bean genotypes and checks under drought stress and non-drought stress condition at GART and UNZA.

Location Year	SoV	DF	PN	SDW	Yield	PHI	HI	100SW
GART	Gen	57	60.8*	6176 *	286946*	87.3***	240***	47483
2021	MR	1	1888.5***	378489**	100316146***	1928.6***	10373***	103286
	Replication	2	163.9*	17521.	5582994***	485.2***	1004***	45906
	Gen: Treatment	57	43.8	6363	163423	62.9.	113	47270
	Residuals	226	49.0	6359	182537	46.5	96	48339
GART	Gen	57	36.0**	2880*	23683+7***	95.7	153.	87341
2022	MR	1	33.8	42929***	31756348***	2504.6***	3510***	3728
	Replication	2	242.0***	1215	139399	465.2**	441*	4632
	Gen: Treatment	57	31.2*	2426	98117	119.1.	148.	91838
	Residuals	226	21.4	1835	128347	91.3	112	92639
UNZA	Gen	57	51.9***	9913*	353092***	312.9*	595***	61
2022	MR	1	421.2***	80294**	39394504***	955.1*	3901***	3067.3
	Replication	2	10.4	8127	2363331***	2476***	4082***	92.8
	Gen: Treatment	57	32.6*	12307	97329	173	310	47
	Residuals	226	23.4	10137	171082	195.6	268	15.7

Significance codes: *** = $p \leq 0.001$, ** = $p \leq 0.01$, * = $p \leq 0.05$, . = $p \leq 0.1$; SoV = Source of Variation, MR = Moisture regime, DF = Degree of freedom

Gen = Genotype, PN = Pod number, SDW = Shoot dry weight, PHI = Pod harvest index, HI = Harvest index, 100SW = Hundred seed weight

4.1.2 Shoot Dry Weight (SDW)

Combined data analysis showed significant ($P<0.05$) genotypic effects on shoot dry weight (SDW). Under DS, PT11-13 had the highest mean SDW at 108 g compared to PT16-20, which had the lowest mean SDW at 50 g. The drought tolerant check SER 16 had a mean SDW of 75 g under DS. The drought susceptible check Kabulangeti had a mean SDW of 60 g under DS. Under NS, SDW ranged from 80 g (Grand Mesa) to 289 g (PT16-4), with an average of 130 g. Water treatment effects on SDW were also significant ($P<0.05$).

4.1.3 Pod Number (PN)

Combined data analysis showed significant ($P<0.05$) genotypic effects on pod number (PN). The drought tolerant check SER 16 consistently had a highest mean PN at 16.2 across trial sites and moisture regimes. Among the pinto genotypes, PT11-13-B recorded the highest PN (15.7) under DS while CO-32330-14 had the lowest mean PN (7.5).

4.1.4 Harvest Index (HI)

There was significant ($P<0.001$) genotypic effect on HI. Under DS, HI ranged from 34.3% (CO-52646-14) to 60.2% (NDZ14048-2). The HI for the drought tolerant check SER16 was 67.5%, which was the highest among genotypes under DS. The HI for the drought susceptible check was 52.4%. The water treatment effect on HI was significant. The mean Harvest index for all genotypes under NS was 55.2 compared to 47.1 under DS.

4.1.5 Pod Harvest Index (PHI)

The genotypic effect on PHI was significant ($P<0.05$). Under DS, the highest mean PHI was recorded for NDZ14048-2 (75.3 %), while the lowest was for CO-52646-14 (57.9 %). The PHI for the drought tolerant check SER 16 was 74.3 % while, the drought susceptible check Kabulangeti recorded a PHI of 70.63 % under DS. The genotypes NDZ14048-2 (75.3 %) and NE2-18-20 (74.8 %) recorded higher PHI than the drought tolerant check SER16 under DS.

4.1.6 Hundred Seed Weight (HSW)

Genotypic effects on HSW at GART were not significant ($P>0.05$), but where significant for UNZA ($P<0.05$). The highest HSW at UNZA under DS was recorded for Kabulangeti (43.6

g) while the lowest HSW was recorded for PR1572-26 (21.14 g). The HSW under DS for the drought tolerant check SER 16 was 30.9 g.

4.1.7 Genetic Correlations

Genetic correlation analyses under DS revealed strong correlation between seed yield and the partitioning indices PHI (r=0.55***) and HI (r=0.68***) as indicated in Table 4.2. Under NS, correlations between seed yield and PHI, HI were also significant, but with reduced correlation coefficients. YPR was significantly correlated with partitioning indices PHI (r=0.51***), YPR and HI (r=0.53***). Under DS, there was a significant, but weak correlation between seed yield and SDW and seed yield was not significantly correlated with HSW.

Table 4.2. Correlation coefficients for morpho-physiological traits under drought stress

	PN	*SDW*	*YIELD*	*PHI*	*HI*	*100 SW*	*YPR*	*GM*
PN	1.00							
SDW	0.23**	1.00						
YIELD	0.55***	0.20**	1.00					
PHI	0.26***	0.14+	0.55***	1.00				
HI	0.46***	0.03ns	0.68***	0.79***	1.00			
100 SW	0.08ns	0.05ns	0.08ns	0.12ns	0.15+	1.00		
YPR	-0.26***	-0.06ns	-0.64***	-0.51***	-0.53***	-0.06ns	1.00	
GM	0.40***	0.10ns	0.86***	0.58***	0.61***	0.05ns	-0.49***	1.00

Significance code: ns = not significant, + = $P \leq 0.1$, * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$

Where; PN = Pod Number; SDW = Shoot dry weight; PHI = Pod Harvest Index; HI = Harvest index; 100 SW = Hundred seed weight; YPR = Yield percentage reduction; GM = Geometric mean

4.1.8 Relationship Between Seed Yield under Drought Stress and Non-Stress

The relationship between seed yield under DS and NS was explored to identify genotypes that had higher seed yield under both DS and NS. The four quadrants in Figure 4.1 illustrates the response of the pinto genotypes and checks under DS and NS, with the genotypes that were considered drought tolerant and responsive to ideal moisture conditions falling in the upperright quadrant. Nineteen genotypes (Figure 4.1) had higher seed yield than average yields of all genotypes under DS and NS. Among these 19 genotypes, the genotypes NDZ14048-2 and NDZ14048-5 were particularly outstanding in their performance under both DS and NS conditions. The drought-tolerant check SER16 also recorded higher seed yield than averages seed yields under DS and NS.

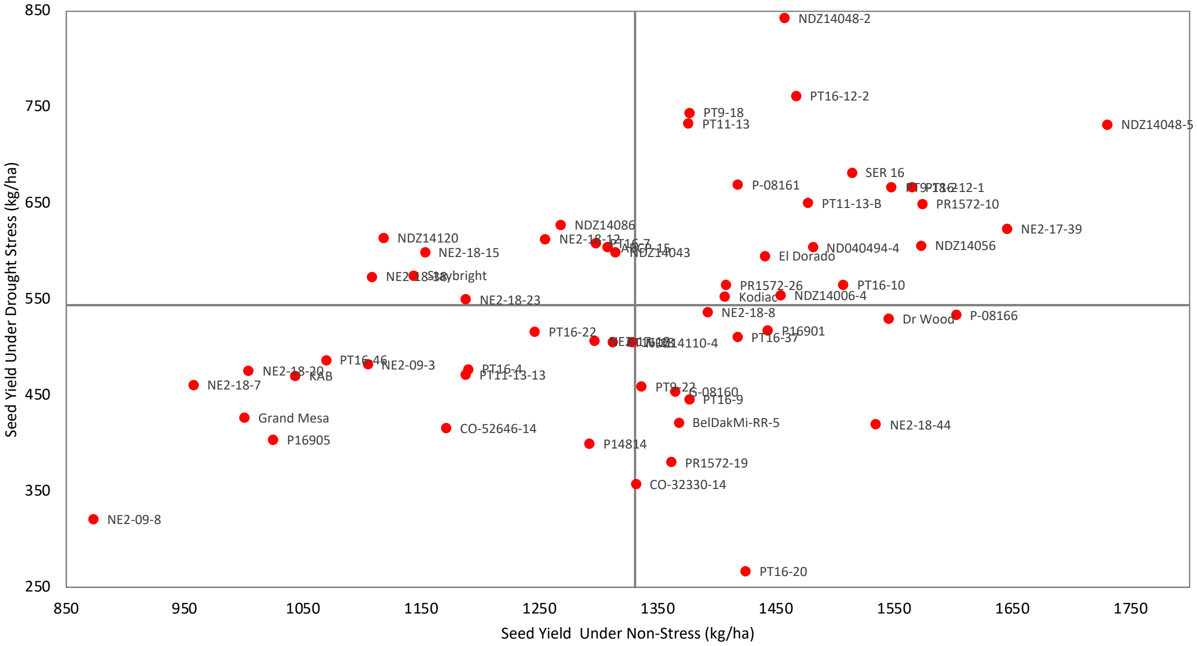

Figure 4.1. Seed yield for the 56 pinto genotypes and checks under drought stress and non-stress conditions.

4.1.9 Electrolyte leakage (EL)

The results for Electrolyte Leakage (EL) are reported in Table 4.3 under DS- EL for drought stress EL and NS-EL for non-drought stress EL. EL ranged from 18.6 (P16901) to 83.7 (PT9-22)) under NS and between 35.15 (Kodiac) to 100.21 (PR1572-26) under DS. The drought-tolerant check SER 16 ranked second for the lowest EL (19.5) whilst the drought susceptible check Kabulangeti ranked 38th for EL at 60.4 compared to other genotypes under NS.

Under DS, the genotype Kodiac had the lowest electrolyte leakage at 35.1 while PR1572-26 had the highest (100.2). The drought tolerant check SER 16 ranked second for lowest electrolyte leakage at 36.8 whilst the drought susceptible check Kabulangeti ranked 20th at

82.5 under DS. Water treatment had a significant effect (p<0.001) on the mean differences in EL under NS (53.9) and DS (82.1).

4.1.10 Canopy temperature depression (CTD)

The results for Canopy temperature depression (CTD) are indicated in Table 4.3 under DS-CTD for drought stress CTD and NS-CTD for Non drought stress CTD. CTD ranged from 1.4°C cooler (P16901) to 5.9°C cooler (NE2-18-15) compared to ambient temperature under NS. The CTD for the drought-susceptible check Kabulangeti and drought-tolerant check SER 16 were 1.6°C cooler and 2.8°C cooler compared to ambient temperature under NS. CTD for the drought-susceptible check Kabulangeti and drought tolerant check SER 16 were 1°C cooler and 4.8°C cooler compared to ambient temperature under DS. Among the pinto genotypes, PT16-20 had the highest CTD (3.2°C cooler) while NE2-18-38 had the lowest (0.9°C cooler). The CTD ranged from 4.77 °C cooler to 0.02 °C cooler compared to ambient temperature under DS.

Table 4.3. Means for electrolyte leakage (EL) and canopy temperature depression (CTD) for the 56 pinto genotypes and checks evaluated under drought and non-stress conditions in the green house at the University of Zambia**.**

Genotype	DS_EL	NS_EL	DS_CTD	NS_CTD
NE2-18-44	78.26	33.18	-2.96	-3.81
NE2-18-7	86.40	51.14	-1.83	-2.80
NE2-18-8	86.48	48.43	-1.74	-4.62
NE2-18-38	82.28	72.97	0.95	-4.31
NE2-18-12	94.03	66.08	-1.56	-3.61
NE2-18-15	68.70	57.14	-1.34	-5.89
NE2-18-20	98.90	74.77	-1.31	-3.37
NE2-18-23	98.21	61.66	-0.84	-1.71
PT11-13-B	96.47	60.14	-1.63	-3.72
PT16-12-1	93.60	80.61	-0.51	-2.45
PT16-12-2	75.50	82.89	-0.70	-2.54
PT16-18	87.84	71.39	-2.16	-1.80
PT11-13-13	87.96	72.72	-0.55	-3.63
PT16-4	85.15	41.94	-1.44	-2.40
PT16-7	81.49	67.61	-2.67	-2.42
PT16-46	75.93	67.61	-0.82	-2.11
PT16-37	96.06	50.13	-1.83	-2.43
PT16-22	85.99	62.05	-1.62	-2.30

PT16-20	86.57	57.74	-3.18	-2.48
PT9-22	92.97	83.66	-0.45	-2.54
PT9-18	86.77	38.79	-0.70	-2.95
PT9-18-2	70.42	34.31	-0.14	-2.73
PT16-9	88.42	42.34	-2.13	-2.45
PT16-10	90.00	43.43	-1.61	-2.71
PT11-13	89.32	54.03	-1.00	-2.74
NDZ14006-4	83.13	43.58	-1.84	-1.86
NDZ14043	54.65	63.72	-0.98	-3.83
NDZ14048-2	95.20	46.19	-2.81	-3.37
NDZ14048-5	99.57	28.61	-2.56	-1.80
NDZ14120	96.93	25.82	-1.32	-2.23
NDZ14056	92.64	81.16	-0.21	-2.52
NDZ14086	90.83	73.35	-0.27	-2.70
NDZ14110-4	83.69	65.69	-0.76	-1.91
PR1572-10	93.59	59.44	-1.29	-1.90
P-08166	95.29	29.34	-0.99	-1.99
PR1572-19	87.45	50.06	-0.90	-2.06
PR1572-26	100.21	61.12	-0.48	-1.89
P14814	89.59	44.77	-0.03	-2.37
P16901	91.41	18.64	-1.34	-1.40
P16905	56.48	53.68	-0.84	-1.55
P-08161	85.22	40.55	-0.84	-1.65
NE2-09-3	60.89	49.67	-2.65	-1.51
NE2-09-8	68.71	77.65	-2.33	-1.97
NE2-17-18	89.35	28.88	-2.12	-3.20
NE2-17-39	97.08	48.35	-2.74	-2.44
CO-32330-14	79.29	48.60	-1.49	-2.19
CO-52646-14	97.76	46.24	-0.83	-2.02
G-08160	93.65	56.55	-2.15	-1.87
ND040494-4	89.23	54.38	-2.07	-2.36
Staybright	96.25	43.52	-2.33	-2.27
ABCP-15	44.16	56.31	-2.93	-1.88
El Dorado	50.43	81.49	-0.88	-2.20
Dr Wood	52.78	40.02	-0.64	-2.07
BelDakMi-RR-5	75.81	75.57	-1.62	-2.13
Grand Mesa	50.12	32.35	-1.03	-2.32
Kodiac	35.13	46.08	-0.66	-2.75
SER 16	36.78	19.49	-4.77	-2.76
KAB	82.46	60.35	-1.00	-1.57
Mean	**82.06**	**53.93**	**-1.44**	**-2.53**

Note:

DS_EL = Drought and Heat Stress Electrolyte Leakage
NS_EL = Non-Drought and Heat Stress Electrolyte Leakage
DS_CTD = Drought and Heat Stress Canopy Temperature Depression
NS_CTD = Non-Drought and Heat Stress Canopy Temperature Depression
KAB (Kabulangeti) = Drought susceptibility check
SER 16 = Drought tolerant check

4.2 Evaluation for resistance to Anthracnose

The resistant check G2333 showed consistent resistant reaction (<2.0) to all seven races. As for the susceptible checks the reaction to the seven races was variable. The first susceptible check Kabulangeti was highly susceptible to three races (51, 65 and 247), moderately resistant to two races (3.7, 5.7) and resistant to two races (73 and 263). The second susceptible check Lusaka was highly susceptible to three races (51, 73 and 247), moderately resistant to three races (253, 263 and 1085) and resistant to one race (65).

There were significant differences among the 56 pinto genotypes in their reaction to races 51, 65, 73, 247, 253, 263 and 1085. For the 56 pinto genotypes, the highest average virulent score (7.8) was for race 263 while the lowest was for race 51. The average virulent scores for races 65, 73, 247, 253 and 1085 where 7.2, 4.5, 7.4, 6.3 and 7.3, respectively. In general, the distribution of the severity scores was skewed towards susceptibility except for races 51 and 73 (Middle American race). There was no pinto genotype that showed resistance to all seven races. The reactions of the genotypes to individual anthracnose races are presented in Figures 4.2 to 4.8.

4.2.1 Race 51

The disease scores of the 56 genotypes for reaction to race 51 were skewed towards resistant (Figure 4.2). The results revealed that 35 (63.3%) genotypes were resistant to race 51, with 12 (21.7%) being moderately resistant and 9 (16.0%) were susceptible. The two local susceptible checks Kabulangeti and Lusaka were both susceptible to race 51 while the anthracnose resistant check G2333 was resistant. The range of scores for reaction to race 51 was 1 to 9, with an average score of 3.5. Eleven genotypes (NDZ4048-2, NDZ14048-5, NDZ14043, PR1572-26, PT11-13, PT16-10, PT16-9, PT9-18, PT9-18-2, PT9-22 and G2333) had a score of 1.

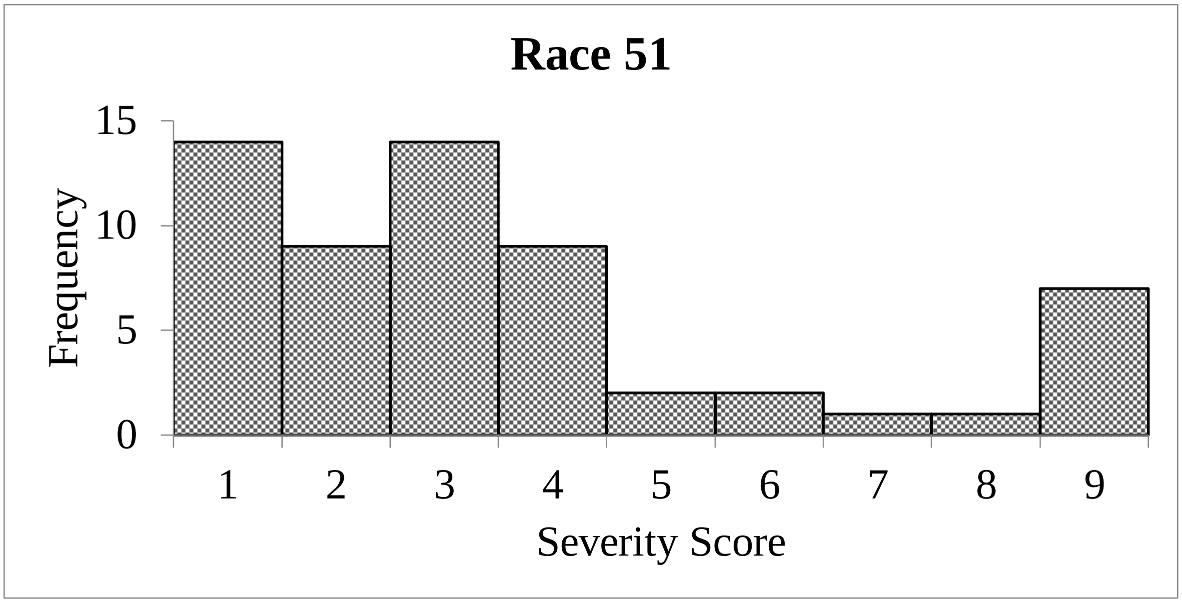

Figure 4.2. Frequency distribution of severity scores for race 51 for the 56 pinto genotypes and checks.

4.2.2 Race 65

The disease scores of the 56 genotypes for reaction to race 65 were skewed towards susceptible (Figure 4.3). Of the 56 genotypes, 11 (20%) genotypes were resistant, one (1.8%) was moderately resistant and 44 (78.3%) were susceptible to race 65. The two local susceptible checks Kabulangeti and Lusaka were both susceptible to race 65 while the anthracnose resistant check G2333 was resistant. The range of scores for reaction to race 65 was 1 to 9, with an average score 7.3. Twelve genotypes (NDZ4048-2, BelDakMi-RR-5, G2333, Lusaka, NDZ14006-4, NDZ14043, NDZ14043, NDZ14043, NDZ14048-5, NDZ14056, NDZ14086, NDZ14110-4 and PT9-18 had a score of 1.

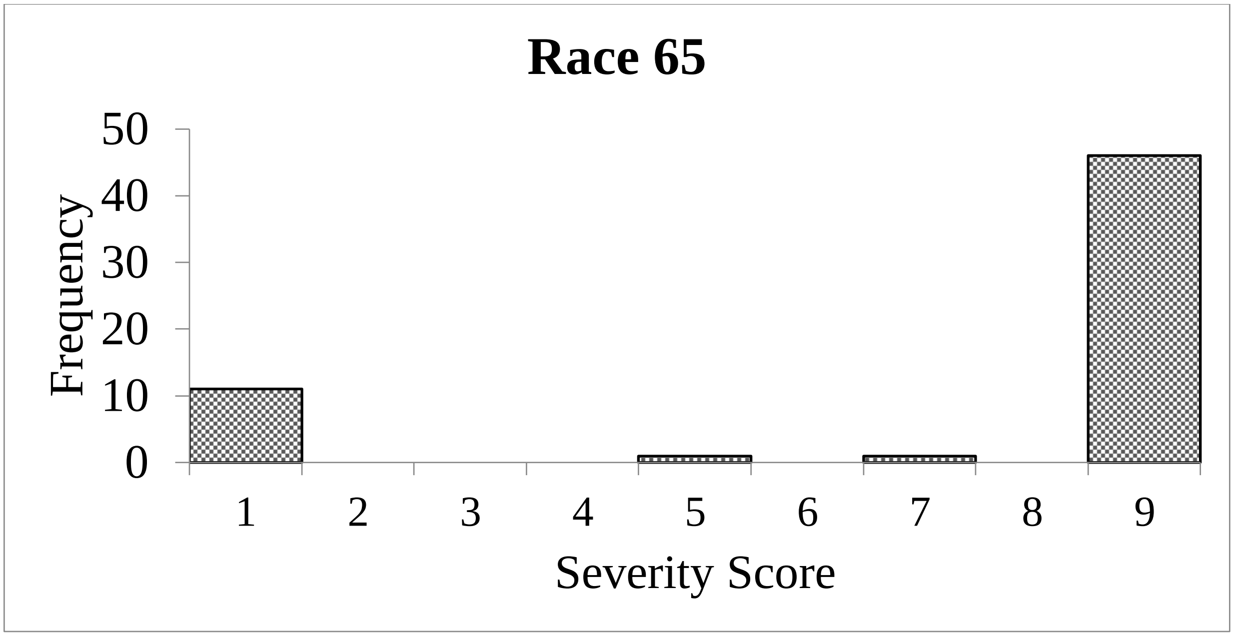

Figure 4.3. Frequency distribution of severity scores for race 65 for the 56 pinto genotypes and checks.

4.2.3 Race 73

The disease scores of the 56 genotypes for reaction to race 73 were skewed towards resistant (Figure 4.4). Of the 56 evaluated, 29 (48.3 %) were resistant, 10 (16.67 %) moderately resistant and 21 (35 %) were susceptible to race to the Middle American race 73. The local susceptible check Kabulangeti was resistant while the other susceptible check Lusaka was susceptible. The resistant check G2333 was resistant. The range of scores for reaction to race 65 was 1 to 9, with an average score 4.5. Six genotypes (NDZ14110-4, G2333, NDZ14056, NE2-18-12, PT9-22 and NDZ14086) had a score of 1.

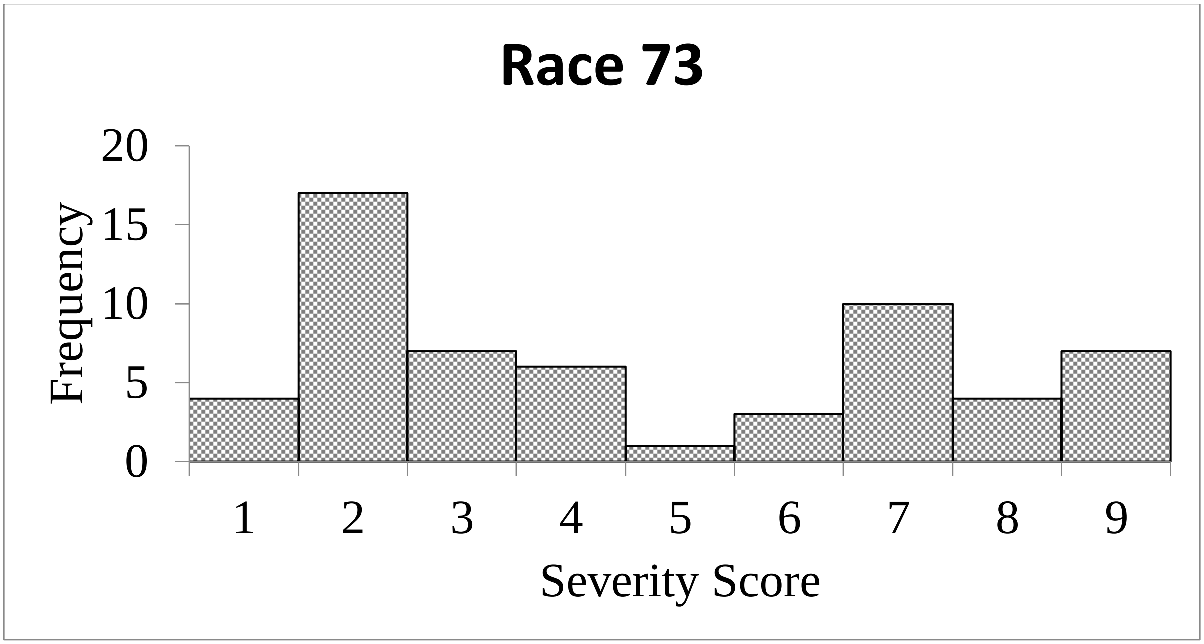

Figure 4.4. Frequency distribution of severity scores for race 73 for the 56 pinto genotypes and checks.

4.2.4 Race 247

The disease scores of the 56 genotypes for reaction to race 247 were skewed towards susceptible (Figure 4.5). The results revealed that 5 (8.3%) genotypes were resistant, 7 (11.7%) were moderately resistant and 48 (80%) were susceptible to race 247. The two local susceptible checks Kabulangeti and Lusaka were both susceptible to race 247 while the anthracnose resistant check G2333 was resistant. The range of scores for reaction to race 247 was 1 to 9, with an average score of 7.5. Three genotypes (NDZ14086, NDZ14006-4 and G2333) had score of 1.

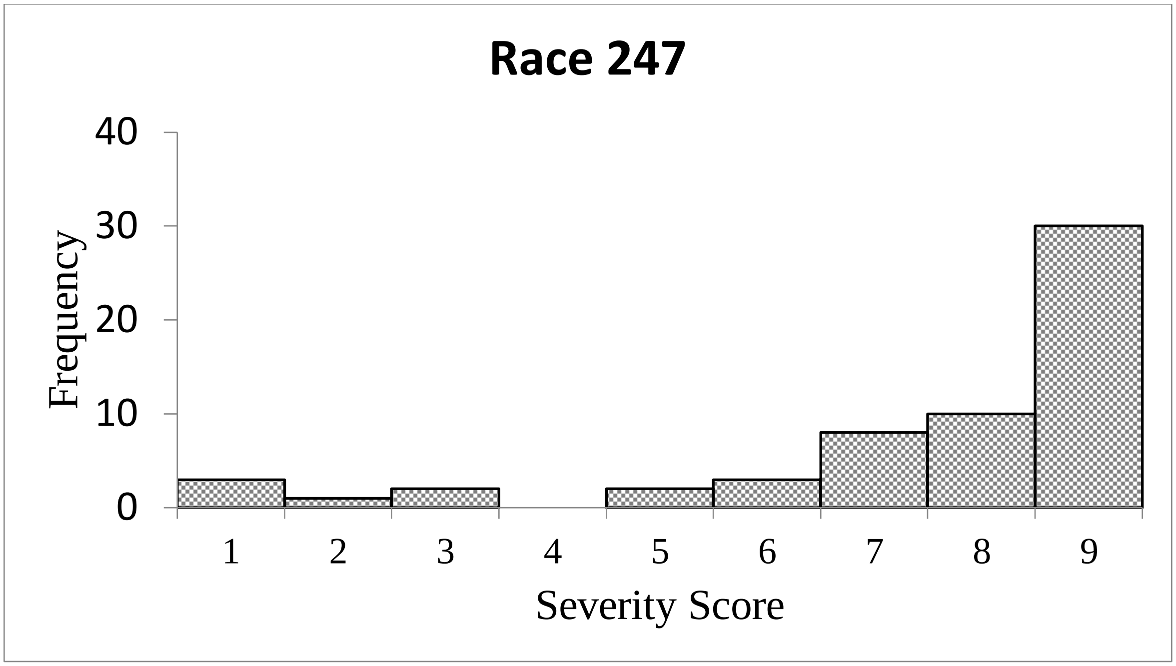

Figure 4.5. Frequency distribution of severity scores for race 247 for the 56 pinto genotypes and checks

4.2.5 Race 253

The disease scores of the 56 genotypes for reaction to race 253 were skewed towards susceptible (Figure 4.6). Of the 56 evaluated, 13 (21.7 %) were resistant, 8 (13.3 %) moderately resistant and 39 (69.6 %) were susceptible to race to the Middle American race 253. The local susceptible check Kabulangeti was resistant while the other susceptible check Lusaka was susceptible. The resistant check G2333 was resistant. The range of scores for reaction to race 253 was 1 to 9, with an average score 6.3. Seven genotypes (G2333, NDZ14006-4, NDZ14056, NDZ14086, NDZ14110-4, PT9-18 and PT9-18-2) had a score of 1.

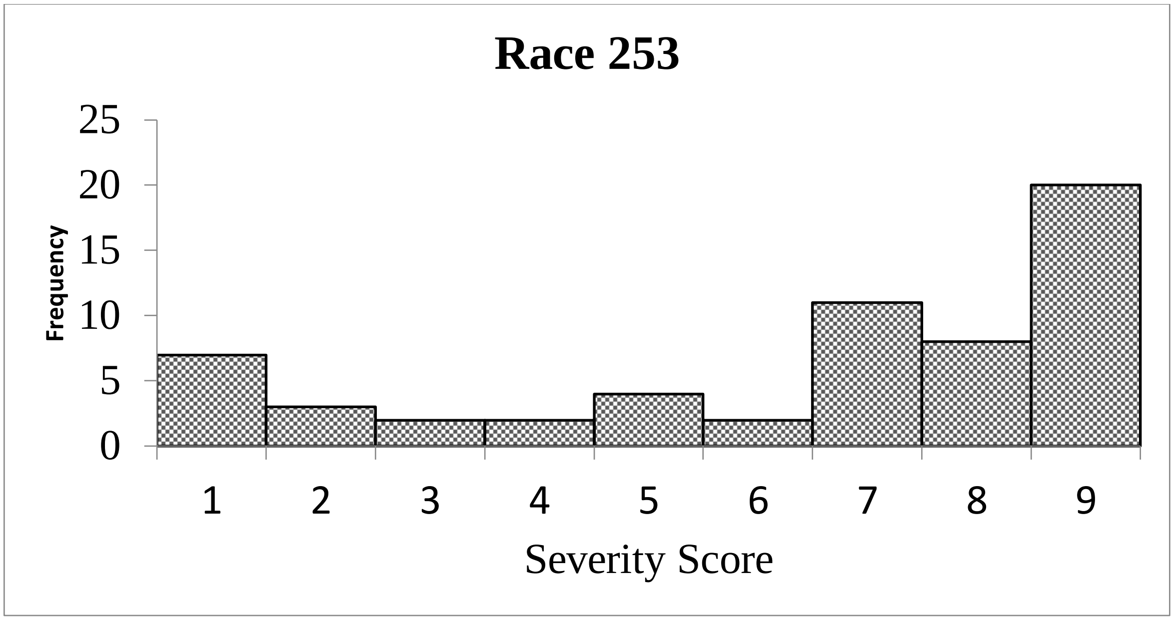

Figure 4.6. Frequency distribution of severity scores for race 253 for the 56 pinto genotypes and checks**.**

4.2.6 Race 263

The disease scores of the 56 genotypes for reaction to race 263 were skewed towards susceptible (Figure 4.7). The results revealed that five (8.3%) genotypes were resistant, one (1.7%) were moderately resistant and 50 (90%) were susceptible to race 263. The two local susceptible checks Kabulangeti and Lusaka were both moderately resistant to race 263 while the anthracnose resistant check G2333 was resistant. The range of scores for reaction to race 263 was 1 to 9, with an average score of 7.8. Three genotypes (G2333, Kabulangeti and NE2-18-12) had score of 1**.**

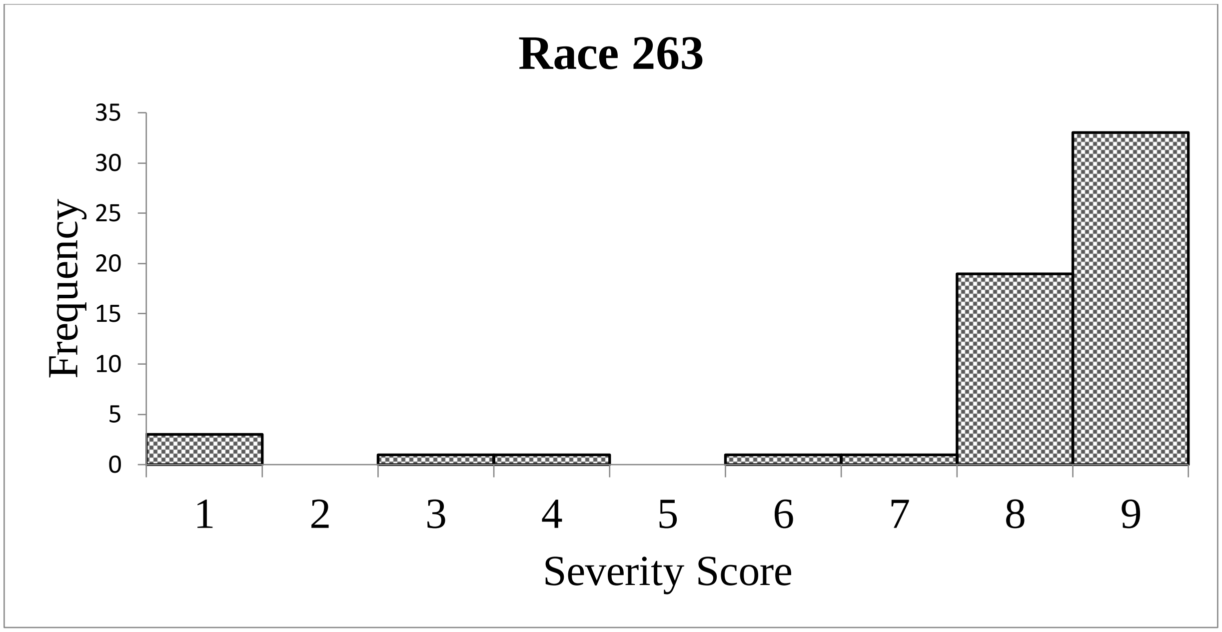

Figure 4.7. Frequency distribution of severity scores for race 263 for the 56 pinto genotypes and checks

4.2.7 Race 1085

The disease scores of the 56 genotypes for reaction to race 1085 were skewed towards susceptible (Figure 4.8). Of the 56 evaluated, 7 (13.3 %) were resistant, 11 (18.3 %) moderately resistant and 41 (68.3 %) were susceptible to race 1085. The local susceptible check Kabulangeti was resistant while the other susceptible check Lusaka was susceptible. The resistant checks G2333 was resistant. The range of scores for reaction to race 1085 was 1 to 9, with an average score 7.3. Four genotypes (BelDakMi-RR-5, G2333, Grand Mesa and Kodiac) had score of 1.

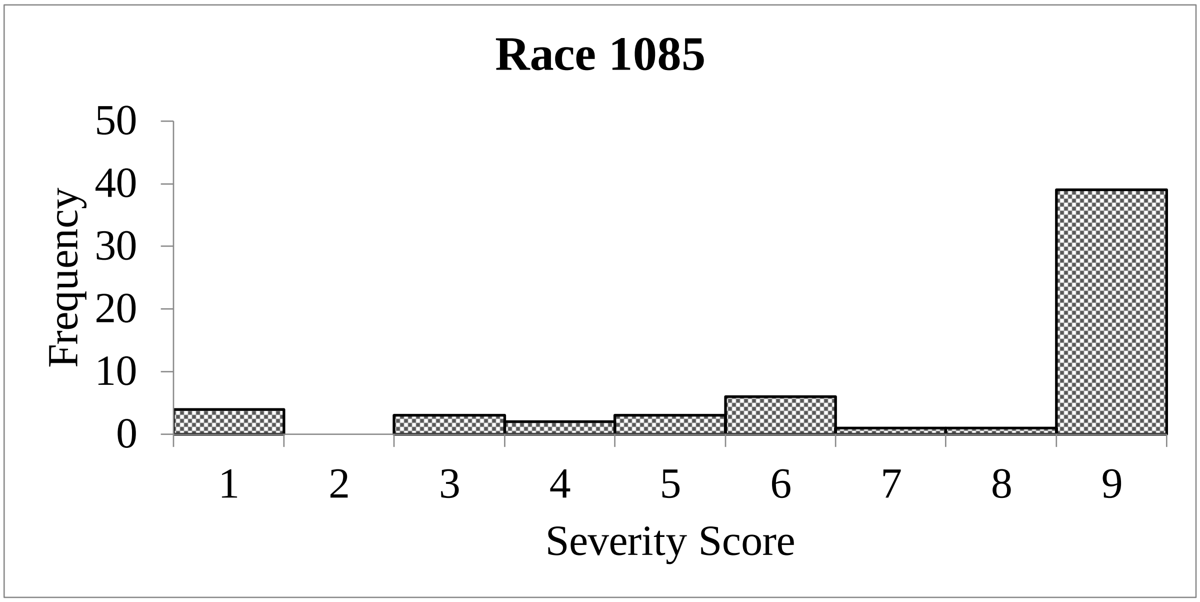

Figure 4.8. Frequency distribution of severity scores for race 1085 for the 56 pinto genotypes and checks

The reaction of the 56 pinto genotypes and checks to the seven races and status of genotypes that carry anthracnose resistance loci CO-4^{-2} are also shown in Table 4.4. The results show that nine pinto genotypes carry the CO-4^{-2} and these are NDZ140064-4, PT9-18, NDZ14110-4, NDZ14056, PT9-18-2, NDZ14086, NDZ14043, NDZ14048-5 and NDZ14048-2. In addition to resistant loci CO-4^{-2}, the anthracnose resistant genotype G2333 carry CO-5 and CO-7 resistant locus

Table 4.4. Average severity scores of the 56 pinto genotypes reaction to seven races of *C. lindemuthianum* and status of genotypes that carry Co-4^{-2}, Co-5^{-2} and Co-7

Genotype	**Race 51**	**Race 65**	**Race 73**	**Race 247**	**Race 253**	**Race 263**	**Race 1085**	**Co-4^{-2}**	**Co-5^{-2}**	**Co-7**
G2333	1	1	2	1	1	1	1	+	+	+
NDZ14006-4	1	1	2	1	1	9	3	+	-	-
PT9-18	1	1	2	5	1	6	4	+	-	-
NDZ14110-4	2	1	2	2	1	3	5	+	-	-
NDZ14056	2	1	1	6	1	9	5	+	-	-
PT9-18-2	1	5	3	7	1	8	6	+	-	-
NDZ14086	3	1	1	1	1	9	6	+	-	-
NDZ14043	1	1	2	3	2	8	3	+	-	-
NDZ14048-5	1	1	2	6	2	8	4	+	-	-
G-08160	1	9	8	8	2	9	9	-	-	-
NDZ14048-2	1	1	3	7	3	8	6	+	-	-
PR1572-26	1	9	7	8	3	9	9	-	-	-
KAB	9	9	2	8	4	1	6	-	-	-
NE2-18-12	8	9	1	8	4	1	6	-	-	-

LSK	9	1	9	9	5	4	6	-	-	-
PT16-9	1	7	3	7	5	8	9	-	-	-
NE2-18-38	4	9	8	9	5	9	9	-	-	-
PR1572-10	7	9	2	8	5	9	9	-	-	-
NE2-18-8	3	9	5	8	6	7	9	-	-	-
PT16-7	4	9	7	9	6	8	9	-	-	-
PT11-13	1	9	4	9	7	9	3	-	-	-
NDZ14120	2	9	3	6	7	9	5	-	-	-
PT16-46	3	9	7	7	7	9	8	-	-	-
NE2-17-39	2	9	2	5	7	8	9	-	-	-
PT11-13-B	2	9	3	8	7	8	9	-	-	-
ND040494-4	3	9	7	9	7	9	9	-	-	-
NE2-18-7	3	9	6	7	7	9	9	-	-	-
PR1572-19	3	9	4	7	7	9	9	-	-	-
PT16-20	2	9	9	9	7	9	9	-	-	-
PT16-22	2	9	4	8	7	9	9	-	-	-
PT9-22	1	9	1	9	7	9	9	-	-	-
CO-32330-14	2	9	2	9	8	8	9	-	-	-
NE2-17-18	2	9	7	9	8	8	9	-	-	-
P16901	3	9	3	7	8	8	9	-	-	-
P16905	4	9	2	7	8	8	9	-	-	-
PT16-4	4	9	7	9	8	8	9	-	-	-
NE2-18-23	3	9	8	9	8	9	9	-	-	-
PT11-13-13	9	9	2	9	8	9	9	-	-	-
Staybright	4	9	7	9	8	9	9	-	-	-
Kodiac	3	9	2	8	9	8	1	-	-	-
BelDakMi-RR-5	3	1	4	3	9	9	1	-	-	-
Grand Mesa	3	9	9	9	9	9	1	-	-	-
P14814	3	9	6	9	9	8	7	-	-	-
El Dorado	6	9	8	9	9	8	9	-	-	-
NE2-09-8	3	9	4	9	9	8	9	-	-	-
P-08161	9	9	2	8	9	8	9	-	-	-
PT16-12-1	5	9	6	9	9	8	9	-	-	-
ABCP-15	4	9	7	9	9	9	9	-	-	-
CO-52646-14	9	9	9	9	9	9	9	-	-	-
Dr Wood	9	9	9	9	9	9	9	-	-	-
NE2-09-3	1	9	2	9	9	9	9	-	-	-
NE2-18-15	4	9	9	9	9	9	9	-	-	-
NE2-18-20	6	9	7	9	9	9	9	-	-	-
NE2-18-44	4	9	2	9	9	9	9	-	-	-
P-08166	5	9	3	9	9	9	9	-	-	-
PT16-10	1	9	4	9	9	9	9	-	-	-
PT16-12-2	3	9	7	9	9	9	9	-	-	-
PT16-18	9	9	9	9	9	9	9	-	-	-
PT16-37	4	9	2	9	9	9	9	-	-	-

Note:

+	refers to positive resistance allele C0-4^{-2}, CO-5^{-2} or C0-7
-	refers to negative resistance allele C0-4^{-2}, CO-5^{-2} or C0-7

Ten genotypes (G2333, NDZ140064-4, PT9-18, NDZ14110-4, NDZ14056, PT9-18-2, NDZ14086, NDZ14043, NDZ14048-5 and NDZ14048-2 had C0-4^{-2}, whereas additionally G2333 had C0-5^{-2} and Co-7.

4.2.8 The effect of CO-4^{2} status on the anthracnose severity score of pinto genotypes and checks

In general, figure 4.9 revealed that the genotypes with Co-4^{2} had a relatively lower anthracnose severity score compared to those without Co-4^{2}. The overall severity score for genotypes with C0-4^{-2} was 2.9 and the race specific average severity scores were race 51 (1.4), race 65 (1.4), race 73 (1.8), race 247 (3.5), race 253 (1.2), race 263 (6.7) and race 1085 (4.1). Whereas, the overall average severity score for genotypes without Co-4^{2} was 7.1 with race specific average severity scores being race 51 (3.9), race 65 (8.5), race 73 (5.1), race 247 (8.3), race 253 (7.4), race 263 (8.2) and race 1085 (8.0).

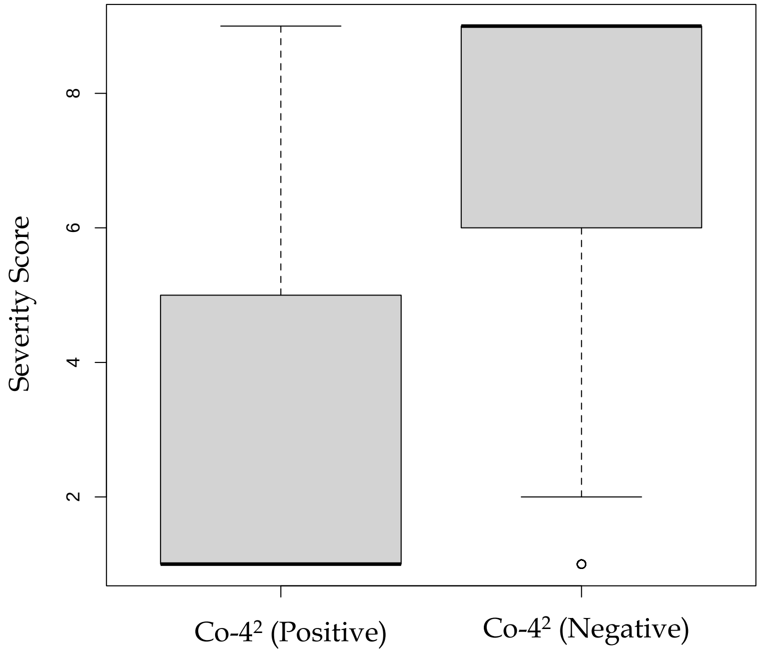

Figure 4.9: Box plot of anthracnose severity score for genotypes with and without Co-4^{2}

4.2.9 Reaction of the 56 pinto genotypes and checks to seven races of *Colletotrichum lindemuthianum*

The results in Table 4.5 revealed a highly significant differential reaction of the 56 pinto genotypes to the seven races of *Colletotrichum lindemuthianum*. The mean severity scores for race 51 and 73 were skewed towards resistant while the mean severity scores for races 65, 247, 253, 263 and 1085 were skewed towards susceptibility.

Table 4.5. Means and ranges for anthracnose severity scores measured on 56 pinto genotypes and checks after being inoculated with seven races of *Colletotrichum lindemuthianum* in the green house at University of Zambia.

	Checks			Pinto Genotypes		
	Susceptible		Resistant			
Race	Kabulangeti	Lusaka	G2333	Mean	Range	ANOVA
51	9	9	1	3.5 ± 0.3	1 – 9	***
65	9	1	1	7.2 ± 0.4	1 – 9	***
73	2	9	1.6	4.5 ± 0.4	1 – 9	***
247	8	9	1	7.4 ± 0.3	1 – 9	***
253	3.7	5	1	6.3 ± 0.4	1 – 9	***
263	1	3.7	1	7.8 ± 0.3	1 – 9	***
1085	5.7	5.7	1	7.3 ± 0.3	1 – 9	***

**Significant at the level 0.05 probability level

***Significant at the level 0.001 probability level

CHAPTER FIVE

DISCUSSION

5.1 Objective One: Evaluation for Resistance to Terminal Drought

Drought and anthracnose are major constraints of common bean production worldwide. In Zambia both stresses are wide spread and devastating, therefore evaluation of common bean genotypes for tolerance to drought and resistance to anthracnose is important.

There was significant variation among the 56 genotypes of the parameters measured such as yield, YPR and GM. The differences could be attributed to the differential response of genotypes to applied moisture regimes and variation in drought intensity indices (GART 2021 = 0.67, GART 2022 = 0.47 and UNZA 2022 = 0.60). Drought caused a 48.59 % reduction in yield across the field trials. This reduction is consistent with previous studies that have reported seed yield reduction of 60 to 99 % due to terminal drought stress (Ambachew et al,2015). Genotypes including NDZ14048-2, NDZ14120, PT16-12-2, PT11-13, NE2-18-15, NE2-18-12 and PT9-18 showed smaller YPR (< 37.6 %) than the drought tolerant check SER16 (37.6 %). These genotypes were considered as being drought tolerant. These genotypes can be considered for use to improve the adaptation of pinto beans to drought. A desirable genotype is one that produces high seed yield under both drought stress or non-drought stress conditions. In the current study, eighteen (18) pinto genotypes had higher yield than average seed yields under DS or NS conditions. These genotypes were well adapted to both DS and NS conditions. The genotypes NDZ14048-2 and NDZ14048-5 were particularly outstanding in their yield performance under both DS and NS and recorded higher yields than the drought resistant check SER 16. The genotype NDZ4048-2, which recorded a low YPR (34.6%) and had the highest GM (1,102 kg ha^{-1}) and could therefore be selected for germplasm improvement based on the two indices. Ramirez and Kelly (1998) recommended a selection strategy for drought tolerance in common bean that is based on the selection for high geometric mean followed by selection for low DSI. This study affirms the selection strategy for common bean drought tolerance that is based on high geometric mean, lower YPR and DSI. Interestingly, NDZ4048-2 was one of the genotypes that showed high HI, suggesting that selecting for partitioning efficiency in addition to geometric mean and DSI (recommended by Ramirez and Kelly (1998)) could result even in more effective selection for drought tolerance in common bean. Identification of agronomic and physiological traits that are strongly related with seed yield and possess high heritability is necessary for developing selection indices to make phenotypic selection for drought tolerance more effective. In this study, seed yield strongly correlated with partitioning indices PHI and HI suggesting that assimilate remobilization to the seed from the pod wall and the rest of the plant played a significant role in the observed variation for drought tolerance among the 56 pinto genotypes. Previous studies have also recommended the use of partitioning indices (PHI and HI) to indirectly selection for drought tolerance in other market classes of common beans. The report that selection for partitioning indices (PHI and HI) in drought could result in yield gain in both drought and non-drought condition makes a very attractive selection criterion for yield improvement (Assefa et al., 2013). Ramirez-Vallejo and Kelly (1998) reported a high heritability for HI. Selection for HI in addition to geometric mean would result in more effective selection for drought tolerance in common bean.

There was a significant correlation (r=0.73***) between seed yield and SDW under non-drought stress, however this correlation was weak (r=0.16***) under drought stress. This suggests lower prediction value of SDW under drought stress than under NS for the 56 genotypes used in the current study. This result could have been caused by genotypes that could have accumulated shoot biomass but lacked partitioning efficiency of the biomass to seed. The weak correlation between seed yield and SDW but strong correlation between seed yield and partitioning indices highlights the importance of jointly selecting for SDW and partitioning indices in breeding for adaptation to drought. This further demonstrates that selection based on SDW alone may not be effective. There was no significant correlation between seed yield and hundred seed weight in the current study indicating the lack of usefulness of seed weight in selecting for drought tolerance in the current study. Although there was significant correlation between seed yield and PN, the low heritability for PN may negate its usefulness in selection for drought tolerance

The difference in electrolyte leakage between DS (82.1%) and NS (54.2%) was significant confirming the significant damage of drought stress on the cell membrane. Additionally, there was significant variation among the 56 genotypes in their cell membrane damage caused by drought. In the current study, two pinto beans (Kodiac and ABCP-15) and the drought tolerant check SER16 had the lowest electrolyte leakage and highest CTD. A cool canopy is an adaptive mechanism of plants to moisture stress and has previously been reported to be significantly associated with increased seed yield in wheat under drought stress conditions (Blum et al., 1989; Rashid et al., 1999).

5.2 Objective Two: Evaluation for resistance to Seven Races of *C. lindemuthianum*

Of the 56 pinto genotypes evaluated in this study, nine had the Co-4^2 anthracnose resistance allele. The average severity score (2.4) for these nine genotypes was significantly lower than the average score (6.2) for the 47 genotypes that did not have the Co-4^2. The nine genotypes with Co-4^2 provided variable levels of resistance to the seven races suggesting that other host resistance genes could have been involved the resistance they showed. It is important to note, however, that the resistance allele Co-4^2 was not effective against all seven races. All nine genotypes with Co-4^2 allele were susceptible to races 251 and 1085 except genotypes

NDZ14110-4 and NDZ14006-4, which were moderately (severity score of 3) resistant to races 251 and 1085, respectively. The resistant check G2333, which also carries Co-4^2 was the only one among genotypes with Co-4^2 that was resistant to all seven races used in the current study. This broad-spectrum resistance in G2333 could be attributed to additional resistance loci Co-5 and Co-7 that it carries. The susceptibility to races 263 and 1085 of some of the genotypes that carries Co-4^2 underscores the need for pyramiding additional resistance loci such as Co-5 to confer broad-spectrum resistance to *C. lindemuthianum* similar to the one observed in the resistant check G2333. Race 263 was highly virulent on all the 56 pintos except NE2-18-38 and the resistant check (G2333). Surprisingly, the susceptible check (Kabulangeti) was resistant NE2-18-38. The genotype NE2-18-38, therefore, would be a valuable source of resistance for genetic improvement of pinto beans in geographic areas that have race 263. However, in areas that have races 51, 65, 247 and 1085 NE2-18-38 would not be an effective source of resistance.

The genotype BelDakMi-RR-5 was interesting because it was resistant to five races including the highly virulent race 1085, and yet it does not carry Co-4^2 or any other known major anthracnose loci. Future studies aimed at understanding the genetic basis of this observed resistance would be worthwhile.

CHAPTER SIX

CONCLUSIONS

6.1 Conclusion for Objective One

This study identified significant genetic variation among the 56 pinto genotypes for morpho-physiological traits under drought stress conditions. The genotype NDZ4048-2 was identified as being highly tolerant to drought stress based on its low YPR and its high yield geometric mean. The partitioning indices (PHI and HI) were strongly correlated with seed yield under drought stress suggesting the important role that partition efficiency played in the observed variation for drought tolerance among the 56 genotypes. The high correlation of the partitioning indices (PHI and HI) with seed yield and their high heritability (Assefa et al., 2013, Ramirez-Vallejo and Kelly., 1998), suggests they could be reliably be used in combination with other traits to select for drought tolerance.

6.2 Conclusion for Objective Two

The genotypes presented variable reaction to the seven races. The most virulent race was 263 while the least virulent was 51. Except for races 51 and 73 the reaction of the 56 pinto genotypes was skewed to susceptibility, which highlighted the lack of resistance in most of the pinto beans to the races used in the current study. In general, the genotypes that showed resistance to most of the races were those that carried Co-4^2, thereby highlighted the importance of this locus. The three genotypes NDZ14006-4, NDZ14110-4 and NDZ14043 showed superior resistance among the 56 pinto genotypes used in the current study as each was resistant to six of the seven races used in the current study. These three genotypes, which all carry Co-4^2 can be used for genetic improvement of pinto beans for anthracnose resistance. None of the 56 pinto genotype that showed resistance to all seven races of *C. lindemuthianum* like the resistant check G2333. This result underscored the importance of pyramiding major loci Co-4^2 and Co-5. Pyramiding the Andean locus Co-1 can also be considered because most of the beans grown in southern African including Zambia are Andean and Andean races or mixed races are prevalent.

CHAPTER SEVEN

RECOMMENDATIONS

Based on the high correlation of the partitioning indices with seed yield, and their higher heritability than seed yield, PHI and HI can be used to indirectly select for drought tolerance or can be used in combination with other traits to develop a reliable selection index for drought tolerance.

The genotypes NDZ14048-2 and NDZ14048-5 showed superior seed yield under both DS and NS, therefore recommend genotype NDZ14048-2 as drought tolerant, and genotype NDZ14048-5 as drought tolerant and highly responsive to ideal moisture condition. Additionally, these two genotypes carry Co-4^2 anthracnose resistance locus, and were resistant to five of the seven races used in the current study and could be used for direct introduction or for genetic improvement of pinto beans for adaptation to drought and resistance to anthracnose.

Three genotypes NDAZ14006-4, NDZ14110-4 and NDZ14043 that carry the CO-4 resistance loci showed superior resistance to six of the seven races in the study and could be used for genetic improvement of pinto bean for anthracnose resistance. There is also need to pyramid anthracnose major resistance loci Co-1, Co-4 or Co-5 in pinto genotypes to provide them with durable resistance to multiple races of *C. lindemuthianum* found in Zambia.

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CHAPTER EIGHT

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APPENDICES

Appendix 1: Germplasm

Genotype names
NE2-18-44
NE2-18-7
NE2-18-8
NE2-18-38
NE2-18-12
NE2-18-15
NE2-18-20
NE2-18-23
PT11-13-B
PT16-12-1
PT16-12-2
PT16-18
PT11-13-13
PT16-4
PT16-7
PT16-46
PT16-37
PT16-22
PT16-20
PT9-22
PT9-18
PT9-18-2
PT16-9
PT16-10
PT11-13
NDZ14006-4
NDZ14043
NDZ14048-2
NDZ14048-5
NDZ14120
NDZ14056
NDZ14086
NDZ14110-4
PR1572-10
P-08166
PR1572-19
PR1572-26
P14814
P16901
P16905

P-08161
NE2-09-3
NE2-09-8
NE2-17-18
NE2-17-39
CO-32330-14
CO-52646-14
G-08160
ND040494-4
Staybright
ABCP-15
El Dorado
Dr Wood
BelDakMi-RR-5
Grand Mesa
Kodiac
NDZ14110-4
G2333
LSK
KAB
SER 16