

**PHYSIOLOGICAL RESPONSES OF COMMON BEAN (*Phaseolus vulgaris* L.)
GENOTYPES TO WATER STRESS**

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LUSAKA

DECLARATION

I **PHILLIP SALYULA KALIMA** 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 a degree at this or any other University.

Signed.....

Date.....

APPROVAL

The University of Zambia approves this dissertation of Phillip Salyula Kalima as fulfilling the requirements for the award of the degree of Master of Science in Plant Breeding and Seed Systems.

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ABSTRACT

Common bean (*Phaseolus vulgaris* L.) is highly constrained by water deficit especially occurring during the reproductive development which significantly reduces the grain yield. A late planted crop, is likely to experience water stress. The purpose of the study was to determine the association of the morphophysiological traits with water stress and how they relate to grain yield in common beans. A field experiment involving eight common bean genotypes (CAR-ZAR, KE 3, ZM 4488, KE 4, SER 76, SER 180, GADRA, and SER 89) and three water regimes (50 %, 75 %, & 100 % of crop evapotranspiration) was conducted at National Irrigation Research Station (NIRS) in Mazabuka district during the 2012 growing season. A 3 x 8 split plot design with four replications was used; with water stress as the main plot factor and genotype as the subplot factor. Water stress was imposed at pre-flowering stage and was discontinued after 43 days when the crop was in its late reproductive stage. The results showed that significant differences were found among genotypes for relative injury percentage, chlorophyll a, chlorophyll b, total chlorophyll, relative water content, grain yield, number of pods per plant, hundred seed weight, number of seeds per pod and days to fifty percent flowering for the three water regimes. Grain yield in normally irrigated treatment (2.19 ton ha⁻¹) was 60 % higher than in 50 % water stress treatment (0.87 ton ha⁻¹), while in the 75 % water stress treatment (1.08 ton ha⁻¹), the reduction in grain yield was 50.8 %. There was significant genotype by environment (GXE) interactions. These interactions were observed in relative injury percentage, chlorophyll a, chlorophyll b, total chlorophyll, relative water content, grain yield, number of pods per plant, hundred seed weight, number of seeds per pod and days to fifty percent flowering in the high water stress condition. The genotypes GADRA, KE 4, ZM 4488, and SER 180 were identified as water stress tolerant while the genotypes SER 89, CAR-ZAR, KE 3 and SER 76 were identified as water stress sensitive genotypes based on GM, DSI and PR. It was postulated that positive association between yield reduction (PR) and drought susceptibility index (DSI) renders either trait useful in selecting water stress tolerant genotypes. All the traits measured were positively and significantly correlated to grain yield apart from chlorophyll b which had a negative correlation ($r = -0.41^{**}$). A strong positive correlation was recorded for hundred- seed- weight ($r = 0.41^{**}$), chlorophyll a content ($r = 0.57^{**}$) and number of pods per plant ($r = 0.36^{*}$). A stepwise multiple regression showed that hundred- seed- weight, chlorophyll a, chlorophyll b and number of pods per plant contributed significantly to the total variation of grain yield. These results suggest that a selection method based on hundred seed weight, chlorophyll a,

chlorophyll b and number of pods per plant can be used in breeding for bean genotypes to water stress.

DEDICATION

I dedicate this piece of work to my wife (Bathsheba Kalima), children (Carol, Cleopatra, Bridget and Loveness) and my late father (Muselekenu Kalima) and mother (Mukosai Kalima). I drew a lot of inspiration from them and the encouragement from my wife and children was great.

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Chapter 1

1. INTRODUCTION

Common bean (*Phaseolus vulgaris L.*) is one of the widely cultivated plants. It is considered one of the most important grains for human alimentation and is planted worldwide on approximately 26 million hectares (CIAT, 2001, Emam *et al.*, 2010). In Zambia, common beans (here after referred to as beans) are an important legume food crop which is widely consumed countrywide by many households. Its importance is especially high in low income households due to its affordability (CSO, 2008). In terms of economic importance, beans are second to groundnuts among the food legume crops grown in Zambia (Series, Crop, and Support 2009). With over 25 % protein content, bean is a major source of protein in cereal-based diets of smallholder farmers (Manjeru *et al.*, 2007). The vegetable protein in beans can easily be a substitute for animal protein such as meat and poultry and is affordable for most of the small-scale households.

Legumes the group of plants to which beans belong, fix atmospheric nitrogen in the soil in association with *Rhizobium* bacteria (Kay, 1979). The fixation of atmospheric nitrogen improves the soil nitrogen level benefiting the crop that are grown thereafter, thereby reducing production costs. The common bean residues left on the field improve the soil structure (Barrett, 1990). Like other plants, the development and productivity of beans is adversely affected by biotic and abiotic stress factors (Jaleel *et al.*, 2009). With the evolving phenomena of climate change, it is anticipated that these stress factors will exert increasing impacts on crop productivity (Man *et al.*, 2011). Among the abiotic factors, water stress is perhaps the major factor limiting crop production worldwide (Jones and Corlett, 1992). Water stress is ranked second from pests and diseases that cause grain yield losses with about 60 % of world bean production areas being prone to drought.

The production of beans is usually under rainfed conditions and consequently insufficient or unpredictable rainfall may limit the yield. (Acosta-gallegos and Domingo, 2009). In Zambia, the bulk of bean production is by small scale farmers who depend entirely on rainfall and stored soil moisture water supply. During the growing season, intermittent and or terminal droughts are sometimes experienced. Improved management practices (e.g. supplementing with irrigation during drought periods) can contribute to improved yields in water-deficient environments though irrigation facilities are costly for most small scale farmers . It is known that major gains in stabilising or improving yield can be achieved through genetic

improvements because the improved genotype would be resilient to drought without the farmer providing extra inputs (White *et al.*, 1994; Singh, 2001). The availability of crops with tolerance to water deficits is thus critical in areas where intermittent or terminal drought are common.(Lizana *et al.*, 2003)

Increasingly, studies point to the fact that climate change associated with increased emissions of greenhouse gases will probably increase the frequency and intensity of droughts (Oldfield, 2005; Rosenzweig and Colls, 2005). Furthermore, there is widespread agreement that increasing anthropogenic related climate changes will exacerbate the present shortages of water, and is likely to increase drought (IPCC *et al.*, 2007). In Sahelian countries, increasing temperature in combination with rainfall reduction has led to a reduced length of vegetative period, no longer allowing present varieties to complete their cycle (Mohamed *et al.*, 2002).

A better understanding of the effects of both intermittent and terminal types of drought on plants is vital for breeding efforts in agriculture under this unpredicted climate change (Khan *et al.*, 2010). Developing water- stress tolerant bean genotypes appears to be a more practical and economical approach than use of improved management practices to lessen the negative effects of drought on the productivity of the crops (Larcher, 2001).

Drought tolerance is defined as relative yield of a genotype compared to other genotypes subjected to the same drought stress (Larcher, 2001). Improving the drought tolerance in cultivated species has been, for long time, a major objective for most of the plant breeding programs (Acevedo and Ceccarelli, 1989; Sánchez *et al.*, 1998). Intensive studies have been carried out in order to identify factors involved in drought tolerance, which can be used as criteria for selection (Acevedo and Ceccarelli, 1989; Blum, 1996) for example: the osmotic adjustment (OA) in wheat (Bajji *et al.*, 2001) and sorghum (Girma and Krieg, 1992), the water use efficiency (WUE) in barley (Acevedo and Ceccarelli, 1989) and wheat (Condon *et al.*, 2002) or the cellular wall elasticity (CWE) in soybean (Sinclair and Venables, 1983).

Progress in improving common bean cultivars for dry environments of the tropics has been achieved by yield testing of large collections over several locations and years (Blum, 1988). Such empirical approaches are, however, slow, laborious, and expensive because of the need to assess the yield of large numbers of lines across several locations and years, and the substantial variation from the effects of environment, error, and genotype-environment

interactions (Blum, 1988). Success in developing drought- resilient bean cultivars has further been limited due to the lack of efficient screening techniques and practical selection criteria other than yield (Ramirez-Vallejo and Kelly, 1998; Acosta-Gallegos and Adams, 1991). Singh (2001) reported that tolerance to drought seems to have been inadvertently reduced in modern bean varieties probably because the emphasis of breeding has been focused on introducing better resistance to biotic (insect pests and diseases) rather than to abiotic stresses.

In dryland or rain-fed environments weather fluctuations, primarily the amount, duration, frequency, and timing of rainfall in relation to crop growth stages, are primary determinants of the level of terminal or intermittent drought stress. Significant variation for these seasonal factors, and their interaction with genotypes complicate the selection process in field-grown nurseries (Acosta *et al.*, 1999). Acosta-Gallegos and Adams, (1991) reported that in common bean where seed yield is the important economic trait, the main selection criteria for drought tolerance are parameters of plant growth and grain production. They, however, pointed out that although these parameters may reflect plant performance under stress, most are difficult and time-consuming to measure, therefore, the identification of main physiological processes determining yield by comparing genotypes differing in drought tolerance has been proposed as the most reliable and sound approach to identifying the potential secondary traits (Araus *et al.*, 2002; Jat *et al.*, 1991; Bohnert and Jensen, 1996). Examples of successful use of indirect selection criteria (physiological traits) in breeding for better yields under dry conditions for important crop plants including common beans are rare (Ober *et al.*, 2005; Slafer *et al.*, 1994; White and Singh, 1991). However, few examples such as selection for low carbon-isotope discrimination (^{13}C) (Passioura, 2002), osmotic adjustment (Chimenti *et al.*, 2002; Morgan, 2000), and introgressing QTLs (loci stretches of DNA containing or linked to the genes that underlie a quantitative trait) associated with deeper rooting into high-yielding cultivar (Babu *et al.*, 2003; Shen *et al.*, 2001) have proven the merit of the indirect selection approach. It, therefore, means that by understanding the key adaptive morphophysiological traits/mechanisms linked to growth and yield of common bean under drought stress may contribute to concerted efforts presently under way to develop drought-resistant cultivars. As noted the use of secondary plant characteristics related to enhanced production in water-limited environments has often been suggested to complement phenotypic selection (Lafitte *et al.*, 2003).

Therefore, in developing new bean genotypes with high productivity under water stress conditions, determining the response of key morphophysiological traits to water stress and elucidating the relationship between these traits and yield can be useful in developing quick and cost effective selection systems.

The purpose of this study therefore, was to determine morphophysiological traits associated with water stress and how they relate to grain yield in common beans.

Specifically the study addressed the following:

Determined the effect of water stress on seed yield and morphological traits of common bean.

Determined the relationship between morphophysiological traits and grain yield under water stress conditions in bean genotypes in the field.

Evaluated the performance of the different bean genotypes under varying water stress levels in the field.

Chapter 2

2. LITERATURE REVIEW

2.1. Botanical and taxonomical characteristics of Common beans

Common bean (*Phaseolus vulgaris* L), also referred to as dry bean, is an annual leguminous plant that belongs to the genus, *Phaseolus*, with pinnately compound trifoliolate large leaves (Katungi *et al.*, 2009). It is cultivated on all continents except Antarctica, under very diverse cultivation conditions (Chaco, 2005).

2.1.1. Origin, domestication and distribution

The genus *Phaseolus* L. includes numerous wild and cultivated species that originated in the New World, the exact number is still unknown (Debouck, 1999). Only five species are important as cultivated food crops; *P. vulgaris*, *P. polyanthus* Greenm., *P. coccineus*, *P. lunatus*, and *P. acutifolius* A. Gray (Chaco, 2005). *P. vulgaris* is the most widely cultivated species owing to its high nutritional value. There are four major gene pools; namely Mesoamerican, Andean, Northern Andean and Columbia. Two major gene pools of common bean were first recognized in the wild form, Mesoamerican and Andean (Gepts, 1998). Evidence of this distribution was based on morphological traits (Singh, 1989; Singh *et al.*, 1991a), phaseolin seed protein (Gepts *et al.*, 1986), isozymes (Koenig and Gepts, 1989), and molecular markers (Freyre *et al.*, 1996; Tohme *et al.*, 1996; Velasquez and Gepts, 1994). A third, genetically unique gene pool was later described in the northern Andes (Debouck, 1999; Tohme *et al.*, 1996). The northern Andes gene pool is located in Ecuador and northern Peru and is considered to be the nucleus of diversity, from where wild beans dispersed both northward and southward (Broughton *et al.*, 2003). A fourth gene pool in Colombia might also exist, but it is still poorly understood (Debouck, 1999; Tohme *et al.*, 1996). However, recent work done by Bitocchi *et al.*, (2011) identifies the origin of *P. vulgaris* as Mesoamerican.

The first domestication of *P. vulgaris* by humans is said to have started slightly more than 4,000 years ago in Mesoamerica and South America (Kaplan and Lynch, 1999). The work of Kami *et al.*, (1995) suggested that, starting from the core area of the western slopes of the Andes in northern Peru and Ecuador, the wild bean was dispersed north (Colombia, Central America, and Mexico) and south (southern Peru, Bolivia, and Argentina), which resulted in the Mesoamerican and Andean gene pools, respectively. The common bean then spread to Europe and Africa (Gepts *et al.*, 1986, 1988).

2.1.2. Morphology and Botany

Common bean is a warm-season crop that does not tolerate frost or long periods of exposure to near-freezing temperatures at any stage of its growth (NDSU, 1997). It is largely a self-pollinated plant though cross-pollination does occur if the stigma is exposed to foreign pollen. Seeds are non- endospermic and vary greatly in size and colour from the small black wild type to the large white, brown, red, black or mottled seeds of cultivars, which are 7-16 mm long (Cobley and Steele, 1976). Common bean shows variation in growth habits from determinate bush to indeterminate, extreme climbing types. The bushy type bean is the most predominant type grown in Africa (Buruchara, 2007). The common bean was taken to Africa and other parts of the world by Spaniards and the Portuguese (Rania *et al.*, 2010). Bush varieties form erect bushes 20–60 cm tall, while pole or running varieties form vines 2–3 m long. All varieties bear alternate, green or purple leaves, divided into three oval, smooth-edged leaflets, each 6–15 cm long and 3–11 cm wide. The white, pink, or purple flowers are about 1 cm long, and give way to pods 8–20 cm long, 1–1.5 cm wide, green, yellow, black or purple in colour, each containing 4–6 beans. The beans are smooth, plump, and kidney-shaped, up to 1.5 cm long, range widely in colour, and are often mottled in two or more colours.

2.1.3. Plant Development

The development of bean (determinate and indeterminate plant types) pass through two main stages of vegetative (V), (7 to 40 days) and reproductive (R), (40 to 94 days) as indicated in (Table 1). Vegetative stages are determined by counting the number of fully expanded trifoliolate leaves on the main stem while the reproductive stages are described by pod and seed characters. The first pod developing on the plant is described and followed to full size. At the time of first bloom (R stage), secondary branching begins in the axis of lower nodes which will produce secondary groups of blooms or pods. To determine the growth state, the main stem is followed, which is readily discernible on both determinate and indeterminate plants. A trifoliolate is counted when it is fully unfolded (Kandel, 2010). The different vegetative and reproductive stages of development of bean are outline in Table 1.

During the vegetative stage of the bean growth, it requires adequate water for germination and root development. Inadequate soil water during the early growth stages results in reduced plant populations and biomass yield, which, in turn, reduces final yield (Efetha, 2011). A

study to compare drought-resistant and drought-sensitive cultivars of field bean and field peas to water stress was conducted and significant decrease in the number of the developed laterals, their total length and dry matter was observed. The reduction was reported to be smaller in drought tolerant cultivars than in the drought sensitive cultivars (Grzesiak *et al.*, 1997).

Table1. Stages of vegetative and reproductive development in an indeterminate (Type III) dry beans

Stage	General description/vegetative stages	Days from planting
VE	Hypocotyl emergence.	7
VC	Cotyledonary and unifoliolate leaves visible.	10
V1	First trifoliolate leaf unfolded	14
V2	Second trifoliolate leaf unfolded.	20
V3	Third trifoliolate leaf unfolded Secondary branching begins to show from branch of V1.	26
V(n)	n trifoliolates on the main stem, but with blossom clusters still not visibly opened.	A new node each 3 days
V8	Vine (indeterminate) plants may begin to exhibit blossom and become stage R1.	40
	Reproductive Stages	
R1	One blossom opens at any node. Tendril will begin to show	40
R2	Pods 1/2 inch long at first blossom position (node 2 to 5 most plants).	43
R3	Pods 2.54 cm long at first blossom position.	46
R4	Pods 5.1 cm long at first blossom position.	50
R5	Pods 7.6 cm long, seeds discernible by feel.	56
R6	Pods 11.4 cm long with spurs (maximum length). Seeds at least 0.6 cm long axis.	60
R7	Oldest pods have fully developed green seeds. Other parts of plant will have full length pods with seeds near same size. Pods to the top and blossom on tendril, nodes 10-13.	70
R8	Leaves yellowing over half of plant, very few small new pods/blossom developing, and small pods may be drying. Point of maximum production has been reached.	82
R9	Mature, at least 80% of the pods showing yellow and mostly ripe. Only 30% of leaves are still green.	94

Modified from Kandel, (2010)

The leaf area depends on cell number and cell size. Lecoœur *et al.*, (1995), reported that the changes with time occurred for cell number and leaf area for individual leaves of pea (*Pisum sativum* L.) and that they showed two distinct and successive phases: phase 1, during 2/3 of leaf development, involves cell division, while phase 2, during the last 1/3 of leaf development, and involves cell expansion. The effect of water deficit on leaf area is two folds. Early deficit during phase 1 reduces leaf area through an effect on cell number, while deficit during phase 2 reduces leaf area by reducing cell area (Jeuffroy and Ney, 1997).

The bean is a warm-season crop. However, high temperatures (>30°C) can cause flower blasting (dropping of buds and flowers); Fageria *et al.*, (1997), which reduces yield. Higher temperatures and photoperiod reduces the crop cycle. Confalone *et al.*, (2009) conducted a study on faba bean (*Vicia faba* L.) in which a wide range of environmental conditions were provided in order to understand the growth and yield response of faba bean. The faba bean plants were exposed to large variations in temperature and photoperiod. Both temperature and photoperiod increased as the sowing date was delayed and this resulted in the reduction of the crop cycle from 209 to 87 days from the first to the last sowing date. The bean requires moderate amounts of water (300-600 mm). Adequate amounts early in the season is essential, but particularly so during the pod-filling stage (during and immediately after flowering). Water stress during other stages of growth and development of the bean other than during and immediately after flowering has little effect on grain yield (Science, 2004). Jeuffroy and Ney (1997) reported that the main effect of water stress was an earlier cessation of flowering, and thus a smaller number of flowering nodes, which potentially bear pods and seeds.

The effect of drought on the seed yield components: pod number per plant, seed number per pod, 100-seed weight (g) in the bean was evaluated by Szilagyi (2003) and showed that drought stress reduced seed yield by 80%, and this was associated with reduction in pods number per plant by 60 %, seeds number per pod by 26 %, 100-seed weight by 13 %.

2.2. Importance of Common Beans in Global Agriculture

Common bean is the world's most important grain legume for direct human consumption (Broughton *et al.*, 2003), with 20.3 million tons of dry beans harvested from 27.9 million ha worldwide in 2008 (FAOSTAT, 2010). The annual production value of common bean is estimated to be over U.S \$ 10 billion (Rao, 2001). The leading bean producer and consumer is Latin America, where beans are an important traditional, food, especially in Brazil,

Mexico, the Andean Zone, Central America, and the Caribbean. In Africa, beans are grown mainly for subsistence, where the Great Lakes region has the highest per capita consumption in the world. Beans are a major source of dietary protein in Kenya, Tanzania, Malawi, Uganda, and Zambia (Centro *et al.*, 1999). It is also an excellent low-fat source of complex carbohydrates, fibre, folate, potassium and B vitamins. (Canada, 2003). It also serves as a break crop in Maize-based and rice-based farming systems to reduce decline in soil fertility.

2.2.1. Importance of Common Bean in Eastern and Southern Africa

It has been reported by Lunze (2001) that common bean is an important grain legume grown on over 3.7 million of hectares every year in Eastern, Central and Southern Africa (ECSA) where bean consumption per capita exceeds 50 kg a year and is perhaps the highest in the world, reaching over 66 kg in densely populated western Kenya (Wortmann *et al.*, 1998). Common bean is mainly produced by the small scale farmers that are resource poor.

Apart from the high protein content, common bean is also a good source of energy and provides folic acid, dietary fibre and complex carbohydrates (Platt, 1962, Cited in Edje *et al.*, 1980). Common bean protein is high in lysine, which is relatively deficient in maize, cassava and rice, making it a good complement to these staples in the diet. It is grown for its green leaves, green pods, and immature and/or dry seeds. Beans are appreciated throughout the Eastern and Southern Africa because they have a long storage life, good nutritional properties and can be easily stored and prepared for eating (Centro, Agricultura, and Ciat, 1999). The cost of common bean is low as compared to meat products thus its high consumption in Africa. Beyond promoting food, health and nutritional security, beans provide a steady and lucrative source of income for many rural households, with the value of bean sales exceeding US \$ 500 million annually (FAO, 2011).

The cost of inorganic fertilizer keeps on increasing and particular in the Eastern and Southern Africa making the poor farmers unable to access these inputs which they need to raise their productivity. Common bean is usually grown either in a pure stand, in rotation with cereals or in crop mixtures usually with maize and the bean fixes nitrogen which benefits the next crop. In this way, the poor resource farmer is able to increase their productivity. The yield of common bean in Eastern and Southern African ranges from 0.60 to 0.80 ton ha⁻¹ though the potential yield of some improved varieties can go up to 2.00 ton ha⁻¹

2.3. Production Constraints

Bean production is constrained by both abiotic and biotic factors that adversely affect the grain yield. Almost all the bean production occurs under low input agriculture on small-scale farms in developing countries (Miklas *et al.*, 2006). Beans produced by these resource-poor farmers are more vulnerable to attack by disease and insect pests and to abiotic stresses including water stress and low soil fertility. Commercial farmers can minimize these stresses through the use of external inputs such as fertilizers and pesticides. Utilization of such inputs, however, can seriously reduce profitability and threaten the environment, and many pests are not effectively controlled with chemicals. Thus, biotic and abiotic stresses continue to be the major constraints on subsistence production and economic yield of common bean.

2.3.1. Biotic Stress

Common bean production is affected by a number of biotic factors notably field and post-harvest pests and diseases.

Insects

The most common insects that affect bean plants are aphids (*Aphis fabae*), bean stem maggot (*Ophiomyia Spp*), flower thrips (*Frankliniella occidentalis*), *Oothea bennigseni* (a beetle which eats the leaves of bean plants) and different types of insects which eat pods and leaves. Insects also cause significant losses in yield and an estimate in yield loss of 297,100 ton yr⁻¹ is attributed to bean stem maggot alone (Wortmann *et al.*, 1998). However, not all insects, cause damage to the bean plant, as some, such as spiders, lady birds and ants, are natural enemies of harmful insects (Buruchara *et al.*, 2011).

Diseases

Common bean is susceptible to a number of diseases. The common diseases in the tropics and sub-tropics that affect bean production are: Common blight (*Xanthomonas axonopodis pv. phaseoli*), Fusarium root rot (*Fusarium solani f. sp. phaseoli*), Rust (*Uromyces appendiculatus var. appendiculatus*), Anthracnose (*Colletotrichum lindomuthianum*), The bean common mosaic virus (BCMV), Angular leaf spot (*Phasoisariopsis griseola*), Halo blight (*Pseudomonas syringae pv. phaseolicola*), Powdery mildew (*Erysiphe polygoni*) and Root knot nematodes (*Meloidogyne spp.*) Wortmann *et al.*, (1988) reported yield losses due to angular leaf spot and anthracnose of 384,200 and 328,000 ton yr⁻¹ respectively.

2.3.2. Abiotic stress

Common bean is often grown on marginal lands under unfavourable environmental conditions (Rao, 2001; Broughton *et al.*, 2003) with minimal soil and crop management inputs (Rao, 2001; Beebe *et al.*, 2008, 2009). Low soil fertility due to phosphorus (P) deficiency or aluminium (Al) toxicity (Beebe *et al.*, 2009) and high risk of intermittent or terminal drought (White and Izquierdo, 1991; Munoz Perea *et al.*, 2006) are among the most common abiotic stresses. These production constraints explain why average yields in many countries of Africa and Latin America did not exceed 0.70 ton ha⁻¹ in 2008 (FAOSTAT, 2010), although experimental dry bean yield potential exceeds 3.00 ton ha⁻¹ (Beebe *et al.*, 2006).

Stresses such as poor soil fertility are long term and predictable (Lunze *et al.*, 2011); while others like drought, some pests and diseases spurred by climate change could be short term, but acute in nature. Soil degradation and drought are serious threats to agriculture and, hence, a frequent cause of crop failure and hunger. These threats are exacerbated by the effects of climate change (Christensen *et al.*, 2007) and crop intensification that leads to soil degradation, fertility decline and surges in pest and disease pressure. According to the Atlas of common bean production in Africa (Wortmann *et al.*, 1998), P is deficient in 65 to 80 % of soils and N in 60 % of soils in bean production areas of Eastern and Southern Africa, while about 45 to 50 % of soils are acidic with a pH less than 5.2, containing high levels of either Al or Mn.

2.4. Characterization of drought prone environments

Wortman *et al.*, (1998) reported that bean growing regions of Africa receive different amount and distribution of rainfall and hence the region experiences different drought scenarios. Four categories of drought scenarios were identified as follows;

Scenario I: represents terminal drought. In this case there could be enough water for early establishment and growth, but later phenological stages are exposed to soil water deficit. This is typically the case in relay cropped beans of the Rift Valley and in rice-based systems of Africa (e.g. Madagascar). In practices of relay planting beans in the Maize field or in systems where beans should follow rice, bean crop is commonly exposed to terminal drought. It is also a common phenomenon, in regions that fully depend on irrigation to produce the major cash and food crops, to grow beans on the residual moisture. Hence beans are exposed to terminal drought starting from early pod filling stages. Sudan and Egypt could be typical examples of this scenario. The short life cycle of common bean makes it an ideal crop to

grow twice, first at the beginning of the first cropping season which starts at the end of November to mid of December and as a second crop planted in January to February in Zambia. The second crop in some years is affected by the diminishing soil moisture if the rains stop towards the end of the rainy season ultimately creating a terminal drought stress. This is typical of the Zambian situation.

Scenario II: represents intermittent drought. This is typical of regions with relatively good rainfall amount but poor distribution during the growing period. There could be enough water for the crop throughout the life cycle except for some short dry spells that may happen at any time of the year. This is also common in Zambia.

Scenario III: represents relatively predictable drought. In this case the total amount of precipitation could be comparable to good years, but most of the rain falls within a short time of the growing season. Bean plants could be exposed to stress at early stage of growth but could receive enough water at later stages if the planting date is adjusted accordingly. This is a common phenomenon in most part of the Great Rift Valley of East Africa.

Scenario IV: represents dry semi -arid climates whereby the amount of rainfall is relatively low to cover the physiological demand of the crop at any stage of growth despite its fair distribution throughout the growing period. Typical of this type of agro ecology could be found in southern and South-western Africa.

2.5. Climatic requirements and Adaptation

Common bean is adapted to deep well drained, sandy loam, sandy clay loam or clay loam soils with clay content of between 15 and 35% with no nutrient deficiencies (Thung and Rao, 1999). The optimum soil pH range is pH 4.6 - 5.0 (CaCl₂). Heavy clay soils with poor oxygenation and capping clay sands are not suitable. Thus, it will not grow well in soils that are compacted, too alkaline or poorly drained (Lunze, 2001). The common bean thrives well in a warm climate. It grows optimally at temperatures of 18 to 24 °C. The maximum temperature during flowering should not exceed 30 °C. High temperatures during the flowering stage lead to abscission of flowers and a low pod set, resulting in yield loss. Day temperatures below 20 °C will delay maturity and cause empty mature pods to develop. Cultivated under rain-fed conditions, the crop requires moderate amounts of rainfall (300 –

600 mm) but adequate amounts are essential during and immediately after the flowering stage (Katungi *et al.*, 2009). Generally, common bean is considered a short-season crop with most varieties maturing in a range of 65 to 110 days from emergence to physiological maturing (Buruchara, 2007). Maturity period can continue up to 200 days after planting amongst climbers that are used in cooler upland elevations (Graham and Ranalli, 1997 in Gomez, 2004).

In Africa, common bean crop cultivation is concentrated at altitude above 1000 metres above sea level, with adequate amounts of precipitation (> 400 mm of rain) during crop growing season and soil pH above 5.5

(Table 2). These are the cooler highlands and the warmer mid-elevation areas of East, Central and Southern Africa.

In Zambia, most of the bean crop is produced in the higher altitudes, cooler and high rainfall zones (Chinsali, Isoka and Mbala areas of Region III) followed by the medium warm zone (Region II), because of reliable distribution of annual rainfall and relatively cool day time temperatures ranging from 17 – 22 °C as shown in (Table 3). (Series *et al.*, 2009 and Muthoni *et al.*, 2011)

Table 2: Important agro-ecological environments of common bean production areas in Africa based on altitude

Altitude (m)	Area share (%)	Percentage of beans produced	Percentage of beans produced
>1500 masl**	51.8	80	64
1000-1500 masl	42.7	79	89
< 1000 masl	5.6	NA*	NA*

*Data not available

** masl: Meters above Sea Level

Modified from Wortmann *et al.*, 1988

Table 3: Bean production in Zambia according to altitudes

Agro ecological zone	Area under Production	
	(HA)	%
High altitude	76,659	90
Medium altitude	7,666	9
Low altitude	852	1
Total	85,177	100

Modified from Muthoni *et al.*, 2011

2.6. Effect of water stress on bean production

Generally, legumes are highly sensitive to water deficit stress (Labidi *et al.*, 2009). Water deficit affects many morphological features and physiological processes associated with plant growth and development (Toker and Cagirgan, 1998). The processes affected include photosynthesis, respiration, translocation, ion uptake, nutrient metabolisms, biosynthesis of proteins, carbohydrates and growth promoters (Sadeghipour and Aghaei, 2012). CIAT Occasional Publication Series, No. 38 reported that crop plants when exposed to drought may alter their cell solute concentration by re-allocating resources so that the osmotic potential of the cell is reduced, and turgor is maintained (CIAT, 2004). Drought stress is characterized by reduction of water content, diminished leaf water potential and turgor loss, closure of stomata, and decrease in cell enlargement and growth (Morgan, 1994 and Jalee *et al.*, 2008).

2.6.1. Effect of water stress on phenotypic traits of Common Bean

Among the abiotic stress, water stress is said to be the second most important constraint after diseases to seed yield (Rao, 2001). Water deficit affects plant growth significantly if the quantity of water supplied is insufficient to meet the basic needs of plants (Seki *et al.*, 2002). Almost the bulk of Common bean in Zambia is grown by small scale farmers as a rain-fed crop, and most of them cannot afford to irrigate the crop. The growth, development and performance of common bean are adversely affected by water stress as the country experiences both intermittent and terminal droughts. There is an urgent need for cultivars that are tolerant to water stress in order to minimize yield losses as well as reduce the amount of water needed for irrigation.

Drought induced changes in plants are mainly related to altered metabolic functions, such as reduced synthesis of photosynthetic pigments, accumulation of osmoprotectants like proline in the cell, reduced growth, loss of membrane stability and integrity and alterations in physiological parameters including plant height and leaf area (Baroowa and Gogoi, 2012).

A number of studies have reported the complex responses of plants at physiological, biochemical and molecular levels when exposed to drought and depending on the different species as well as varieties within the same species, differential adaptation and tolerance mechanisms are shown (Baroowa & Gogoi, 2012; Costa-Franca *et al.*, 2000; Lin & Markhart, 1996; Lizana *et al.*, 2006; Martinez *et al.*, 2007). The responses of plants to stresses depended on many factors, such as the phenological stage and the duration and degree of stress (Torres *et al.*, 2006).

The reduction in bean grain yield as a result of water stress is attributed to adverse effects of water stress on individual yield components (number of pods per plant, number of seeds per pod, seed weight and harvest index), however, their relative importance as determinants of grain yield varies from experiment to experiment (Ramirez-Vallejo & Kelly, 1998; Shenkut & Brick, 2003). Nielsen and Nelson (1998) reported that bean grain yield was reduced due to reductions in pods per plant or seeds per pod, when water stress occurred during the reproductive stage. Singh, (1995) also reported that water stress during flowering and grain filling reduced seed yield and seed weight and accelerated maturity of dry bean. Emam *et al.*, (2010), reported that plant height, number of leaves, leaf area, number of pods, pod dry weight and total dry weight of two common bean cultivars with contrasting growth habits decreased significantly due to water stress. Akıncı and Lösel (2009, 2010) also reported that water stress caused major reductions in height, leaf number, leaf area index, fresh and dry weight of cotton plants and some *Cucurbitaceae* members. Water stress has also been reported to reduce the expression of many characteristics in faba beans (*Vicia faba* L.) except days to flowering and moisture retention in the leaf. In common beans, accelerated maturity of crop along with reducing grain yield and mean weight of hundred seeds following water stress, have been reported (Singh, 1995). Large variations in yield in French bean (*Phaseolus vulgaris* L.) production have been reported due to irregular occurrence of drought periods accompanied by high temperature due to climatic changes (Remenyik and Nemeske, 2010). Ramirez-Vallejo and Kelly (1998) reported that both the quality and the yield of beans are negatively affected by brief periods of water shortage.

2.6.2. Impact of water stress on physiological factors

Water stress reduces photosynthesis by decreasing both leaf area and photosynthetic rate per unit leaf area (McCree, 1986). Photosynthesis by crops is severely inhibited and may cease altogether as water deficits increase. The decrease in leaf growth, or increasing senescence of leaves under drought conditions, may also inhibit photosynthesis in existing leaves (Akıncı and Lösel, 2006). Decreasing water content is accompanied by loss of turgor and wilting, cessation of cell enlargement, closure of stomata, reduction in photosynthesis, and interference with many other basic metabolic processes (Kramer and Boyer, 1995). Photosynthesis by crops is severely inhibited and may cease altogether as water deficits increase. The decrease in leaf growth, or increasing senescence of leaves under drought conditions, may also inhibit photosynthesis in existing leaves (Boyer, 1976).

Ehleringer (1980) pointed out that leaf pubescence, which increases under water stress, can decrease the photosynthesis by reflecting quanta that might have been used in photosynthesis. In the field, plants are normally not deprived of water rapidly. The simplest explanation for the inhibition of photosynthesis during water stress would be that the stomatal closure and internal CO₂ concentration decrease (Farquhar & Sharkey, 1982; Schulze 1986), since stomatal limitation is more severe when a plant is stressed than when it is not (Farquhar & Sharkey, 1982). Therefore, it is rather surprising that photosynthesis often decreases in parallel with, or more than, stomata conductance (Wong 1985; Huber *et al.*, 1984; Raschke & Resemann, 1986; Cornic *et al.*, 1989). The photosynthetic rate in higher plants decreases more rapidly than respiration rate with increased water stress, since an early effect of water reduction in leaves is usually a partial or complete stomata closure, markedly decreasing the movement of carbon dioxide into the assimilating leaves and reducing the photosynthetic rate up to ten times, according to the amount of water removal and the sensitivity of the plant (Levitt, 1972). In terms of the relationship between photosynthesis and leaf water status, Quick *et al.*, (1992) reported that, under field conditions, photosynthesis in ambient CO₂ reached a maximum value in the morning and declined later in the day when water potential decreased and leaf- to-air water vapour pressure deficits increased. In non-watered plants the decline was larger, and occurred earlier. In most cases stomatal conductance followed a diurnal pattern similar to that of photosynthesis.

Water stress inhibits photosynthesis and decreases growth and productivity of plants. At a whole-plant level, soil drought and leaf water deficit lead to a progressive suppression of photosynthesis, and is associated with alterations in carbon and nitrogen assimilation (Chaves, 1991; Mwanamwenge *et al.*, 1999; Yordanov *et al.*, 2000). Decreased photosynthetic rate is a result of stomatal and non-stomatal (biochemical) limitations (Wise *et al.*, 1992; Yordanov *et al.*, 2003). The first reaction of plants to water stress is rapid closure of stomata to avoid further loss of water through transpiration (Cornic, 1994; Lawlor, 1995), which leads to the restriction of diffusion of CO₂ into the leaf (Chaves, 1991, Flexas *et al.*, 2006). The decrease in net photosynthetic rate (A_n) observed in many studies under water stress is usually explained by reduced internal CO₂ concentration (C_i) which limits photosynthesis at the acceptor site of ribulose-1-5-bisphosphate carboxylase/oxygenase (Rubisco) (Cornic *et al.*, 1992) or by the direct inhibition of photosynthetic enzymes like Rubisco (Haupt- Herting and Fock, 2000) or ATP synthase (Tezara *et al.*, 1999; Nogués and Baker, 2000). The non-stomatal limitation of photosynthesis has been attributed to reduced

carboxylation efficiency (Jia and Gray, 2004), reduced ribulose-1,5-bisphosphate (RuBP) regeneration (Tezara and Lawlor, 1995), reduced amount of functional Rubisco (Kanechi *et al.*, 1995), or to inhibited functional activity of PSII. Concomitantly inhibition or damages in the primary photochemical and biochemical processes may occur (Lawlor, 2002).

Assessment of pigment content has also become an effective means of monitoring plant growth and estimating photosynthetic productivity (Chen *et al.*, 2007). Ahbarian *et al.*, (2011) reported that plants also show response to drought stress in the change in photosynthetic pigment content. The photosynthetic pigments play important roles in harvesting light. The content of both chlorophyll a and b changes under drought stress (Farooq *et al.*, 2009). Drought stress inhibits Chlorophyll a, b synthesis and decreases the content of Chlorophyll a/b binding proteins, leading to reduction of the light-harvesting pigment protein associated with photosystem II (Sayed, 2003). The effects of drought stress on chlorophyll content have been investigated in cotton (Mssacci, 2008) and *Catharanthus roseus* (Jaleel *et al.*, 2008a).

2.6.2.1. Cell Membrane Thermo-stability

Cell Membrane Thermo-stability (CMT) is the ability of a plant to resist cellular membrane modification as a result of environmental stress such as drought. Drought stress damages the cell membrane which leads to increased electrolyte leakage. The relative rate of this electrolyte leakage is used as a measure of the cell membrane stability. The electrolyte leakage is estimated by measuring the electrical conductivity of the medium in which the leaf sample is equilibrated. Cell membrane modification, which results in total dysfunction, is a major factor in plant environmental stress. The exact structural and functional modification caused by stress is not fully understood. However, the cellular membrane dysfunction due to stress is well expressed in its increased permeability for ions and electrolytes (Ruter, 1993). Chu-Yung *et al.*, (1985) and Espevig, *et al.*, (2012) suggested that increased solute leakage is attributed to the loss of membrane integrity through lipid phase transitions (principally, altered phospholipid and fatty acid composition) and to the effect on membrane bound transport proteins. These proteins play a role in preventing leakage.

The estimation of membrane dysfunction under stress by measuring cellular leakage from affected leaf tissue into an aqueous medium is finding a growing use as a measure of CMT and as a screen for stress tolerance and has been demonstrated in several studies involving

agronomic, fruit, and vegetable crops (Chen *et al.*, 1982; Ingram and Buchanan, 1984; Lester, 1985; Martineau *et al.*, 1979; Saadalla *et al.*, 1990; Sullivan and Ross, 1979). CMT was found to be a sensitive and rapid method to evaluate heat and drought tolerance in plants (Sullivan, 1972; Wu and Wallner, 1993). Tripathy *et al.*, (2000) used cell membrane stability to determine drought tolerance of 104 rice genotypes and found the method to be very effective. Ruter (1993) reviewed electrolyte leakage as an effective means of measuring membrane thermostability in leaves and followed sigmoidal response curves. Blum and Ebercon (1981) reported that wheat genotypes grown under conditions of moisture stress significantly vary in their membrane injury levels. They also noted that injury level ranged from 16.8 % to 70 % when the genotypes were screened in the laboratory using a 40 % PEG solution as a dehydration medium. Mark *et al.*, (1991) recommended that cellular rupture due to leaked substances is important for assessing freezing injury in alfalfa.

Using the cell membrane stability test, Blum and Ebercon (1981) found that younger wheat leaf tissues are more tolerant to drought than the older leaf tissues. They also found a variation between bread wheat and durum wheat cultivars on the level of their cell injury percentage under drought stress and concluded that bread wheat cultivars consistently suffered greater injury than durum wheat cultivars. Sullivan *et al.*, (1979) used the cell membrane stability test as a selection method for drought and heat tolerance in grain sorghum. However, there is little information in literature on use of CMT testing method to evaluate drought tolerance in common bean. A notable example is the study conducted to determine the effect of induced drought on different growth and biochemical attributes of black gram (*vigna mungo*) and green gram (*vigna radiata*) and showed a considerable decrease in the membrane stability in the plants grown under drought stress condition as compared to the control plants for both the cultivars. (Baroowa and Gogoi, 2012).

2.6.2.2. Relative Water Content, Transpiration and Water Use Efficiency

A satisfactory basis for relating cellular water status to metabolism is relative water content (RWC), an easily measured, robust indicator of water status for comparison of tissues and species, which ‘normalizes’ water content by expressing it relative to the fully turgid (hydrated) state (Lawlor and Cornic, 2002). Sinclair and Ludlow (1985) proposed that leaf relative water content (RWC) is a better indicator of water status than water potential. RWC is a measure of relative change in cell volume; is the resultant of cell turgor and osmotic potential, and thus depends both on solute concentration and cell wall rigidity and does not

relate directly to cell volume (Kramer and Boyer, 1995; Lawlor, 1995; Kaiser, 1987). RWC as an integrative indicator of internal plant water status under drought conditions and has successfully been used to identify drought-resistant. The measurement of RWC under low soil moisture is of importance since high RWC appears to be a common trait in drought resistant species as species which exhibit restricted changes in RWC per unit reduction in water potential are often considered to be relatively drought resistant (Rahaman *et al.*, 2000). Schonfeld *et al.*, (1988) showed that wheat cultivars having high RWC were more resistant against drought stress. Ramos *et al.*, (2003) stated that RWC of bean leaves under drought stress significantly was lesser than control. Lazacano-Ferrat and Lovat (1999) found that RWC of the stem of bean plant was significantly lower comparing with control when the bean plants were subjected water stress. Blackman *et al.*, (1995) attributed the reduction of RWC of plants subjected to water stress to damages to the cell including cleavage in the membrane and sedimentation of cytoplasm content.

Transpiration is the process by which moisture is carried through plants from roots to small pores on the underside of leaves, where it changes to vapour and is released to the atmosphere. Transpiration is essentially evaporation of water from plant leaves. Under moisture-limiting environments, productivity in crop plants may be increased by improving water-use efficiency (WUE) (Ehleringer *et al.*, 1993). WUE is defined as the ratio of shoot biomass production to the total amount of transpired water.

2.6.2.3. Harvest Index, Respiration and Assimilate partitioning

Harvest index is defined as the ratio of economic (grain) yield to shoot biomass at maturity. Harvest index depends inter alia on the relative proportion of pre and post anthesis biomass and on the re-mobilization of the pre-anthesis assimilates to the grain. Ghafoor *et al.*, (2000) reported that high harvest index trait is very important for increasing yield potential in crops; however, they stated that it was a complex parameter in legumes, largely due to high sensitivity to environmental variations. A severe water deficit at a crucial stage, e.g. flowering greatly decreases seed numbers and harvest index. This was also reported by De Costa *et al.*, (1999) who observed that water stress during the reproductive stage reduced harvest index.

It was reported by (Lawler and Cornic, 2002; Flexas *et al.*, 2005) that respiration is an equally important factor controlling productivity, unlike photosynthesis which is limited temporally (i.e., daytime hours) and spatially (i.e., to green biomass), respiration occurs

continuously in every plant organ, particularly when photosynthesis is largely depressed due to water stress.

Respiration is an essential metabolic process that generates Adenosine Tri- Phosphate (ATP) and several carbon skeletons - metabolites that are used in many synthetic processes essential for growth and maintenance of the cell homeostasis, including under water stress conditions (MacCabe *et al.*, 2000; Bartoli *et al.*, 2000). Several authors have found different results on the effect of water stress on respiration, ranging from decrease (Brix, 1962; Brown and Thomas, 1980; Palta and Nobel, 1989; Escalona *et al.*, 1999; Ghashghaie *et al.*, 2001; Haupt-Herting *et al.*, 2001) to stimulation (Upchurch *et al.*, 1955; Shearmann *et al.*, 1972; Zagdanska, 1995). Ghashghaie *et al.*, (2001), showed in sunflower (*Helianthus annuus*) that leaf respiration decreased at early stages of water stress and then increased even above control values at later stages.

Water stress has been shown to affect the relationship between the carbon content in photosynthetic organs, such as leaves (source), and the carbon content in heterotrophic organs, such as seeds and roots (sink), indicating that the processes related to carbon partitioning are sensitive targets of this adverse environment (Cuellar-Ortiz *et al.*, 2008). Chaves *et al.*, (2002), stated that the ability of genotypes to partition stored vegetative biomass to reproductive organs to a larger extent determines sink establishment and economic yield under drought stress. Cuellar-Ortiz *et al.*, (2008), in their study showed that carbohydrate partitioning is affected by drought in common bean, and that the modulation of the partitioning towards seed filling has been a successful strategy in the development of drought- resistant cultivars. Setter, (1990), reported that during water stress photosynthate partitioning is altered to increase root/shoot ratio. Several authors (Pelleschi *et al.*, 1997; Pinheiro *et al.*, 2001; Yang *et al.*, 2001), have reported that under water deficit, there is a strong reduction in levels of inactive osmotically solutes (starch) and increase in active osmotically solutes (soluble sugars) and as a consequence the osmotic potential decreases, contributing to the maintenance of leaf water status. Rosales-Serna *et al.*, (2000, 2003), showed that drought resistant bean cultivar displayed a small reduction in harvest index under water stress and it was hypothesized that the cultivar was able to improve carbon partitioning into the pods as part of its drought adaptation mechanism.

2.7. Adaptation to drought

Hall *et al.*, (1979) reported that crop plants use various adaptive mechanisms to cope with drought stress, but the responses are complex, and adaptation is attributable to a plant's

ability to exercise one of the mechanisms. Levitt (1980); Ludlow and Muchow (1990) reported the classification of the components of response to water deficit as escape, avoidance or postponement, and tolerance.

2.7.1. Drought escape

Turner *et al.*, (2001) reported that in regions where the growing season is short and terminal drought stress predominates, drought can be escaped by earliness, matching phenological development with periods of soil moisture availability. These strategies of drought escape are not as successful when transient drought occurs, with unpredictable timing, earlier in the growing season. Generally, drought affects seed yield by reducing total biomass production and therefore the plant's capacity to sustain a high yield depends on its stage of growth.

2.7.2. Dehydration avoidance

Dehydration avoidance relates to the maintenance of high tissue water potential under varying soil water tension and consists of mechanisms that reduce water loss while maintaining water uptake. Reduction of water loss generally depends on stomatal control of transpiration while increased water uptake depends on a deep and prolific root system. The general risk thus under declining water status is increased root: shoot ratio as a result of altered assimilate partitioning with the result of reduced grain yield. Stomatal closure as a drought avoidance mechanism and is one of the first steps in a plant's adaptation to water deficit, allowing the water status to be maintained. Stomatal closure may reduce transpiration losses but reduces CO₂ uptake (C_i) negatively affecting photosynthesis (Chaves, 1991, Flexas *et al.*, 2006).

The plant escapes periods of drought, particularly during the most sensitive periods of its development.

2.7.3. Dehydration tolerance

Dehydration tolerance is the ability of the plant to endure or withstand a dry period by maintaining a favourable internal water balance under drought conditions. Khanzada *et al.*, (2001) showed that yield and relative water content in different guar (*Cyamopsis tetragonoloba*) genotypes were reduced under different water stressed conditions.

In summary, studies have shown variable plant responses to water deficits depending on the development stages and degree of stress applied. Additionally, genotypic differences

significantly affected yield and morphophysiological responses to water stress- Osmotic potential, water potential and turgor potential were differentially affected by water stress.

Chapter 3

3. MATERIALS AND METHODS

3.1. Study location

The study was conducted at the National Irrigation Research Station (15° 45' S and 27° 56' E) in the Southern Province of Zambia from September to December 2012. The Research Station falls in Agro-ecological region IIa and the ecological sub zone 9 (Veldkamp, 1987). The length of the growing period for the plants is about 120 days with the early planting dates occurring between 20th to 30th November, a normal start for planting occurring between 1st to 10th December and ending the plant growing season about 10th to 20th March in the year. During the cold season (June/July) slight ground frost may occur. The occurrence of drought condition is also considered frequent in the area taking between 20 to 30 days.

The soils were generally classified as Alfisols (Sokotela *et al.*, 2009). They were well drained, deep, yellowish red to strong (5-7.5 YR), slightly hard to hard and friable to slightly firm sandy clayey soil with medium activity clay, medium base saturation, with a loamy top soil and a silty/clay ratio of less than 9.0. In the FAO/UNESCO world Soil Map legend classification, these soils are equivalently known as ferric luvisols or chronic luvisols. According to USDA soil taxonomy; this series is typic kanhaplustalf, clayey, kaolinitic, isohyperthermic. This is the Nakambala series that can develop from limestone dolomite, gneiss and granulite parent material. The laboratory soil analysis results of the National Irrigation Research Station are indicated in Table 6.

3.2. Plant materials

Eight bean genotypes were planted in the field. The genotypes used in the study came from the Bean Improvement Programme (BIP) of the University of Zambia and some from International Centre for Research in Semi-Arid Tropics (ICRISAT). Selection of the genotypes were based on the drought susceptibility index (DSI) values for the bean genotypes from BIP, the genotypes that had DSI value lower than unit were selected as drought tolerant genotypes and those whose DSI values higher than a unit, were selected as drought sensitive genotypes, Nathan (2012). The genotypes from ICRISAT were specifically selected for being drought tolerant. Details of genotypes used are presented in (Table 4).

Table 4: Common bean (*Phaseolus vulgaris* L) materials used in the experimental trial

Line	Status	Origin
SER 89	Drought tolerant	ICRISAT
SER 76	Drought tolerant	ICRISAT
SER 180	Drought tolerant	ICRISAT
GADRA	Drought tolerant	ICRISAT
ZM 4488	Drought tolerant	LOCAL
CAR-ZAR	Sensitive	BIP-UNZA
KE 3	Drought tolerant	ICRISAT
KE 4	Drought tolerant	ICRISAT

Table 5: Water regimes and schedule applied at 7 day interval (mm)

Irrigation	50 %	75 %	100 %
Date	Etc (mm)	Etc (mm)	Etc (mm)
12/10/12	23.4	35.0	46.7
19/10/12	32.4	48.5	64.6
26/10/12	32.6	49.0	65.2
2/11/12	31	46.7	62.2
9/11/12	29.3	43.8	58.4
16/11/12	27.2	40.9	54.4
23/11/12	25.3	38	50.7
30/11/12	23.6	35.5	47.1
Total	224.8	337.4	449.3

Table 6: Laboratory Soil Chemical Analytical data for National Irrigation Research Station

Horizon (layer)	Depth (cm)	pH	Exchangeable bases (me/100g)					N	Org C	Av. P-1 CEC	
			Ca	Mg	Na	K	mg Kg			Me/100g	
Ap	0-10	6.37	5.68	7.20	0.01	1.33	0.16	1.12	7.21	9.20	
BA	10-27	6.49	5.91	7.28	0.02	1.21	0.15	0.67	2.45	10.53	
Bt	27-60	7.00	5.37	7.94	0.02	0.61				11.73	
Btz ₁	60-83	6.71	5.22	8.07	0.03	0.46				12.27	
Btz ₂	83-120	6.78	5.19	8.02	0.02	0.38				12.40	
BC	120-190	6.69	5.91	8.39	0.03	0.44				11.47	
Critical Value		4.5	0.1	0.1		0.1	0.1	2.0		14 %	

Modified from Sokotela *et al.*, 2009.

Table 7: Laboratory Soil Chemical Analytical data for the experimental site

pH	C	NH ₄ ⁺	NO ₃ ⁻	P	K ⁺	Ca ²⁺	Mg ²⁺	Fe	Mn
CaCl ₂	%	mg/kg	mg/kg	mg/kg	cmol/kg	cmol/kg	cmol/kg	mg/kg	mg/kg
6.3	0.98	1	2.94	26	0.03	61.04	0.01	32.77	23.04

Table 8: Parameters measured on common bean (*Phaseolus vulgaris*) genotypes.

Characters	Abbreviation
Grain yield	GYLD
Days to 50% flowering	50% DF
Number of pods per plant	NP/P
Number of seeds per plant	NS/P
Number of nodes per plant	NN/P
Chlorophyll a, b and total	Chla, Chlb and Total Chl
Drought Susceptibility Index	DSI
Hundred Seed Weight	100 SW
Relative Injury %	RI %

3.3. Experimental Design and treatments

Three water regimes namely, 50 % ETc, 75 % ETc, and 100 % ETc (ETc - the maximum water requirements for dry bean production) were used. The water regimes were determined basing on specific weather parameters for Nanga and common bean crop coefficient value (kc) (Appendix II). Water stress was imposed at pre-flowering stage (V 8), on the 12th October 2012 and discontinued on the 30th November 2012 when the plants were in their late reproductive stage (R 8), (Table 6). Stressing was done for 43 days. Normal irrigation and rains resumed after pod filling stage.

3.3.1. Experimental design

The trial was laid out in a split plot design replicated four times. Each replication had three blocks. The main plot measured 2 x 1.6 m long. The Subplots measured 2 x 0.2 m long and had 3 rows spaced at 0.5 x 0.15 m. Water regime treatments were assigned to main plots, and common bean genotypes were assigned to the subplots. Sowing was done on the 6th September 2012 with a basal dressing fertilizer applied at the time of planting at the rate of 20 kg N, 40 kg P₂O₅ and 20 kg K₂O (D Compound) ha⁻¹. Thirteen seeds were planted per 2 m row giving a total of 39 plants per plot. Normal agronomical practices for growing common bean were performed. An irrigation interval of seven days was used. Ordinary water meter was connected to the main water line and water was applied using a ¾” hosepipe using flooding method.

3.3.2. Data Analysis

Data were analyzed using the statistical package GenStat Discovery Version edition 14. Data were subjected to analysis of variance (ANOVA) to determine significant differences among treatments for various parameters. Means separation was done using the least significant difference (LSD) test. Relationships between selected parameters were determined using the Pearson’s simple correlation test. In order to determine the relationship between morphophysiological traits and yield components, stepwise multiple regression analysis was carried out using SPSS (SAS Institute, 1988).

3.4. Analytical procedures

Data was collected on morphological and physiological traits as well as on yield and yield components (Table 9).

3.4.1. Cell Membrane thermo-stability

Leaf discs each measuring about 1.3 cm² were cut using a cork borer from fully expanded photosynthesing leaves obtained from randomly selected plants in the middle row of each plot and evaluated for CMT following procedures described by Martineau *et al.*, (1979) and Karlidag *et al.*, (2013).

Two sets of 10 leaf discs each were placed in test tubes and thoroughly rinsed 3 times with deionised water to remove electrolytes adhering to the surface. After rinsing, all the test tubes were filled by 10 ml of deionised water and capped with aluminium foil to prevent water evaporation. One set of the test tubes was incubated at 55°C for 20 minutes in a temperature controlled water bath. After incubation, the set of test tubes were brought to 25°C. The other set of test tubes was left at room temperature of about 25°C and initial measurement of conductance of the control (CEC1) and treatment (TEC1) was measured using a conductivity meter (model pH/EC/TDS, Hanna Instruments, Mauritius). All the test tubes were then autoclaved at 0.1 Mpa for 12 minutes to kill tissues, cooled to 25 °C and final conductance was measured (CEC2 and TEC2). Cell membrane stability was expressed as relative injury percentage (RI %) and was calculated using the equation;

$$RI (\%) = (1-(TEC1/TEC2))/(1-CEC1/CEC2) \times 100 \quad [1]$$

3.4.2. Chlorophyll content

Chlorophyll was extracted by grinding the small pieces of leaves from fully expanded photosynthesing leaves at mid flowering period obtained from randomly selected plants in the middle row of each plot. The pieces were weighed and about 0.1 g of material was left for grinding. The total fresh weight of each sample was recorded. The pieces were ground in 10 ml of 80% acetone (acetone:water, 80:20 v:v). The leaf homogenate was then filtered through a filter paper. The retentate was removed by the filter paper and discarded while the extract was collected in a test tube. The absorbance of the extract was determined at 663 and 645 nm wavelength. Total chlorophyll contents, chlorophyll a and chlorophyll b were calculated by use of the equations of Arnon (1949).

$$(i) \text{ Chl a (mg g}^{-1}\text{)} = [(12.7 \times A_{663}) - (2.6 \times A_{645})] \times \text{ml acetone} / \text{mg leaf tissue} \quad [2]$$

$$(ii) \text{ Chl b (mg g}^{-1}\text{)} = [(22.9 \times A645) - (4.68 \times A663)] \times \text{ml acetone} / \text{mg leaf tissue} \quad [3]$$

$$(iii) \text{ Total Chl} = \text{Chl a} + \text{Chl b.} \quad [4]$$

3.4.3. Relative Water Content

Relative water content was determined by collecting two fully expanded leaves of randomly selected plants from the middle row of the plot (Karlidag *et al.*, 2013). The leaves were weighed to get the fresh weight (FW). Then, the leaves were soaked in distilled water in a petri dish and kept in the dark at 10 °C for 24 hrs. They were then weighed to get the turgid weight (TW). The leaves were then dried in an oven at 70 °C for 48 hrs to get the dry weight (DW). RWC was calculated as;

$$\text{RWC} = (\text{FW}-\text{DW})/(\text{TW}-\text{DW}) \times 100 \quad [5]$$

3.4.4. Drought Susceptibility Index

The drought susceptibility index (DSI) predicts the performance of a line grown under stressed and non-stressed conditions. DSI for each genotypes used in the study was calculated according to Fischer and Maurer (1978).

$$\text{DSI} = (1-\bar{Y}_s/\bar{Y}_p)/\text{DII}, \quad [6]$$

where $(1-\bar{Y}_s/\bar{Y}_p)$ is the stress index and \bar{Y}_s and \bar{Y}_p are mean of all genotype under stress and non-stress conditions, respectively.

Geometric mean was calculated according to Kristin *et al.*, (1997); Fernadez (1992).

$$\text{GMP} = \sqrt{(Y_p/Y_s)} \quad [7]$$

Chapter 4

4. RESULTS

The results of the analysis of variance presented in Table 9 on the physiological responses of common bean genotypes to water stress show that there were significant differences in treatment responses among the eight genotypes used in this study. Water regimes were significant different for most parameters measured except for plant height (PHT), number of pods per plant (NPP) and number of seeds per pod (NSP). Significant differences among genotypes were observed for all traits measured. Highly significant interaction between genotypes and water regimes for all traits measured were also observed.

4.1.1. Effect of water stress on morphophysiological traits of 8 bean genotypes

Highly significant differences among genotypes for water regime ($p \leq 0.001$) were observed for relative injury percentage, chlorophyll a, chlorophyll b, total chlorophyll contents and relative water content, however, plant height was non-significant (Table 9).

4.1.2. Effect of water stress on grain yield, yield components of 8 bean genotypes

There were highly significant differences among genotypes for grain yield (Table 9). The mean grain yield across all genotypes was 2.19 ton ha^{-1} (Table 13). The yield varied from 1.74 ton ha^{-1} to 4.36 ton ha^{-1} . The highest grain yield was obtained from SER 76 (4.36 ton ha^{-1}) followed by KE 3 (2.49 ton ha^{-1}), then KE 4 (2.24 ton ha^{-1}) and the lowest grain yield was obtained from CAR-ZAR (1.74 ton ha^{-1}), followed by SER 180 (1.43 ton ha^{-1}).

The differences among genotypes for yield components were highly significant for hundred seed weight, days to fifty percent flowering and number of pods per plant and they were significant differences for number of seeds per plant (Table 9). KE 4 recorded the highest hundred seed weight of 43 g while SER 76 recorded the lowest hundred seed weight of 24 g in 50% water regime. SER 180 recorded the highest number of pods (23), followed by SER 76 (22) and GADRA (22) and the lowest number of pods was obtained from CAR-ZAR (12) in 50 % water regime. The highest number of seeds per pod was 5, and the lowest number of seeds per pod was 4 and was obtained from seven genotypes in 50% water regime (Table 12).

There were highly significant differences among the genotypes for days to 50% flowering. KE 4, CAR-ZAR, GADRA, KE 3 and SER 76 took the longest period (50days) to reach 50% flowering. SER 180 took the shortest period (35 days) to reach 50% flowering, followed by SER 89 (36 days), ZM 4488 (36 days) in 50% water regime (Table 12).

Table 9: Summary of ANOVA of treatments effects on 8 genotypes of common bea subjected to various levels of water stress

Source of variation	DF	Relative injury (%)	Chlorophyll a	Chlorophyll b	Total Chlorophyll	Relative water content	Grain yield (t/ha)	Plant height	NPP ^z	NSP ^y	100 seed weight	DTF ^x 50%	NN ^s
Replication	3	ns	Ns	ns	ns	ns	Ns	ns	ns	ns	ns	ns	ns
Water regime (A)	2	***	***	***	***	***	***	ns	ns	ns	***	***	***
Error (a)	5												
Genotype (B)	7	***	***	***	***	***	***	***	***	**	***	***	***
Water x genotype	14	*** ^w	***	***	***	***	***	***	***	***	***	***	***
Error (b)	62												
CV%		2.8	5.7	5.3	3.9	1.9	2.7	4.5	4.2	10.3	2.2	2.3	7.0

^zNPP: Number of pods per Plant;

^yNSP: Number of seeds per Pod;

^xDTF 50%: days to 50% Flowering;

^sNN: Number of nodes per plant;

^wLevel of significance ns, **, *** denoting non- significant, significant at $p \leq 0.05$ and at $p \leq 0.001$.

Table 10: Means of Water stress Regimes and Genotypes for morphophysiological traits of common bean grown at Nanga, Zambia during the 2012 season

Genotype	Stress level ^z	Relative Injury (%)	Cell membrane stability (%)	Chl a (mg g ⁻¹)	Chl b (mg g ⁻¹)	Total Chl (mg g ⁻¹)	Relative water content (%)	Plant height (cm)
CAR-ZAR	WR 1	55.8	44.2	8.7	7.1	15.8	77	12
	WR 2	41.5	58.5	8.6	17.2	25.8	84	20
	WR 3	22.9	77.1	9.8	21.9	31.7	83	20
GADRA	WR 1	68.3	31.7	4.9	5.4	10.3	77	14
	WR 2	52.1	47.9	9.0	13.9	22.9	83	19
	WR 3	43.2	56.8	11.9	12.4	24.3	89	19
KE 3	WR 1	42.5	57.5	9.3	20.0	29.3	76	24
	WR 2	38.2	61.8	10.4	23.7	34.1	83	26
	WR 3	37.9	62.1	17.2	22.2	39.4	88	33
KE 4	WR 1	40.3	59.7	5.0	6.4	11.4	72	22
	WR 2	38.6	61.4	9.1	9.4	18.5	80	30
	WR 3	28.7	71.3	17.8	16.7	34.5	85	40
SER 180	WR 1	62.5	37.5	8.4	16.5	24.9	80	22
	WR 2	46.5	53.5	9.6	22.2	31.8	90	25
	WR 3	42.2	57.8	16.9	25.0	41.9	93	40
SER 76	WR 1	47.5	52.5	9.4	8.8	18.2	78	23
	WR 2	42.3	57.7	11.1	18.6	29.7	89	25
	WR 3	37.3	62.7	15.9	19.0	34.9	85	25
SER 89	WR 1	48.3	51.7	5.6	5.8	11.4	82	20
	WR 2	45.4	54.6	10.9	20.8	31.7	90	33
	WR 3	38.0	62.0	14.4	26.2	40.6	91	38
ZM 4488	WR 1	42.7	57.3	9.5	13.3	22.8	59	16
	WR 2	34.7	65.3	10.6	16.9	27.5	87	19
	WR 3	35.3	64.7	14.8	37.9	52.7	85	32
LSD@ 5%		1.13	1.13	0.56	0.76	1.66	1.4	0.5
CV(%)		3.0	3.0	5.8	5.1	3.9	7.1	2.0

^zWater stress level indicated as evapo transpiration .^yRelative Injury Percentage, determined from membrane stability index;

^xChl a: Chlorophyll content a;

^wChl b: Chlorophyll content

Table 11: Means of water stress regimes for morphophysiological traits of common bean genotypes, grown at Nanga.

^z Water Regime	RI ^y (%)	Cell membrane stability (%)	Chl a ^x (mg g ⁻¹)	Ch lb ^w (mg g ⁻¹)	Total Chl (mg g ⁻¹)	Relative water content (%)	Plant height (cm)
^p WR 1(50 %)	51.0	49.0	7.6	10.4	18.0	75.0	19.0
^r WR 2 (75 %)	42.4	57.6	9.9	17.8	27.7	86.0	24.0
^q WR 3(100 %)	35.7	64.3	14.8	22.7	37.5	87.0	31.0
LSD (5 %)	1.1	1.1	0.6	0.8	3.9	1.4	0.5
CV (%)	3.0	3.0	5.8	5.1	3.9	2.0	2.0

^zWater stress level indicated as evapo transpiration.

^yRelative Injury Percentage, determined from membrane stability index;

^xChl a: Chlorophyll content a;

^wChl b: Chlorophyll content b;

^pHigh water stress level;

^rLow water stress level;

^qNormally irrigated.

Table 12: Effect of Water Regime and Genotype on grain yield, yield components of common bean

Genotype	Stress level^z	Yield (kg ha⁻¹)	NN^y	Number of pods per plant	Number of seeds per plant	100 seed weight (g)	DTF^x
CAR-ZAR	WR 1	559.8	6	12	4	27	50
	WR 2	493.2	8	25	4	45	48
	WR 3	1741	8	37	7	51	61
GADRA	WR 1	1320	5	22	4	36	50
	WR 2	1610.6	6	20	4	38	49
	WR 3	1631.9	8	35	6	46	59
KE 3	WR 1	616.2	7	15	4	38	50
	WR 2	860.7	8	16	5	41	48
	WR 3	2489.6	9	22	5	42	53
KE 4	WR 1	1499.3	6	18	4	43	50
	WR 2	1506	7	21	5	47	55
	WR 3	2236.3	10	23	6	47	62
SER 180	WR 1	645.8	5	23	4	27	35
	WR 2	855.8	6	32	4	29	38
	WR 3	1433.8	9	36	6	41	54
SER 76	WR 1	812.2	6	22	5	24	50
	WR 2	902.9	7	27	5	35	52
	WR 3	4363.7	8	42	5	47	62
SER 89	WR 1	672.1	6	14	4	23	36
	WR 2	1137.3	8	23	4	42	47
	WR 3	1904.5	9	31	5	43	61
ZM 4488	WR 1	803.9	7	16	4	32	36
	WR 2	1259.4	8	21	4	39	34
	WR 3	1729.7	9	34	6	47	54
LSD (5%)		32.3	0.5	0.9	0.9	0.8	0.9
CV(%)		2.6	7.1	4.3	10.7	2.3	2.0

^zWater stress level indicated as evapo transpiration; WR1= 50 %, WR 2 = 75 % and WR 3 = 100 %.

^yNN; Number of nodes per plant.

^xDTF 50%: days to 50% Flowering.

Table 13: Comparison of mean seed yield of eight genotypes in stressed and non-stressed environments. Plants were stressed up to 50 %

Genotypes	Yield of non- stressed treatment (ton ha ⁻¹)	Yield of stressed treatments (ton ha ⁻¹)	Percentage yield reduction (%)	Drought susceptibility Index	Geometric mean (ton ha ⁻¹)
GADRA	1.63	1.32	19.1	0.3	1.47
KE 4	2.24	1.50	33.0	0.5	1.83
ZM 4488	1.73	0.80	53.5	0.9	1.18
SER 180	1.43	0.65	55.0	0.9	0.96
SER 89	1.90	0.67	64.7	1.1	1.13
CAR-ZAR	1.74	0.56	67.9	1.1	0.99
KE 3	2.49	0.62	75.3	1.2	1.24
SER 76	4.36	0.81	81.4	1.3	1.88
Grand mean	2.19	0.87	56.2		

4.2. Effect of water regimes on morphophysiological traits

Water stress significantly reduced chlorophyll a and b content, relative water content, plant height and increased the relative injury percentage (Table 9). The relative injury percentage in 50% water regime (51 %) was 42.9 % higher than in the optimum water regime (35.7%). In the 75 % water regime (42.4 %), the increase in relative injury percentage from the optimum water regime was 18.8 %. The chlorophyll a content in 50 % water regime (7.6 mg g^{-1}) was lower by 48.7 % than in the optimum water regime (14.8 mg g^{-1}). In the 75 % water regime (9.9 mg g^{-1}), the reduction in chlorophyll a content to the optimum water regime was 33.1 %. The chlorophyll b content in 50 % water regime (10.4 mg g^{-1}) was lower by 54.2 % than in the optimum water regime (22.7 mg g^{-1}). In the 75 % water regime (17.8 mg g^{-1}), the reduction in chlorophyll b content to the optimum water regime was 21.6 %. The total chlorophyll content in 50 % (18 mg g^{-1}) water regime was 52 % lower than in optimal water regime (37.5 mg g^{-1}). In the 75 % (27.7 mg g^{-1}) water regime, the total chlorophyll content was 26 % lower than in the optimal water regime.

The plant height in 50 % water regime (19 cm) was shorter by 38.7 % than in the optimum water regime (31 cm). In the 75 % water regime (24 cm), the reduction in plant height to the optimum water regime was 22.6 %.

4.3. Effect of water regimes on grain yield and yield components

The grain yield in optimum water regime (2.19 ton ha^{-1}) was 60.3 % higher than in 50 % water regime (0.87 ton ha^{-1}) (Table 13). In the 75 % water regime (1.08 ton ha^{-1}), the reduction in grain yield was 50.8 %, (data not shown). The reduction in the amount of water applied to the plants did not significantly affect plant height, number of pods per plant and number of seeds per pod. However, it affected the hundred seed weight significantly (Table 9). In the 50 % water regime (31 g), the reduction in hundred seed weight was 31.1 % from the optimal water regime (100 %), while in the 75 % water regime (45 g), the reduction in hundred seed weight was 11.1 %, data not shown.

4.4. Effect of Water Regime and Genotype on morphophysiological traits of common bean

The result of the study indicates that there were significant differences among genotypes for Chlorophyll a, Chlorophyll b, Total Chlorophyll, Relative water content percentage and Relative injury percentage (Tables 9).

Significant differences for Chlorophyll a content among the three water stress regimes were observed. The 100 % water stress regime showed the highest Chlorophyll a content across all the genotypes, while the 50 % water stress regime showed the lowest Chlorophyll a content across all the genotypes. The 75 % water stress regime showed Chlorophyll a content values lying in between those of 100 % and 50 % water stress regimes.

Differences were observed among the genotypes for chlorophyll a content, with genotypes ZM 4488, SER 76 and SER 89 having the highest chlorophyll a content of 9.5 mg g⁻¹, 9.4 mg g⁻¹ and 9.3 mg g⁻¹ respectively (Table 10) while GADRA, KE 4 and SER 89 had the lowest chlorophyll a content of 4.9 mg g⁻¹, 5.0 mg g⁻¹ and 5.6 mg g⁻¹ respectively in the 50 % water stress regime. In the 100 % water stress regime KE 4, KE 3, and SER 180 had the highest chlorophyll a content of 17.8 mg g⁻¹, 17.2 mg g⁻¹ and 16.9 mg g⁻¹ respectively. The genotypes CAR-ZAR, GADRA and SER 89 had the lowest chlorophyll a content of 9.8 mg g⁻¹, 11.9 mg g⁻¹ and 14.4 mg g⁻¹ respectively. In the 75 % water stress regime, SER 76, SER 89 and ZM 4488 had the highest chlorophyll a content of 11.1 mg g⁻¹, 10.9 mg g⁻¹, and 10. mg g⁻¹) respectively. The genotypes CAR-ZAR, GADRA and KE 4 had the lowest chlorophyll a content of 8.6 mg g⁻¹, 9.0 mg g⁻¹ and 9.1 mg g⁻¹) respectively.

Differential response of the genotypes to the varying water stress regimes was also observed for chlorophyll a content (Table 10).

Significant differences were observed for Relative Injury Percent among the three water stress regimes (Table 9). The 100 % water stress regime showed low relative injury percent across all the genotypes, while 50 % water stress regime showed high relative injury percent across all the genotypes. The 75 % water stress regime showed moderate relative injury percent.

Significant differences were also observed for relative injury percent among genotypes for the three water stress regimes. The highest relative injury percent was observed in the genotype GADRA (68.3 %) while the lowest relative injury percent was observed in KE 4 (40.3 %) in the 50 % water stress regime.

Differential response of the genotypes to the varying water stress regimes was observed for relative injury percent (Table 10).

The relative injury percentage in 50% water regime (51 %) was 42.9 % higher than in the optimum water regime (35.7%). In the 75 % water regime (42.4 %), the increase in relative injury percentage from the optimum water regime was 18.8 % (Table 11).

Significant differences for chlorophyll b content among the three water stress regimes were observed (Table 9). The 100 % water stress regime showed the highest chlorophyll b content across all the genotypes, while the 50 % water stress regime had the lowest chlorophyll b content across all the genotypes. The 75 % water stress regime showed chlorophyll b content values lying in between those of 100 % and 50 % water regimes.

Differences were observed among the genotypes for chlorophyll b content, with genotypes KE 3, SER 180 and ZM 4488 having the highest chlorophyll b content of 20 mg g⁻¹, 16.5 mg g⁻¹ and 13.3 mg g⁻¹ respectively while GADRA and SER 89 had the lowest chlorophyll b content of 5.4 mg g⁻¹ and 5.8 mg g⁻¹ respectively in the 50 % water stress regime. In the 100 % water stress regime ZM 4488, SER 89, and SER 180 had the highest chlorophyll b content (37.9 mg g⁻¹, 26.2 mg g⁻¹ and 25.0 mg g⁻¹) respectively. The genotypes GADRA, KE 4 and SER 76 had the lowest chlorophyll b content (12.4 mg g⁻¹, 16.7 mg g⁻¹ and 19.0 mg g⁻¹) respectively. In the 75 % water stress regime, KE 3, SER 180 and SER 89 had the highest chlorophyll b content (23.7 mg g⁻¹, 22.2 mg g⁻¹, and 20.8 mg g⁻¹) respectively. The genotypes KE 4, GADRA and ZM 4488 had the lowest chlorophyll b content (9.4 mg g⁻¹, 13.9 mg g⁻¹ and 16.9 mg g⁻¹) respectively. KE 3, SER 180 and ZM 4488 had the highest total chlorophyll content (29.3 mg g⁻¹, 24.9 mg g⁻¹ and 22.8 mg g⁻¹) respectively while the lowest total chlorophyll content was obtained from GADRA (10.3 mg g⁻¹) data not shown.

Differential response of the genotypes to the varying water stress regimes was also observed for chlorophyll b content (Table 10).

The chlorophyll b content in 50 % water regime (10.4 mg g⁻¹) was lower by 54.2 % than in the optimum water regime (22.7 mg g⁻¹). In the 75 % water regime (17.8 mg g⁻¹), the reduction in chlorophyll b content to the optimum water regime was 21.6 %. The total chlorophyll content in 50 % (18 mg g⁻¹) water regime was 52 % lower than in optimal water regime (37.5 mg g⁻¹). In the 75 % (27.7 mg g⁻¹) water regime, the total chlorophyll content was 26 % lower than in the optimal water regime.

Significant differences for relative water content among the three water stress regimes were observed (Table 9). The 100 % water stress regime showed the highest relative water content across all the genotypes, while the 50 % water stress regime had the lowest relative water

content across all the genotypes. The 75 % water stress regime showed some genotypes having relative water content values similar to those of 100 % water regimes.

Differences were observed among the genotypes for relative water content, with genotypes SER 89, SER 180 and SER 76 having the highest relative water content of 82 %, 80 % and 78 % respectively while ZM 4488, KE 3 and KE 4 had the lowest relative water content of 59 %, 76 % and 72 % respectively in the 50 % water stress regime. In the 100 % water stress regime SER 180, SER 89 and GADRA had the highest relative water content (93 %, 91 % and 89 %) respectively. The genotypes CAR-ZAR, KE 4, SER 76 and ZM 4488 had the lowest relative water content (83 %, 85 %, 85 % and 85 %) respectively. In the 75 % water stress regime, SER 180, SER 89 and SER 76 had the highest relative water content (90 %, 90 %, and 89 %) respectively. The genotypes KE 3, GADRA and CAR-ZAR had the lowest relative water content (83 %, 83 % and 84 %) respectively (Table 10).

Differential response of the genotypes to the varying water stress regimes was observed for relative water content.

There were no significant differences for plant height among the three water stress regimes (Table 9). The 100 % water stress regime had the tallest plants for three genotypes, while the 50 % water stress regime had the shortest plants for three of the genotypes. The 75 % water stress regime showed plants of similar height to genotypes in 100 % water stress regime.

Differences were observed among the genotypes for plant height, with genotypes KE 3, SER 76, KE 4 and SER 180 having the tallest plants (24 cm, 23 cm, 22 cm and 22 cm respectively while CAR-ZAR, GADRA and ZM 4488 had the shortest plants (12 cm, 14 cm and 16 cm respectively in the 50 % water stress regime. In the 100 % water stress regime KE 4, SER 180 and SER 89 had the tallest plants (40 cm, 40 cm and 38.3 cm) respectively. The genotypes GADRA, CAR-ZAR and SER 76 had the shortest plants (19 cm, 20 cm, and 25 cm) respectively. In the 75 % water stress regime, SER 89, KE 4 and KE 3 had the tallest plants (33 cm, 30 cm, and 26 cm) respectively. The genotypes CAR-ZAR, ZM 4488 and GADRA and had the shortest plants (20 cm, 19 cm and 19 cm) respectively.

Differential response of the genotypes to the varying water stress regimes was observed for plant height (Table 10).

The plant height in 50 % water regime (19 cm) was shorter by 38.7 % than in the optimum water regime (31 cm). In the 75 % water regime (24 cm), the reduction in plant height to the optimum water regime was 22.6 % (Table 11).

4.5. Effect of Water Regime and Genotype on grain yield and yield components

The response by genotypes to water stress differed (Table 12). SER 76 with grain yield of 4.36 ton ha⁻¹ in 100 % water regime, performed poorly in 50 % water regime with grain yield of 0.81 ton ha⁻¹. Genotypes KE 4 and GADRA with grain yields of 2.24 ton ha⁻¹ and 1.63 ton ha⁻¹ in 100 % water regime performed well in 50 % water regime with grain yields of 1.50 ton ha⁻¹ and 1.32 ton ha⁻¹ respectively. These two genotypes showed stability in grain yield when moisture content is reduced from 100 % to 50 % (Table 12).

The results of the study indicate that there were significant differences in grain yield among the three water stress regimes (Table 12). The 100 % water stress regime showed the highest grain yield across all the genotypes, while the 50 % water stress regime had the lowest grain yield across all the genotypes. The 75 % water stress regime showed some genotypes having grain yield values lying in between the 50 % and the 100 % water regimes.

Differences were observed among the genotypes for grain yield, with genotypes KE 4, GADRA, ZM 4488 and SER 76 having the highest grain yield (1.5 ton ha⁻¹, 1.3 ton ha⁻¹, 0.8 ton ha⁻¹ and 0.8 ton ha⁻¹) respectively while CAR-ZAR, KE 3 and SER 180 had the lowest grain yield (0.6 ton ha⁻¹, 0.6 ton ha⁻¹ and 0.6 ton ha⁻¹) respectively in the 50 % water stress regime. In the 100 % water stress regime SER 76, KE 3, and KE 4 had the highest grain yield (4.4 ton ha⁻¹, 2.5 ton ha⁻¹ and 2.2 ton ha⁻¹) respectively. The genotypes SER 180, GADRA, CAR-ZAR and ZM 4488 had the lowest grain yield (1.4 ton ha⁻¹, 1.6 ton ha⁻¹, 1.7 ton ha⁻¹ and 1.7 ton ha⁻¹) respectively. In the 75 % water stress regime, GADRA, KE 4 and ZM 4488 had the highest grain yield (1.6 ton ha⁻¹, 1.5 ton ha⁻¹, and 1.3 ton ha⁻¹) respectively. The genotypes CAR-ZAR, KE 3, SER 180 and SER 76 had the lowest grain yield (0.5 ton ha⁻¹, 0.9 ton ha⁻¹, 0.9 ton ha⁻¹ and 0.9 ton ha⁻¹) respectively (Table 13).

Differential response of the genotypes to the varying water stress regimes was observed for grain yield.

Significant differences in the number of nodes per plant among the three water stress regimes were observed (Table 12). The 100 % water stress regime showed the highest number of nodes per plant for five of the genotypes, while the 50 % water stress regime had the lowest number of nodes per plant two of the genotypes. The 75 % water stress regime showed some genotypes having number of nodes similar to those in the 50 % and 100 % water regimes.

Differences were observed among the genotypes for number of nodes per plant, with genotypes KE 3, and ZM 4488 having the highest number of nodes per plant (7 each) while GADRA and SER 180 had the lowest number of nodes per plant (5 each) in the 50 % water stress regime. In the 100 % water stress regime KE 4 had the highest number of nodes per plant (10) followed KE 3, SER 180, SER 89 and ZM 4488 (9 each). The genotypes CAR-ZAR, GADRA, and SER 76 had the lowest number of nodes per plant (8 each). In the 75 % water stress regime, CAR-ZAR, KE 3, SER 89 and ZM 4488 had the highest number of nodes per plant (8 each). The genotypes GADRA and SER 180 had the lowest number of nodes per plant (6 each).

Differential response of the genotypes to the varying water stress regimes was observed for number of nodes per plant (Table 12).

Significant differences in the number of pods per plant among the three water stress regimes were observed (Tables 9). The 100 % water stress regime showed the highest number of pods per plant for all the genotypes, while the 50 % water stress regime had the lowest number of pods per plant for seven of the genotypes. The 75 % water stress regime showed genotypes having number of pods per plant lying in between the 50 % and 100 % water regimes except for GADRA which had a low number of pods per plant as compared to the same genotype in 50 % water stress regime.

Differences were observed among the genotypes for number of pods per plant, with genotypes SER 180, GADRA, and SER 76 having the highest number of pods per plant (26, 22 and 22) respectively while KE 3, SER 89, and KE 3, SER 89 and CAR-ZAR had the lowest number of pods per plant (15, 14 and 12) respectively in the 50 % water stress regime. In the 100 % water stress regime SER 76, CAR-ZAR and SER 180 had the highest number of pods per plant (42, 37 and 36) respectively. The genotypes KE 4 and KE 3 had the lowest number of pods per plant (23 and 22) respectively. In the 75 % water stress regime, SER 180, SER 76 and CAR-ZAR had the highest number of pods per plant (32, 27 and 25) respectively. The genotypes KE 4, GADRA, and KE 3 had the lowest number of pods per plant (21, 20 and 16) respectively (Table 12).

Differential response of the genotypes to the varying water stress regimes was observed for number of pods per plant.

There were no significant differences in the number of pods per plant among the three water stress regimes (Tables 9). The 100 % water stress regime showed the highest number of seeds per pod for all the genotypes, while the 50 % water stress regime had the lowest number of seeds

per pod for seven of the genotypes. The 75 % water stress regime showed genotypes having number of seeds per pod lying in between the 50 % and 100 % water regimes except for SER 76 which had the same number of seeds per pod across all the water stress regimes.

There were no differences observed among the genotypes for number of seeds per pod for seven genotypes except SER 76 which had (5), the rest had (4) in the 50 % water stress regime. In the 100 % water stress regime CAR-ZAR had the highest number of seeds per pod (7). The genotypes KE 3, SER 76 and SER 89 had the lowest number of seeds per pod (5 each). In the 75 % water stress regime, KE 3, KE 4 and SER 76 had the highest number of seeds per pod (5 each). The genotypes CAR-ZAR, GADRA, SER 180, SER 89 and ZM 4488 had the lowest number of seeds per pod (4 each).

Differential response of the genotypes to the varying water stress regimes was observed for number of seeds per pod especially for 100 % and 50 % water stress regimes as well as 100 % and 75 % water stress regimes and not for 75 % and 50 % water stress regimes (Table 12).

Significant differences for hundred seed weight among the three water stress regimes were observed (Tables 9). The 100 % water stress regime showed the highest hundred seed weight for all the genotypes, while the 50 % water stress regime had the lowest hundred seed weight for all the genotypes. The 75 % water stress regime showed genotypes having hundred seed weight lying in between the 50 % and 100 % water regimes except for KE 4 which had the same hundred seed weight as that in 100 % water stress regime.

There were differences observed among the genotypes for hundred seed weight for all the genotypes, with KE 4, KE 3 and GADRA having the highest hundred seed weight (43, 38 and 36) respectively while genotypes SER 76 and SER 89 had the lowest hundred seed weight (24 and 23) respectively in the 50 % water stress regime. In the 100 % water stress regime CAR-ZAR, KE 4, SER 76 and ZM 4488 had the highest hundred seed weight (51, 47, 47 and 47) respectively. The genotypes SER 89, KE 3, and SER 180 had the lowest hundred seed weight (43, 42 and 41) respectively. In the 75 % water stress regime, KE 4, CAR-ZAR and SER 89 had the highest hundred seed weight (47, 45 and 42) respectively. The genotypes GADRA, SER 89 and SER 180 had the lowest hundred seed weight (38, 35 and 29) respectively.

Differential response of the genotypes to the varying water stress regimes was observed for hundred seed weight (Table 12).

Significant differences for days to fifty percent flowering among the three water stress regimes were observed (Tables 9). The 100 % water stress regime showed the longest days to fifty percent flowering for all the genotypes, while the 50 % water stress regime had the shortest days to fifty percent flowering for three genotypes. The 75 % water stress regime showed genotypes having days to fifty percent flowering lying in between the 50 % and 100 % water regimes except for CAR-ZAR, GADRA and KE 3 which had shorter days to fifty percent flowering than the 50 % water stress regime.

There were differences observed among the genotypes for days to fifty percent flowering for three of the genotypes, with CAR-ZAR, GADRA, KE 3, KE 4, and SER 76 having the longest days to fifty percent flowering (50 each) while genotypes SER 89, ZM 4488 and SER 180 had the shortest days to fifty percent flowering (36, 36 and 35) respectively in the 50 % water stress regime. In the 100 % water stress regime KE 4 and SER 76 had the longest days to fifty percent flowering (62 each). The genotypes ZM 4488 and KE 3 had the shortest days to fifty percent flowering (54 and 53) respectively. In the 75 % water stress regime, KE 4, SER 76 and GADRA had the longest days to fifty percent flowering (55, 52 and 49) respectively. The genotypes SER 180 and ZM 4488 had the shortest days to fifty percent flowering (38 and 34) respectively (Table 12).

Differential response of the genotypes to the varying water stress regimes was observed for days to fifty percent flowering.

4.5.1. Mean seed yield in stressed and non-stressed environments

Plants were stressed up to 50 %. The genotypes GADRA, KE 4, ZM 4488, and SER 180 had the lowest drought susceptibility index (Table 13) of less than unit (0.3, 0.5, 0.9 and 0.9 respectively), whereas the genotypes SER 89, CAR-ZAR, KE 3 and SER 76 had higher drought susceptibility index values higher than unit (1.1, 1.1, 1.2 and 1.3 respectively). GADRA had the lowest percent yield reduction (19.11 %) followed by KE 4 (32.96 %) and ZM 4488 (53.52 %). GADRA, KE 4 and ZM 4488 had geometric mean of 1.47, 1.83 and 1.18 ton ha⁻¹ respectively

4.5.2. Effect of Relative Injury Percentage on Genotypes

The relative injury percentage of the eight genotypes varied significantly (Table 10), ranging from KE 4 (40.3) having the lowest relative injury percentage to GADRA (68.3), the highest relative injury percentage. The highest relative injury percentage was observed in 50 % water regime (52), followed by 75 % water regime (43) and 100 % water regime (34) (Figure 1)

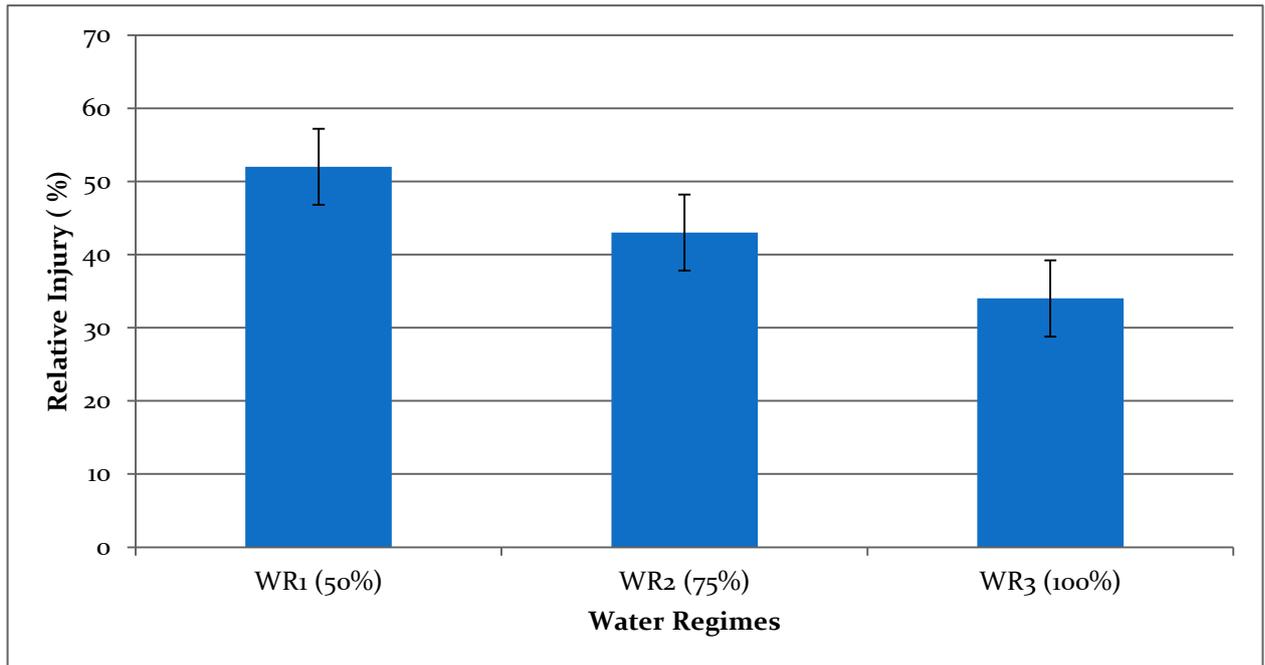


Figure 1: Effect of water regimes on relative injury percent.

Performance of genotypes across water regimes in terms of relative water injury. With highest relative injury in water regime 1, followed by water regime 2 and lastly water regime 3. Vertical bars are standard error of the means.

4.6. Stepwise multiple regression

The morphophysiological traits were used as independent variables and seed yield as the dependent variable. Small and significant contribution to total variations was observed among the independent variables in the study.

Hundred seed weight had a significant influence of grain yield explaining 37.3 % of the total variation (Table 14). Other variables (Chla, Chlb, NPP and NN) showed significant contributions to total variation expressed as R^2 from 37.3 % to 30 %. Further additions of other variables to the model did not show significant differences, thus not included in the model.

Table 14: Stepwise multiple regression of yield on morphophysiological traits

Variable	Partial Square δ^2	R- Model Square r^2	R-F-Value	Pr > F
HSW ^s	0.373	0.373	55.900	0.000
Chla ^w	0.117	0.490	21.354	0.000
Chlb ^x	0.068	0.558	14.217	0.000
NPP ^y	0.090	0.648	23.130	0.000
NN ^z	0.30 0	0.678	8.375	0.005

^sHSW: Hundred seed weight;

^wChla: Chlorophyll a content;

^xChlb: Chlorophyll b content;

^yNPP: Number of pods per plant;

^zNN: Number of nodes per plant.

HSW: Hundred seed weight; Chla: Chlorophyll a; Chlb: Chlorophyll b; NPP: Number of pods per plant; NN: Number of nodes.

4.7. Relationship among morphophysiological traits, grain yield and yield components of eight bean genotypes.

The strength of association for traits measured with yield as well as the inter component correlation amongst the components are presented in Table 15.

The results showed that all the traits measured were positively and significantly correlated to grain yield except for chlorophyll b ($r = -0.41^{**}$) which had a negative correlation. A strong positive correlation was recorded for hundred seed weight ($r = 0.41^{**}$), chlorophyll a content ($r = 0.57^{**}$) and number of pods per plant ($r = 0.36^*$).

The results in Table 14 also showed a strong positively and significantly inter component correlation between components. Moderate positive correlations were observed between hundred seed weight and days to 50% flowering ($r = 0.46^{**}$), chlorophyll b and chlorophyll a ($r = 0.44^{**}$), relative water content and number of pods per plant ($r = 0.47^{**}$), chlorophyll b and hundred seed weight ($r = 0.36^*$), number of seeds per pod and days to 50% to flowering ($r = 0.3^*$), number of pods per plant and chlorophyll b ($r = 0.39^*$), relative water content and plant height ($r = 0.34^*$ and number of seeds per pod and days to 50% flowering ($r = 0.3^*$).

A weak and positive correlation was observed between number of nodes and hundred seed weight ($r = 0.28^*$). A strong negative correlation was observed between chlorophyll a and hundred seed weight ($r = -0.41^{**}$), chlorophyll b and days to 50% flowering ($r = -0.35^*$), relative injury and number of nodes ($r = -0.34^*$) and relative injury and number of seeds per pod (-0.39^*).

Table 15: Partial correlation between each pair of variable

GYha											
DTF50%	0.07ns										
100SW	0.41**	0.46**									
Chla	0.57**	0.12ns	-0.41**								
Chlb	-0.41**	-0.35*	0.36*	0.44**							
NN	-0.24ns	0.01ns	0.28*	0.36*	-0.02ns						
NPP	0.36*	0.07ns	0.05ns	-0.11ns	0.39*	-0.19ns					
NSP	-0.14ns	0.3*	-0.04ns	0.05ns	0.01ns	0.10ns	0.24ns				
PHT	-0.13ns	0.13ns	0.15ns	0.39*	0.11ns	0.06ns	-0.15ns	-0.23ns			
RI%	-0.09ns	0.17ns	-0.29*	-0.02ns	-0.06ns	-0.34*	0.03ns	-0.39*	-0.08ns		
RWC	0.00ns	0.13ns	-0.22ns	0.02ns	0.05ns	0.23ns	0.47**	0.10ns	0.34*	0.17ns	

	GYha	DF_50%	100SW	Chla	Chlb	NN	NPP	NSP	PHT	RI%	RWC
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** Correlation is significant at $P \leq 0.01$; * Correlation is significant at $P \leq 0.05$; GY ha: Grain yield per hectare; DTF 50%: Days to 50% flowering; 100 SW: Hundred seed weight; Chla: Chlorophyll a; Chlb: Chlorophyll b; NN: Number of nodes; NPP: Number of pods per plant; NSP: Number of seeds per pod; PHT: Plant height; RI%: Relative injury percent; RWC: Relative water content.

Chapter 5

5. DISCUSSION

The present study focused on the physiological responses of different bean genotypes to water stress and in particular, the relationship between morphophysiological traits and grain yield under water stress conditions. It was anticipated that findings from the study could enable the identification of traits that could be useful in breeding bean genotypes for water stress conditions. The effects of water stress like all other stresses depend on the plant development stage at which the stress is applied, the degree and the duration of the stress. In this study, plants were subjected to three level of stress for 43 days during reproductive stage. Prior to stressing, normal irrigation was provided to all the treatments following the irrigation schedule prepared and after stress was discontinued the rains took over. Subjecting the beans to water stress reduced grain yields in this study. The results showed wide variation in the responses of the eight genotypes in terms of morphophysiological traits, grain yield and yield components. A marked genotypic variability in traits measured was observed among the different genotypes. Water stress negatively impacted on important morphophysiological traits, grain yield and yield components in all the genotypes tested.

5.1. Relative injury

There was a general increase in the relative injury percentage for all the genotypes grown in high water stress (52 %) and low water stress (43 %) as compared to those grown in the normally irrigated control (34 %). The increase in the relative injury of all bean genotypes was highest in the high water stress (52 %) treatment. The increase in the relative injury percentage of the genotypes was more in GADRA (68.3 %), SER 180 (62.5 %) and CAR-ZAR (55.8 %) while KE 4, KE 3 and ZM 4488 (40.3 %, 42.5 % and 42.7 % respectively) had less relative injury percentage in high water stress condition. It was noted that genotypes with high relative injury also had decreased cell membrane stability. According to Caruso *et al.*, (1999) increase in the permeability or increased leakage of electrolyte from the cell across the membrane of the cell membrane as a result of water stress is attributed to cell membrane damage. . Bajji *et al.*, (2001) found that drought sensitive cultivars of wheat exhibited greater values of cell membrane injury as compared to drought resistant cultivars.

5.2. Chlorophyll content and quality

Photosynthetic efficiency depends to large extent on quantity and quality of photosynthetic pigments such as chlorophyll a and chlorophyll b which play an important role in photochemical reactions of photosynthesis (Taiz and Zeiger, 2002). Water stress can inhibit photosynthesis of plants by affecting chlorophyll components, causing changes in chlorophyll content, and damaging the photosynthetic apparatus in plants (Iturbe Ormaetxe *et al.*, 1998). The current study showed significant differences among genotypes for chlorophyll a, chlorophyll b and total chlorophyll contents especially after being subjected to water stress. There was a general decrease in the leaf chlorophyll content in all the genotype subjected to water stress. The decrease in chlorophyll content across all the genotypes in the study was more in high water stress condition than in the low water stress and normally irrigated condition (Table 10). The highest decrease in total chlorophyll content was obtained from SER 89 (29.2 mg g^{-1}) in the high water stress condition. This finding is in agreement with what Baroowa and Gogoi (2012) found in Black gram and Green gram. These workers found that chlorophyll content decreased with intensity in water stress indicating that photosynthetic pigments are sensitive to water stress conditions. A reduction in chlorophyll content was also reported in drought stressed cotton (Mssacci *et al.*, 2008). Chlorophyll content decreased significantly at high water deficits in sunflower plants (Kiani *et al.*, 2008). The reduction in leaf chlorophyll content under drought stress might be due to the cause of excessive swelling of chloroplast membranes, distortion of the lamellae vesiculation and the appearance of lipid droplets (Kaiser *et al.*, 1981). This degradation is considered as one of the consequences of drought stress which has resulted from sustained photo-inhibition and photo bleaching (Long *et al.*, 1994).

The decrease in chlorophyll content of leaves under water stress can also be alluded to water stress inhibiting chlorophyll synthesis which is said to occur at four consecutive stages: (I) the formation of 5-aminolevulinic acid (ALA); (II) ALA condensation into porphobilinogen and primary tetrapyrrole, which is transformed into protochlorophyllide; (III) light-dependent conversion of protochlorophyllide into chlorophyllide; and (IV) synthesis of chlorophylls a and b along with their inclusion into developing pigment-protein complexes of the photosynthetic apparatus (Lisar *et al.*, 2012). Several authors have reported that higher Chlorophyll content have been associated with the stress tolerance of plants (Pastori and Trippi, 1992; Sairam, 1994; Kraus *et al.*, 1995).

5.3. Leaf relative water content

Water stress decreased the relative water content of all genotypes in the stressed environments by 13.8 % in the high water stress and by 1.1 % in the low water stress (Table 11). The genotypes SER 89, SER 180, SER 76 exhibited higher relative water content while genotypes GADRA, KE 4 had lower relative water content. Ghanbari *et al.*, (2013) reported that relative water content was an integrative indicator of internal plant water status under drought conditions and that it has successfully been used to identify drought-resistant cultivars of common bean. Genotypes with high relative water content under stress condition have the ability to retain more water in the leaves under stress. According to Kumar *et al.*, (2006) high yielding bean cultivars displayed a smaller reduction in leaf water content than the poor yielder. However, in the present study, the genotypes with low relative water content had higher grain yield in water stress environment than those with high relative water content (Table 10). This could probably be attributed to the stage at which water stress was applied- the seed filling phase.

5.4. Days to flowering

There were highly significant differences among genotypes for days to 50 % flowering phase with five genotypes taking the longest period of 50 days to flower (KE 4, CAR-ZAR, GADRA, KE 3 and SER 76) in the high water stress condition. The genotype with the shortest number of days to fifty percent flowering was SER 180 (35) followed by SER 89 and ZM 4488 (36). Days- to- fifty- percent flowering measures the number of days when 50 % of the plants have one or more flowers and this marks the beginning of the reproductive phase of the bean plants. The genotypes with longest period of days to 50 % flowering showed resilience to water stress and the flowering almost corresponded with normal flowering. Messia *et al.*, (2012) reported that some early flowering may be induced by disease or drought and may not correspond with normal flowering period consequently impacting negatively on grain yield. The differences in the duration to fifty percent flowering can be attributed to genetic variability among the genotypes. Bray (1993) found similar results in soybeans.

5.5. Grain yield

There were highly significant differences in grain yield among the different genotypes (Table 9). The mean grain yield across all genotypes was 2.19 ton ha⁻¹. The yield varied from 1.74 ton ha⁻¹ to 4.36 ton ha⁻¹. The highest grain yields were obtained from SER 76 (4.36 ton ha⁻¹), KE 3 (2.49 ton ha⁻¹ and KE 4 (2.24 ton ha⁻¹), while the lowest grain yield came from

CAR-ZAR (1.74 ton ha⁻¹), and SER 180 (1.43 ton ha⁻¹). The high yield exhibited by the genotypes SER 76 and KE 4 for both conditions could be attributed to high number of pods per plant, hundred seed weight, relative water content, chlorophyll a and b content, total chlorophyll as well as low relative injury. The low yield exhibited by genotypes CAR-ZAR and SER 180 could be attributed to negative effects on physiological components- low chlorophyll a and b content, relative water content and increased relative injury. This was translated to low yields arising out of diminished yield components- number of pods per plant and hundred seed weight. The findings in this study are in agreement with Molina *et al.*, (2001); Nielsen and Nelson, (1998); and Emam, (1985). They all reported a reduction in grain yield and mean weight of hundred seeds of common bean following water stress.

Subjecting the genotypes to water stress during the reproductive stage reduced grain yields in this study. Genotype SER 76, gave the highest grain yield of 4.36 ton ha⁻¹ in the normally irrigated environment while in the high stressed environment, the same genotype gave 0.81 ton ha⁻¹. The highest genotype grain yield of 1.50 ton ha⁻¹ in the high stressed environment was recorded from KE 4 (Table 12). This finding is in agreement with what other researchers found. Emam and Seghatoleslami (2005); and Emam, (1985) stated that common bean grain yield is significantly reduced when water stress occurs during the reproductive phase. The reduction in grain yield is attributed to lower percentage of pod production when the water stress occurs during flowering (Emam, 1985) and from embryos abortion when the water stress occurs during pod filling stage (Robins and Domingo, 1956).

5.6. Pod number

The number of pods per plant was significantly influenced by water stress in this study. The reduction in quantity of water from non- stressed to stress (high and low) resulted in the reduction in the number of pods and seed number per pod per plant. This finding is in agreement with what Castaneda- Saucedo *et al.*, (2009) found. The researchers stated that high moisture stress during the reproductive stage exposed the plant to floral abortion and resulted in low seed yield. Other authors (Barrios *et al.*, 2005; Singh (1995); Sponchiado *et al.*, (1989) all reported that water stress imposed during flowering and pod setting causes flower and pod abortion. The reproductive stage is the most sensitive stage to drought stress (Nielsen and Nelson, 1998). This phase includes flower formation (Pedroza and Muñoz, 1993), full flowering (Pimentel *et al.*, 1999), pod formation (Castañeda *et al.*, 2006), or grain filling (Nielsen and Nelson, 1998).

Water deficits cause falling or abortion of reproductive structures in soybeans (Acosta and Kohashi, 1989, Kokubun *et al.*, 2001), reduced pollen formation and pollination in common bean (Shen and Webster, 1986, Dornbos *et al.*, 1989; Boutra and Sanders, 2001, Nielsen and Nelson, 1998). The need to maintain the high pod numbers under water stress conditions in common bean is vital, since it constitutes an important yield component that determines seed yield. Andriani *et al.*, (1991) reported that the number of pods per plant constitute the main yield component which is mostly affected by water deficit during flowering stage and can reduce seed yield up to 70 % depending on the duration and severity of the water stress. Drought susceptible genotypes in high and low water stress conditions showed a slow and weak development of pod and seed setting as water stress continued toward physiological maturity (Szilagyi, 2003). Lopez *et al.*, (1996) reported that total number of flowers in some susceptible varieties may be reduced up to 47 % under drought conditions thereby influencing the number of pods per plant; though pod setting may also vary among different common bean varieties in response to drought.

Water stress affected the seed weight of the bean genotypes and this observation was in agreement with what other researchers found. Teran and Singh (2002) reported that drought stress, on the average reduced common bean hundred seed weight by 13 %. In the present study, high water stress reduced hundred seed weight by about 31.1 % while in low water stress condition the reduction in hundred seed weight was 11.1 %. Barrios *et al.*, (2005) reported that seed yield reduction of up to 60 % observed in common beans under drought stress was attributed to losses of 63.3 % in pods per plant, 28.9 % in seed per pod and 22.3 % in seed weight. Acosta and Kohashi (1989), Nielsen and Nelson (1998) and Nuñez *et al.*, (2005) also identified the number of pods per plant as the principal cause of yield losses of bean subjected to drought stress, followed by the number of seeds per pod and seed weight.

5.7. Drought susceptibility

The genotypes GADRA, KE 4, ZM 4488, and SER 180 in the high water stress condition had the lowest Drought Susceptibility Index (DSI) values, which can be considered as genotypes with low drought susceptibility and high yield stability in the both conditions, whereas the genotypes SER 89, CAR-ZAR, KE 3 and SER 76 with DSI values higher than unit can be identified as high drought susceptibility and poor yield stability genotypes (Table 13). Agili *et al.*, (2012) found similar ranks for Orange Fleshed genotypes using geometric mean and

mean product parameters as well drought susceptibility index, suggesting that these three parameters can be used for selecting genotypes that are drought tolerant.

A strong correlation was observed between yields of drought stress and non-stress conditions which indicated that genotypes which performed well under non-stress also performed well under water stress growing conditions. This result is in agreement with the findings for Cattivelli *et al.*, (2008) that explain that traits maximizing productivity normally in the absence of stress, can still sustain a significant yield improvement under mild to moderate stress. Positive and strong correlations were observed in the present study for yield components and other morphophysiological traits, these were: hundred seed weight with days to fifty percent flowering (0.46**), Chlorophyll b with Chlorophyll a (0.44**), relative water content with number of pods per plant (0.47**), Chlorophyll b with hundred seed weight (0.36*), number of seeds per pod with days to fifty percent flowering (0.3*), number of pods per plant with Chlorophyll b (0.39*), relative water content with plant height (0.34*) and number of seeds per pod with days to fifty percent flowering (0.3*). The Negative correlations that were significant were: Chlorophyll a with hundred seed weight (-0.41*), Chlorophyll b with days to fifty percent flowering (-0.35*) and relative injury percentage with number of nodes (-0.34*). Negative and significant correlations indicate that selection of a trait can decrease the expression of another (Ramalho *et al.*, 1993). Molina *et al.*, (2001), assessed the water stress tolerance in three cultivars and seven lines of common bean and they observed both positive and negative significant correlations among yield components.

Grain yield is a function of morphophysiological traits and their inter-relationships. The relationship between the individual traits and yield was used to develop a regression model to identify traits most closely associated with yield. Five traits were correlated with grain yield and these were; hundred seed weight, Chlorophyll a, Chlorophyll b, number of pods per plant and number of nodes. However, only hundred seed weight, Chlorophyll a, Chlorophyll b and number of pods per plant contributed significantly to the multiple regression model to predict yield (Table 14). When the four traits were combined, they accounted for 64 % of the variation in seed yield. The remaining traits did not improve the model. Shenkut & Brick, (2003), came up with a model using stepwise multiple regressions which included plant biomass and number of pods per plant as the best model that could be used as selection criteria to improve grain yield in dry beans. Akande and Bologun, (2007), using stepwise multiple regressions also showed that, pod weight, hundred seed weight and pod length were the main seed components accounting for 98 % of the total variation.

Chapter 6

6. CONCLUSION

The study measured eleven parameters and the results revealed that all genotypes were different in grain yield and morphophysiological traits. The differences indicated presence of genetic variation for these traits, a key factor in plant breeding and selection for bean crop improvement. Water stress during the reproductive stage of common bean reduced most of the parameters assessed; grain yield, relative water content, chlorophyll a, chlorophyll b and total chlorophyll content, number of pods per plant, number of seeds per pod and increased relative injury percentage. Differences were found in chlorophyll a, chlorophyll b, total chlorophyll contents, relative water content, relative injury percentage, grain yield, hundred seed weight, number of pods per plant, number of seeds per pod and days to fifty percent flowering among bean genotypes tested. Based on the result of geometric mean (GM), drought susceptible index (DSI) and percent yield reduction (PR), genotypes were identified with greater yield potential under the tested environment. The genotypes GADRA, KE 4, ZM 4488, and SER 180 were identified as water stress tolerant while the genotypes SER 89, CAR-ZAR, KE 3 and SER 76 were identified as water stress sensitive genotypes. A stepwise multiple regression showed that hundred seed weight, chlorophyll a, chlorophyll b and number of pods per plant contributed significantly to the total variation of grain yield. These results suggest that a selection method based on hundred seed weight, chlorophyll a, chlorophyll b and number of pods per plant can be used in breeding for water stress tolerant bean genotypes. Some genotypes like KE 4 and ZM 4488 can be recommended for production under water stressed conditions. However, caution should be taken in the use of these results as the study was conducted only in one location and for one season. In order to validate the findings, the study should be conducted for a number of years and in many locations.

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Appendix I: Mazabuka Average Climate Data

CLIMATE PARAMETER/ MONTH	JUL	AUG	SEPT	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	YEAR
Evaporation (mm)	112	143	183	226	189	177	171	146	171	150	124	105	1897
Rainfall (mm)	0	0	2	15	85	250	197	149	55	18	6	0	777
Water balance(mm)	-112	-143	-181	-211	-104	+73	+26	+3	-116	-132	-118	-105	-1120
Relative Humidity (%)	54	47	43	41	59	75	81	81	76	67	62	60	62
Wind Speed (Km/d)	235	245	280	270	180	140	120	110	140	170	215	220	190
Sunshine hours	9.3	10.0	10.1	9.8	6.9	5.7	6.2	6.4	8.2	9.3	9.6	9.1	8.5
Mean Max temp (⁰ c)	24.7	27.4	29.9	33.6	31.3	28.9	28.3	28.3	28.7	28.9	27.2	24.9	28.5
Mean Min Temp (⁰ c)	7.4	9.7	13.4	16.6	18.2	18.2	18.2	18.2	16.3	13.9	10.1	8.5	12.4
Mean Temp (⁰ c)	15.9	18.2	21.9	24.8	23.8	22.7	22.3	22.2	21.7	20.7	18.3	16.1	20.8

Source: Kafue Polder Agro-Met Station, Nanga

