GROWTH, NODULATION AND YIELD OF SELECTED LEGUMES UNDER DROUGHT CONDITIONS IN KITUI COUNTY, KENYA.

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DECLARATION

This thesis is my original work that has never been presented for the award of a degree in any other university.

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DEDICATION

To my late father, Maluki King’etu, who was an ardent believer in the value of education, and to my mother, Franciscah Kang'wele, for her financial and moral support. Lastly, my lovely wife Jamila, and my four children Joyce, Juliet, Gift, and Maluki, for their encouragement, tolerance, and support throughout my study.

ABSTRACT

Loss of fertility in soil is the main limiting factor that affects production of crops in Kenya, especially in the Arid and Semi-arid regions. In the lower parts of Eastern Kenya, unreliable and low rainfall has led to the low yields in crop production. Lack of the use of commercial fertilizers is also a contributing factor to low crop yields. Amongst new solutions that can assist farmers facing this challenge of low yields includes the emerged potential role of *rhizobia* in crop performance under water-scarce conditions. Therefore, this study focused on the analysis of drought affects nodule formation, growth, and yield. The analysis involved four legumes that are cultivated as a norm Arid and Semi-Arid Lands in Kitui County, Kenya. The legumes include; beans, cowpeas, *Dolichos lablab*, and green grams. The trials in the project lasted for two seasons and involved randomized complete block design with drought stress treatment (DST). DST had to be induced using withholding total irrigation, and a well-watered treatment (WWT) maintained to act as the control. Four blocks, each with four plots, were well divided. The four legumes were randomly placed in the plots and maintained under WWT. After a period of thirty days (a month), upon planting, the induction of DST followed which limited irrigation in of the two blocks. On the other hand, WWT was maintained in the other two blocks as controls. After the specified duration of the experiment, the roots for the legumes for both DST and WWT were harvested and analyzed. The root nodules were then taken to the laboratory for isolation of *rhizobia* and an inoculant preparation for specificity assays under greenhouse conditions. The results indicated that plants subjected to DST had less ($p \leq 0.05$), TND, NoP, lower LAI, more WIX, and lower GYD than control or plants under WWT. This implied that the widespread deleterious impact of water deficit on legume nodulation, growth, and yield. The reduced TND under DST could inhibit nitrogen fixation, further lessening GYD in legumes. Amongst the legumes, green grams had significantly higher ($P \leq 0.05$) GYD, TND, and least WIX, *Dolichos lablab*, and cowpeas showed a moderate performance of the three types traits. Beans showed the least TND, GYD, and high WIX under DST. Under DST, Green grams had a higher ($p \leq 0.05$) yield followed by Cowpeas, Dolichos, lablab, and Beans, significantly affected by water stress to give the lowest yield. Generally, TND positively correlated with GYD and negatively with wilting (WIX), potentially implying that higher nodulation might have enhanced nitrogen fixation, thus higher legume YLD and tolerance to water deficit. Based on observed performance, i.e., wilting index, root nodules number per plant, and grain yield, green grams were considered drought tolerant and beans drought susceptible. Therefore this study recommends the adoption and growth of green grams. In conclusion, the present study identified green grams (variety KS-20) as a high yielding and drought tolerant legume that could be adopted or promoted for sustainable food production in Kitui County. The rhizobium isolated from this green gram could also be cultured and potentially used as a bio-fertilizer to enhance yield in other Kenya's ASALs.

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Abbreviations and acronyms

AIDS	Acquired Immunodeficiency Syndrome.
ANOVA	Analysis of variance
ARS	The Agricultural Research Service.
ASALs	Arid and semi-arid lands
BNF	Biological nitrogen fixation
CEC	Cation Exchange Capacity
CIAT	International Centre for Tropical Agriculture Cooperation Agency
DSL	Drought Stressed Leaves
DST	Drought Stressed Treatment
FAO	Food Agricultural Organization.
FAOSTAT	Food and Agriculture Statistical Databases.
GDP	Gross Domestic Product.
GMD	Differences in Grain Germination
GoK	Government of Kenya.
GYD	Grain Yield Difference
HIV	Human Immunodeficiency Virus.
IITA	International Institute of Tropical Agriculture
ISFM	Integrated Soil Fertility Management.
KALRO	Kenya Agriculture and Livestock Organization.
LAI	Leaf area index
LSD	Least Significant Difference
MAP	Months After Planting
MIRCEN	Microbial Resources Centre Network.
NoP	Number of Pods
SAS	Statistical analysis system.
SDG	Sustainable Development goal
SDW	Shoot Dry Weight
SEKU	South Eastern Kenya University
SIDA	Swedish International Development Agency.
TND	Total Number of Nodules
USDA	U.S. Department of Agriculture.
WAP	Weeks After Planting.
WIX	Wilting Index
WWT	Well-Watered Treatment
YMA	Yeast mannitol agar.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Poverty and hunger in Africa are major challenges in dry areas (U.N., 2007), and sadly, in such areas, the main source of food is agriculture. (GoK, 2010). Current approximate data indicate that agriculture comprises of 34.99% of the World's GDP, 39.98% of export earnings, and 71% of job creation. In Kenya, agriculture stands as the pillar of the economic and social progress. Despite Kenya doing economically well in East Africa, hunger is a big problem (Glopolis, 2013; GoK, 2011). Regrettably, more than 82% of Kenta's Land is categorized as Arid and Semi-Arid lands. In such a climate, cases of poor food insecurity, high degree of poverty, and frequent famines are a normal pattern (GoK, 2010). Approximately, over 49.9% of Kenya's population lack enough food which translates to poor nutritional values in their diets. (Anon, 2010). The dwindling fertility of the soil and lack of adequate rainfall, has led to a decline in production in the Kenyan farms and, in turn, insecurity of food in the Kenyan ASAL's (GoK, 2009a). The infertility of soil is attributed to soil leaching, erosion, and mining of nutrients, mainly through continuous monocropping of cereal crops that lacks soil fertility replenishment (Gachimbi *et al.*, 2002).

Food security has been an integral part of global efforts to develop and reduce poverty (Vink, 2012). In Kenya, many types of cereals and legumes are grown to alleviate food insecurity. Philips (1980) noted that leguminous crops continue to play a crucial role in agricultural production throughout history and attribute their success in N-deficient soils results from root nodules containing symbiotic Rhizobium bacteria that reduce N_2 to NH_4. Many farmers do not understand the benefits of root nodules in soil fertility (Woomer *et al.*, 1997). In another study, a small percentage of 1% of farmers in Kenya use inoculants (Karanja *et al*., 2000).

Nitrogen consists of 78 % of the earth's atmosphere are nitrogen gas (Sangakara *et al.*, 2003). Thus, every hectare has substantial N on its surface. Despite the abundance of Nitrogen in the atmosphere, plants cannot use it directly because it is available in an inert form (N_2). Nitrogen in the soil is lost through microbial denitrification, soil erosion, leaching, chemical volatilization, removal of Nitrogen-containing crop residues from land, making it the most unavailable nutrient to African crops (Sangakara *et al.*, 2003).

Naturally, most legumes can biologically convert N_2 through BNF to a more useable form (Mugwe *et al.*, 2007*)*. The amount of Nitrogen fixed varies according to the legume species and variety. Within a species, yield (dry matter) is directly proportional to Nitrogen's amount (Delfin *et al*., 2008). When right *rhizobial* strains are present under optimal conditions (George *et al.*, 2007), they can fix upto200 kg N ha^{-1} $year^{-1}$ (Giller, 2001). The Nitrogen content provided by legumes is considerate to the symbiotic rate of the Nitrogen fixing Bacteria's activity, growth, and most importantly, the Nitrogen harvest index of the legume crops (Zahran, 1999). Nitrogen fixation rate depends a lot on the following; the type of the legume crop, how it is measured, Rhizobia available and several soil factors such as soil moisture, Nitrogen oxide level in the soil, and the acidity of soil (Danga *et al.*, 2010; Zahran, 1999). Nevertheless, biological Nitrogen fixation can be achieved by using other advanced methods like the inoculation with proven strains, detection and selection of better microbial and host-plant materials, and change of farming cultural practices (Zengenia *et al.*, 2006; Giller, 2001).

The desire to advance from home consumption to market production makes farmers improve yields and field practices (Woomer *et al*., 1998). Many farmers continue cultivating legumes, including beans, lablab, cowpeas, and green grams, as increasingly important cash crops (Woomer *et al*., 1998). Most of these grain legumes can obtain between 50 - 80% of their nitrogen concentration requirements through biological fixation (Solomon *et al*., 2012).

Scientific data on nitrogen fixation by these legumes under water-scarce conditions is either nascent or non-existent. This study aimed at analyzing legume growth, nodulation, and yield under drought stress conditions in Kitui County, Kenya**.**

1.2 Statement of the Problem

Crop production in dry areas is limited by the depletion of soil fertility (Gachimbi *et al.*, 2002*).* Continued mining of soil nutrients without adequate replenishment results in loss of fertility (Gachimbi *et al.*, 2002*).* There is low usage of fertilizers in semi-arid eastern Kenya and this is due to the increased prices and low supply of the fertilizers (MoA, 2009). Biological Nitrogen Fixation (BNF) is the alternative method of maintaining the fertility of the soil (Mugwe *et al.*, 2007*).* Legumes like, cow peas and green grams, that are adaptive to climatic conditions with low and unreliable rainfall have been discriminated when it comes to their contribution to nitrogen fixation (GoK, 2009).

Legumes are second only to the cereal grasses as a source of human food and animal forage; the world's population majorly depends on proteins (Katungi *et al*., 2010). The FAO (2015) estimates annual global legume production of some 60 million metric tons per year. FAO continues to note that consumption has increased in the developed economies due to their healthy nutritional levels and ecological sustainability. With a high demand for legumes and the poor conditions of Kenyan soil coupled with unexplored economic and diverse African rhizobia, there is a need to select appropriate Legume-*Rhizobium* symbioses for Kenyan dry environments.

1.3 Justification

Previously, studies on Nitrogen fixation through nodulation by legumes. Bueno *et al.* (1987) studied the effects of drought on the survival of *Rhizobium leguminosarum*, while J.G Streeter (2003) studied water stress on soybean root nodule. Ramos *et al*.1999 worked on the effect of drought on the drought-resistant cultivar of common bean (*Phaseolus vulgaris* L.). In 2006, Marino *et al*. worked on water stress relations in Nitrogen metabolism in pea nodules. Farooq *et al.* (2016) worked on water stress in grain legumes during flowering and yielding. Muhammed *et al*. (2017) did research on progress and perspective on drought stress in legume. In 2017, Muhammad *et al*. worked on drought stress in Legumes during reproduction and grain filling. This project aimed at analyzing an appropriate rhizobia-legume association under drought conditions in Kitui, a County under ASAL of Kenya. Through this study, farmers can be advised on legume species to grow for high or sustainable yields, thus food security under such drought-prone conditions besides offering alternative production and soil amelioration technology. The rhizobium species can be recommended for culturing for use as a potential bio-fertilizer to enhance yield in Kenya's other ASALs.

1.4 Objectives

1.4.1 Broad Objective

The study aimed at evaluating growth, nodulation, and yield of selected legumes under drought stress conditions in Kitui County, Kenya

1.4.2 Specific Objectives

i) To evaluate root nodulation in beans, cowpeas, *Dolichos lablab*, and green grams under drought stress field conditions in Kitui County, Kenya.

ii) To assess selected morphological characteristics and yield response to drought stress in beans, *Dolichos lablab*, cowpeas, and green grams under field conditions in Kitui, Kenya.

iii) To evaluate Nitrogen fixation's specificity by *Rhizobia species* in beans, *Dolichos lablab*, cowpeas, and green grams under Kenya's greenhouse conditions.

1.4.3 Statistical hypothesis

i) Drought stress conditions do not affect legume growth, nodulation, beans yield, *Dolichos lablab*, cowpeas, and green grams in Kitui, Kenya.

ii) Drought stress has no significant effect on morphological differences in beans, *Dolichos lablab*, cowpeas, and green grams under field conditions in Kitui, Kenya.

iii) Nitrogen fixation in beans, *Dolichos lablab*, cowpeas, and green grams under greenhouse conditions is non-Rhizobia specific.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Importance of legumes in soil fertility management

Biological nitrogen fixation can increase soil N (Mburu and Gitari, 2006). Legumes are important in agriculture as they form associations with bacteria in their root nodules and fix atmospheric Nitrogen (Delfin *et al*., 2008). Legumes' ability to biologically fix atmospheric N is beneficial in a cropping system (Chemining'wa *et al.*, 2006; Walley *et al.*, 2007). This makes them richer in proteins than other crops (Broughton *et al*., 2003). Nitrogen fixation contributes about 70 million tonnes of Nitrogen per year and results in increased plant protein levels and reduced depletion of soil nitrogen reserves (Hussein, 1999). Leguminous crop residues and green manures improve soil fertility, increase nutrient supply in the soil through biological Nitrogen

Legumes are important in agriculture as they form associations with bacteria in their root nodules and fix atmospheric Nitrogen (Delfin *et al*., 2008). This makes them richer in proteins than other crops (Broughton *et al*., 2003). Nitrogen fixation contributes about 70 million tons of Nitrogen per year and results in increased plant protein levels and reduced depletion of soil nitrogen reserves (Hussein, 1999). Biological nitrogen fixation reduces production costs and enhances natural resource management due to reduced water pollution caused by runoffs and leaching of nitrogen fertilizers (Giller and Cadisch, 1995). Nitrogen-fixing legumes increase soil fertility and improve soil structure through practices such as green manuring and crop rotation. Green manure legumes are fast-growing, accumulate high biomass, and provide good ground cover, thus minimizing erosion (Mureithi *et al*., 2003). However, the N fixed amount is reported to vary with legume species and rhizobia species. Soils in ASALs have indigenous *rhizobia* strains that can fix atmospheric Nitrogen with the assistance of legume crops.

2.2 The Role of grain legumes in the soil

Small scale farmers can benefit from the nitrogen fixing legume crops regarding the Integrated Soil Fertility Management (ISFM) (Fujita *et al.*, 1992). Lack of Nitrogen in farms is attributed to soil volatilization, leaching of nutrients the discarding of harvest residues that are have high content of nitrogen from the farms (Amba *et al.*, 2013). Thus, the low-content nitrogen in soil should be corrected as a regular measure so as to maintain an adequate crop production level (Zahran, 1999). Thus, Biological Nitrogen Fixation (BNF) is a potentially cheaper solution to small-scale farmers in not only the Kenyan ASALs but also in the lower sub-Saharan Africa (Sangina and Woomer, 2009). BNF is also a remedy to help minimize the urgent need of artificial nitrogen fertilizers (Shisanya and Gitonga, 2007).

Therefore, agro-experts have begun campaigning for the use of BNF for the whole of Africa where soil is relatively fertile (Danga *et al.*, 2010). This potential solution to the deficiency of nitrogen will be achieved by the use of continued crop rotation and intercropping of legumes with other crops in farms (Mafongoya *et al*., 2007). However, the common legumes such as soya-bean, common beans, and cowpeas, are classified as minor intercrops when intercropped with cerals in small-scale systems. This is because small-scale farmers are faced with poor production methods which disadvantage the legumes (Kimiti *et al.*, 2009). Also, most legumes produce root nodules for certain particular *Rhizobial* strains. Such strains may be scattered and not random in the drought regions (Theuri, 2007; Zahran, 1999).

The amount of N contributed to the soil system by the legume crops depends on the rate of symbiotic N fixing activity, growth, and N harvest index of the legume crops (Zahran, 1999). The rate of N fixation varies considerably, depending on the type of legume cultivar, method of measurement, the presence of appropriate *Rhizobia*, and certain soil and environmental variables, including soil moisture, NO_3 level, soil acidity, and P nutrition (Danga *et al.*, 2010; Zahran, 1999).

However, the amount of biologically fixed N can be enhanced by different methods, including inoculation with proven strains, screening for improved microbial and host-plant materials, and introduction of improved cultural practices (Zengenia *et al.,* 2006; Giller, 2001).

2.3 The Productivity and Importance of Cowpeas

The world's yearly cowpea produce is approximated at 7.56 million tons on about 12.76 million ha, and in this value sub-Saharan Africa (SSA) is responsible for over 69% of total (IITA, 2002). In Kenya, cowpea not considered as an important grain legume. Cowpeas are ranked third after beans and pigeon pea (Kimiti *et al.,* 2009). It is estimated that 85% of total cowpea production in Kenyacomes from the ASALs (Onduru *et al.,* 2008; Kimiti *et al.,* 2009). However, the average yield of the cowpea grain is about 0.5 t ha^{-1} in farmers" fields compared to the potential yield of 2.5 t ha^{-1} (Faraj *et al.,* 2012). There is low production of cowpea in small-scale farms because of infertility of the soil, low planting densities, shading by other crops' pests, and disease attacks (Onduru *et al.,* 2008).

On a bright side, Cowpea gives production advantages in a short duration and is given a priority by small-scale farmers for its fast solution to food, and income (Kimiti *et al.,* 2009; Saidou *et al.,* 2007). According to Fatokum and colleagues (2000), cowpea can fix up to 88 kg N/ha, while in an effective cowpea-*Rhizobium* symbiosis, it can fix more than 150 kg N/ha. This can in turn supply 80-90% of plants with enough Nitrogen need. However, P levels are low in the soil and this is a main drawback to the BNF process (Kamanga *et al.,* 2010; Saidou *et al.,* 2007; Singh, 2011). In ASALs, the soils contain very low useful P (Gachimbi *et al.,* 2002). Therefore, P nutrition also has to be replenished tooif cowpeas are to excel in Nitrogen fixation process and potentiality (Asuming-Brempong *et al.,* 2013). If that is not done, it is probable that cowpeas will not be able to be part of the integrated soil fertility management options in the ASALs.

2.4 The Productivity and Importance of *Beans*

Common bean (*Phaseolus vulgaris L.)* is the chief protein source for many farmers in Kenya. Beans play a key role in reducing food insecurity, hunger, and malnutrition (Korir *et al.,* 2003), since it can be utilized as leaves, pods, green and dry seeds and can be prepared in a wide range of recipes. Dry beans can be boiled and consumed, mashed with bananas or potatoes, or mixed with other cereal grains like maize and consumed as "Githeri" (Wortman *et al.,* 2008).

Poor crop establishment can also be attributed to variable moisture in the soil, excessive ambient soil temperatures (Wuebker *et al*., 2001). The low yields of beans in the ASALs are due to the interaction of moisture stress, soil fertility, pests, and diseases (Katungi *et al*., 2010). Bean production constraint is a problem that occurs within the season and among seasons with a 60% probability of occurrence (Katungi *et al*., 2010, Mungai *et al*., 2000). Besides, rainfall is low and variable within and among seasons (Kaggwa *et al*., 2011).

Water loss rates from the soil rate at 4-6 mm per day (Anon *et al*., 2009). Intermittent or mid-season rainfall gaps aggravate the moisture deficit, affecting bean development and yield during the growing period. Evapotranspiration (Beebe *et al*., 2013; Miller *et al*., 2003) together with lack of intrinsic Phosphorus (P) available for plants (Wortman *et al*., 2004) constitute major limitations of common bean production in these areas (Beebe *et al.*, 2013; Boko *et al*., 2007).

2.5 The Productivity and Importance of Green grams

Green grams (*Vigna radiata* L.) is a drought-tolerant, twinning herbaceous plant with several uses like green manure to improve soil fertility, as a pulse crop for human consumption, fodder legume, and a cover crop (Nyambati et al.,2009). It is a climbing or erect perennial herbaceous crop that grows up to one meter tall, with a long stem (Bradley, 2009). The crop tolerates acidic soils better than most legumes and does well in low fertility soils (Karachi, 1997). It is more tolerant to drought than beans and cowpeas despite these two being more preferred by most farmers (Amole et al., 2013).

Among the pulses, green gram *(Vigna radiata* L.) has recently become popular among smallholder farmers in the region, especially in the climatically marginal areas (Hargrave 2007; Purseglove, 2003). The crop serves as an alternative source of non-animal protein, as was the case in some parts of East Africa during the Rift Valley Fever outbreak. Besides, it is easily cooked and does not cause flatulence (Pursglove, 2003). Green gram is drought tolerant and gives reasonable yields with as little as 650 mm of rainfall (CBS Kenya Govt, 2003). Additionally, it is adapted to poor soils because it forms associations with mycorrhiza (Kasiamdari *et al*., 2002) and is a relay crop, hence plays an important role in environmental conservation and food security, respectively.

2.6 *Rhizobia* and Biological Nitrogen Fixation (BNF)

Rhizobia are defined as symbiotic bacteria capable of eliciting and invading root or stem tissues of leguminous plants, differentiated into N fixing bacteroids (Sahgal and Johri, 2003). They are rod-shaped bacteria, aerobic, and do not sporulate (Zakhia and Lajudie, 2001). Many soils are associated with most non-symbiotic *Rhizobia* in bulk and the rhizospheres of legumes and other plants (Saito *et al*., 1998). *Rhizobia* are also found as viable cells in water, where they can infect and nodulate aquatic legumes such as *Aeschynomene* sp. and *Sesbania* sp. (Wang and Martinez-Romero, 2000). Symbiotic *Rhizobial* strains elicit the formation of root and stem nodules on host legumes, which they colonize following a complex infection process that is still poorly understood (Maingi *et al*., 2006). The symbiotic and intracellular form of rhizobial cells is called the bacteroid, a differentiated cell type found within a membrane-bound compartment, the symbiosome (Oke and Long, 1999).

Legume N fixation starts with the formation of a nodule. The plant feeds the bacteria (Sprent *et al*., 1989). In legumes, small nodules can be seen 2-3 WAP, dictated by legume species and germination conditions (Maingi *et al.,* 2006). When nodules are young and not yet fixing N, they are usually inside are white or grey. When nodules become larger in size, they gradually turn pink or reddish, indicating N fixation has started. The pink or red color is caused by leghaemoglobin that controls oxygen flow to the bacteria (Maingi *et al.,* 2006). In favorable conditions, perennial crop nodules will fix N through the entire growing season (Wagner, 2012). Most of the nodules will be centered on the taproot. Annual legumes nodules like beans, green grams, cowpeas, among others, are round and big like a pea. Nodules on annuals are short-lived and will be replaced constantly during the growing season (Wagner, 2012). When the legumes are producing pods, the root nodules develop a deficiency to continue fixing Nitrogen as the attention is focused on the seed generation. The Legume root nodules become in active and the plant begins getting rid of them. Pink or red nodules should predominate on a legume in the middle of the growing season. If White, grey, or green nodules predominate, little N fixation occurs (Wagner, 2012).

2.7 Legume-*Rhizobium* symbiosis

Among plant-microbe interactions, the legume-*Rhizobium* symbiosis forms a unique system (Maingi *et al.,* 2006). This symbiotic relationship leads to root nodule formation in the plant host. Through symbiosis the host plant gains a continuous supply of Nitrogen from *Rhizobia,* and the *Rhizobia,* in return, acquire nutrients from plant (Sprent *et al.,* 1989). In many legumes, the development of root nodules begins with the root hair *Rhizobial* infection (Dart, 1977). *Rhizobia* identify their suitable legume host through energy-rich nutrients released by the roots (Young and Johnson, 1989) and are linked to a root hair by the host plant proteins called lectins. These proteins bind polysaccharides present on the cell surface of *Rhizobia* species. Lectins and polysaccharides are involved in the recognition process (Sprent and Faria, 1988).

Infection proceeds via an infection thread in which the *Rhizobia* penetrate to the cortex's cells of the host root. The infected cortical cells increase in size and divide to form a ball surrounded by uninfected cells and an outer fibrous layer (Sprent and Sprent, 1990). Within the infected cells, the *Rhizobia* differentiate into bacteroids, which always remain confined in vesicles bound by the host plant (Bauer, 1981). Different nodule structures are formed on different plants' infection, varying from cylindrical to spherical and from annular to irregular. Two broad classes are recognized as determinate and indeterminate nodules (Sprent, 1980). Determinate nodules do not have persistent meristems, the vascular system becomes more or less closed, investing the nodule in a continuous system of vascular traces, and little or no involvement of infection threads in the distribution of bacteria to the nodule cells (Bauer, 1981).

Nodules tend to be spherical. Indeterminate nodules have persistent meristems with an open vascular system. Growth occurs at the distal end of the nodule by cell division. Infection threads are major mechanisms for distributing bacteria to the nodule cells (Sprent, 1980). A typical indeterminate nodule tends to be branched and cylindrical initiating new growth from the old nodules' tips. This type of nodule occurs in peas, clover, and alfalfa (Bergersen, 1982). Within a mature and functional nodule, several unique proteins are produced. Two of these are nitrogenase and leghaemoglobin. Nitrogenase is responsible for reducing Nitrogen to ammonia, while leghaemoglobin serves to maintain a rapid flux of oxygen at low concentrations necessary to avoid oxygen inactivation of nitrogenase (Appleby, 1984).

The N fixation is an energy-demanding process (Vance *et al*., 1991) and may require up to 25% of the plant’s net photosynthesis (Minchin *et al*., 1981). The basic structure of the nitrogenase enzyme complex consists of a molybdenum-iron protein and an iron protein. This general structure is common to all N fixing organisms (Sprent and Sprent, 1990). Legume-*Rhizobium* symbiosis provides N through N fixation for the legume and the subsequent crop, as residues returned to the soil are rich in N and are therefore readily mineralized (Hornetz, 1995). If a legume is grown in association with another crop, commonly a cereal, the N nutrition of the associated crop may be improved, either by direct N transfer from the legume to the cereal or by a simple sparing of the available soil N (Zahran, 1999). The legume uses fixed atmospheric N rather than the soil mineral N, which can be exploited by the companion crop. Therefore, productivity is potentially enhanced by adding a legume in the cropping system (Gachimbi *et al.,* 2002).

2.8 Nitrogen fixation efficiency and nitrogen fertilization

Approximately 80% of air is nitrogen gas (N_2) (Garrison, 2006). Microorganisms and plants can wilt due to N deficiency (Vessey *et al*., 2005). Living things use the ammonia form of Nitrogen to manufacture amino acids, nucleic acids, and other N-containing components necessary for life (Chemining'wa *et al.,* 2006; Walley *et al.,* 2007). BNF is the process that changes Nitrogen to biologically useful ammonia (Mburu and Gitari, 2006). When the bacteria die, they release N to the environment or are used through symbiosis (Katungi *et al*., 2010).

The bacteria live in the nodules of legumes and other plants where the two symbiotically benefit (Woomer *et al.,* 1997). BNF can take many forms in nature, including lichens and free-living soil bacteria (Mburu and Gitari, 2006).with the exception of paddy rice, N fixation releases more nutrients to the ecosystem, not to cropping systems (Maingi *et al.,* 2006). For sustainability to be achieved in any cropping system, the replacement of soil mineral nutrients that are removed or lost is of paramount importance (Gachimbi *et al.,* 2002 The removal of plant material and its constituent minerals at harvest is generally one of the largest single factor contributing to the decline in soil fertility (Okalebo *et al.,* 2006).

A large proportion of the N accumulated during the growth of legume crops are removed with the harvested seed, and it is commonly concluded that the net return of fixed N to the soil is likely to be small when the amounts of N fixed by the legumes have been compared with the amounts removed in the seed. For instance, approximately 80 kg N ha^{-1} is removed in the grain of maize grown in the USA (Hauck, 1990), and between 100 and 160 kg N ha^{-1} is removed in the grain of winter wheat in the Netherlands (Dilz, 1988). Therefore, a continued supply of N is fundamental to any cropping system (Sangakara *et al.,* 2003).

A high amount of N input is reported when N fixing systems are used as high as 320-360 kg N ha^{-1} (Ladha *et al*., 1999). Among symbiotic N fixing systems, nodulated legumes have been used in cropping systems for centuries. They can serve several purposes in sustainable agriculture (Sangakara *et al.*, 2003). Symbiotic N fixation is the primary pathway by which inorganic N is made available for living organisms (Shah and Emerich, 2006). Effective symbiosis can only be achieved when the nodules are formed by efficient and effective *Rhizobia* (Sprent, 2001; Giller, 2001; Shah and Emerich, 2006). The term symbiotic effectiveness is used to describe the ability of a nodulated legume to fix N, and this can be expressed qualitatively (as high, moderate, or ineffective) or quantitatively (total N, shoot, or nodule dry weight) (Simms *et al*., 2002). Quantitative symbiotic effectiveness is measured by comparing standard *Rhizobia* strains' performance regarding the legume receiving adequate mineral N, or with non-inoculated legumes.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental sites description

The field experiment was carried out in Kwa-Mulungu farm situated in Kitui County between January- March 2016 (first season) and June to August 2016 (second season). Kwa Mulungu farm is located at latitude 1^0 21’45.78” S, longitude 37^0 52'48.18" E, and altitude 1105m above sea level (Meteorological department Kitui, 2002).

As an ASAL, Kitui County is a drought-vulnerable region in Kenya with an annual rainfall of 500 – 1050 mm and 40% reliability (GOK, 2010). The annual mean minimum temperatures range from 22 – 28ºC, while the annual mean maximum temperatures range from 28 – 32º C (GOK, 2010). The area is semi-arid under AEZ IV with very erratic and unreliable rainfall. The rainfall pattern is bimodal, ‘long rains’ fall in April-May; the ‘short rains' last from October - December and are reliable. (Hoogmoed, 2007).

Failure of rains fall causes food shortage and long drought periods. Indeed rains completely fail at least once a year every four years (Thomas, 1999). The major soil type of the area is lixisols (red soils), with alluvial deposits (Fluvisols) occurring in isolated patches along rivers and on hill slopes (Pauw *et al.,* 2008). The soils are generally poorly drained and easily eroded by runoff (Borst and De Haas, 2006).

A greenhouse experiment was carried out at Kenya Agricultural and Livestock Research Organization (KALRO) sub-station at Kitui County located at latitude 10 36'48.19" S, longitude 380 43'37.86" E, and altitude 1148m above sea level (Management Hand Book Ministry of Agriculture, 2010).

3.2 Selected Legume varieties

The experiment comprised of four legume varieties, namely: KAT 56 (Beans), K80 (Cowpeas), 1001(Lablab), and KS20 (Green grams). They were researched and developed from KALRO in Machakos and adapted well in ASAL field conditions (Karanja, 2006).

3.3. Field experiment

The field with no history of legume growth and which had not been fallowed for long was selected to ensure crops grew in a field free from pests and diseases, according to Lenne (2000). It was then cleared of grasses and other prevalent weeds using mechanical methods, followed by demarcation. Soil sampling was done, according to Zeeshan (2016). The fields were ploughed to a depth of 30 cm using oxen. To ensure high viability and quality, legume seeds to be planted were carefully sorted to increase uniform germination chances. Plots measuring 1.5 m x 4 m were marked out with a one-meter path between the plots. Recommended spacing of 45cm by 15 cm for each legume was followed in sowing.

Before sowing seeds into the plots, water field capacity was determined according to the procedure by Zhen-tao Cong *et al*., 2014. This capacity would be used as a reference when watering crops (Rodríguez-Iturbe and Porporato, 2004). Watering was done every day to maintain the moisture at field capacity.

The two seasonal field-based trials involved RCBD with DST induced through withholding total irrigation and WWT maintained as a control. Four blocks, each with four plots, were demarcated. The four legumes were randomly assigned to the plots and maintained under WWT. One month after planting (MAP), DST was randomly induced in two blocks until the legumes wilted while WWT was maintained in the other two blocks as controls. Once the project was terminated, nodules were harvested and taken to the laboratory for isolation, and rhizobium cultured was used to prepare an inoculant for specificity assays under greenhouse conditions.

3.4 Laboratory experiment

Roots were harvested, carefully, and thoroughly washed to remove soil. Ten nodules were collected from each plant using forceps to reduce the risk of damaging the nodule. The nodules were soaked in ethanol (95%) for 30 seconds and one minute in 6% sodium hypochlorite (Barrett *et al*., 2006). They were then rinsed four times in sterilized water and finally crushed with a flame-sterilized glass rod. The crushed nodule was streaked across the surface of a Petri dish containing YMA (Vincent, 1970) and incubated at 28^0C in the dark. Colonies that isolated well were placed on diagnostic media, adapted from Odee *et al*. (1997).

The incubated bacteria at 28^0C incubated in a shaker (220 rpm) were classified as alkaline, neutral or acidic. Petri dishes streaked with crushed nodules incubated at 28°C in the dark until the colonies were evaluated. They were characterized according to color (white, pink, translucid, yellow, or white with a pink center), the number of extracellular polysaccharides (EPS) production (none to moderate or moderate to copious), and colony size (the colony diameter measured with a ruler, after 3, 6 and 8 days of incubation).

Replicates for each isolate were analyzed, with mean growth rate being used to separate different categories in three days (Odee *et al*., 1997): very fast (colonies $\geq$ 5 mm in diameter) fast (colonies $\geq$ 3 mm diameter) intermediate (colonies $\geq$ 3 mm diameter after six days of incubation) slow (colonies $\geq$ 3 mm diameter after eight days of incubation) and very slow (colonies $\leq$ 3 mm diameter after eight days of incubation).

3.5 Greenhouse experiment

Materials assembled for rhizobium specificity tests included fourteen (14) pots, vermiculite media for the pots, seeds from the four legumes, Petri-dishes for seed pre-germination, and nutrient solutions for watering in the greenhouse. Each pot was planted with three seeds per legume. A Completely Randomized Design was used because greenhouse conditions are homogeneous. The design included one pot for each legume inoculated with the rhizobia isolated from that specific legume; a pot for ALL four legumes but inoculated with *rhizobia* cultured from bean nodules; a pot for ALL four legumes but inoculated *rhizobia* cultured from Cowpea nodules; a pot for ALL four legumes but inoculated with *rhizobia* cultured from Green gram nodules and a pot for ALL four legumes but inoculated with *rhizobia* cultured from Lablab nodules. Two pots for ALL the four legumes that were non-inoculated to act as a control and four pots for standard inoculants for comparison.

The plants were maintained in the greenhouse for 45 days after inoculation (Ferraira and Hungria, 2002). The legumes were replenished with sterile N-free nutrient solution as required (Odee *et al.,* 1995). One week after germination, inoculation was done before the legumes could start forming root nodules. During the experiment, i.e., between 15 days to 45 days, plants were examined for differences in growth, vigor, and nodulation. At the end of the growth period, plants were removed from the rooting medium, and the presence or absence of nodules was noted.

3.6 Data collection and analysis

3.6.1 Days to seed germination

After land preparation, planting for the four legume genotypes was done simultaneously, and all blocks were given the same treatment of watering and drought stress as assigned above. Data on germination was taken for each plot by physically counting the number of seeds germinated in the morning and evening, and the results were tabulated, according to Timson (1965).

3.6.2 Nodule number per plant

Ten plants were randomly selected and carefully dug out from the two middle rows on the experiment's termination to avoid the border effect. The roots were then washed with water through a fine sieve to remove soil particles according to the procedure outlined by Geetha *et al.* (2012). The number of nodules on each plant was counted, and the average nodules per plant calculated.

3.6.3 Determination of dry shoot weight

Ten plants were randomly selected from the two middle rows on each side of the treatment plot and cut at the ground level for shoot dry matter determination at the experiment's termination. Total fresh shoot weight was measured using an electronic weighing balance. Plant materials were then put in brown envelopes and oven-dried at 65^0 C for 72 hours as outlined by Ping Huang (2016). The dry materials were weighed, and the shoot dry weight was recorded.

3.6.4 Leaf Area Index

Leaf area of the third, fifth, and seventh leaf of the ten legume plants selected from the experimental plot were measured and determined against their ground area.

Leaf surface Index = Leaf area /ground area m^2.

3.6.5 Wilting Index

Leaf wilting is a fundamental trait used in drought tolerance evaluation. Signs of wilting were observed after one week of stress. A visual assessment of wilting was done since leaf water potentials cannot be measured in dead leaves. The following visual characteristics were used according to Bettina *et al*. (2007).

Table 1.1: scoring scale for above ground symptoms for wilting.

Severity score	**Severity Rating**	**Visual characteristics**
1	Leaves green	*No signs of wilting* or drought stress
2	Leaves slightly wilted	*Slight leaf angle* changes but no folding, rolling, or changes in leaf surface structure
3	Leaves wilted *Strong*	*leaf angle change* or *protrusion of veins on the leaf surface* but no cell death
4	Leaves severely wilted	Very great change of leaf angle or protrusion of veins on the leaf surface with *beginning necrosis*
5	Yellowing leaves	*Most leaves necrotic*, and some young leaves still green near the midrib, leaf angles mostly near 0◦
6	Vegetative part dead	*ground parts dead*, no re-sprouting after re-watering at the end of the experiment

3.6.6 Pod number per plant and pod yield

Ten plants were randomly selected; pods from the legume plants were collected from the ten harvest plants and counted to obtain the number of pods per plant as outlined by FAO (2017). The pods harvested from ten plants were then averaged to obtain the pod yields in grams.

3.6.7 Grain yield per plot and mean hundred seed weight

After threshing the pods harvested in the harvest area of each treatment plot, the grains were weighed on an electronic balance, according to FAO (2017). A hundred seeds from each treatment were randomly picked and weighed. This was replicated three times, and the average 100-seed weight was determined.

3.7 Rhizobial Specificity and characterization

The root nodules preparation was cultured in Petri dishes and incubated at 28°C in the dark. The colonies were evaluated and were characterized according to color (white, pink, Translucid, yellow, or white with a pink center), and the colony diameter measured with a ruler after three, six, and eight days of incubation).

3.8 Data analysis

The data collected on nodule number per plant, shoot dry weight, leaf area index, wilting index, pod numbers, pod yield or weight, and grain yield were subjected to analysis of variance (ANOVA) using SAS (version 8.0) and Least Significant Difference (LSD) at $P \leq 0.05$ used to separate treatment means of effective treatments.

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CHAPTER FOUR

4.0 RESULTS

4.1 Soil analytical data

The soil's chemical characteristics in the farm were analyzed, and results tabulated and indicated in table 4.1.

Table 4.1 Soil chemical analysis for the farm.

Parameter	**Value**	**Class**
Soil pH	4.4	Adequate
Acidity me %	5.90	Adequate
Total nitrogen %	0.34	Adequate
Organic carbon %	0.62	Adequate
Phosphorus ppm	108.2	Adequate
Potassium me %	1.45	Adequate
Calcium me %	7.90	Adequate
Magnesium me %	4.25	Adequate
Manganese me %	1.2	Adequate
Copper ppm	3.8	Adequate
Iron ppm	35	Adequate
Zinc ppm	8.6	Adequate
Sodium me %	0.85	Adequate

ppm = parts per million; me% = metal percentage in the soil

4.2 Comparison of days to germination

The legumes varied significantly ($P \leq 0.5$) days to seed germination (Fig.4.1). About 27% of Cowpeas (K80) germinated on the 3rd day, while only 5% of Green grams (KS20), 0.5% of *Dolichos lablab* (1001), and no beans germinated by that day. On the 4th day, 93% of planted Cowpeas (K80) had germinated, while 66.2% of G/grams (KS20) and Beans (30.4%) had the lowest seeds that had germinated. Cowpeas took a shorter period of days to germinate, followed by Green grams, *Dolichos lablab*, and Beans in that order (Fig. 4.1).

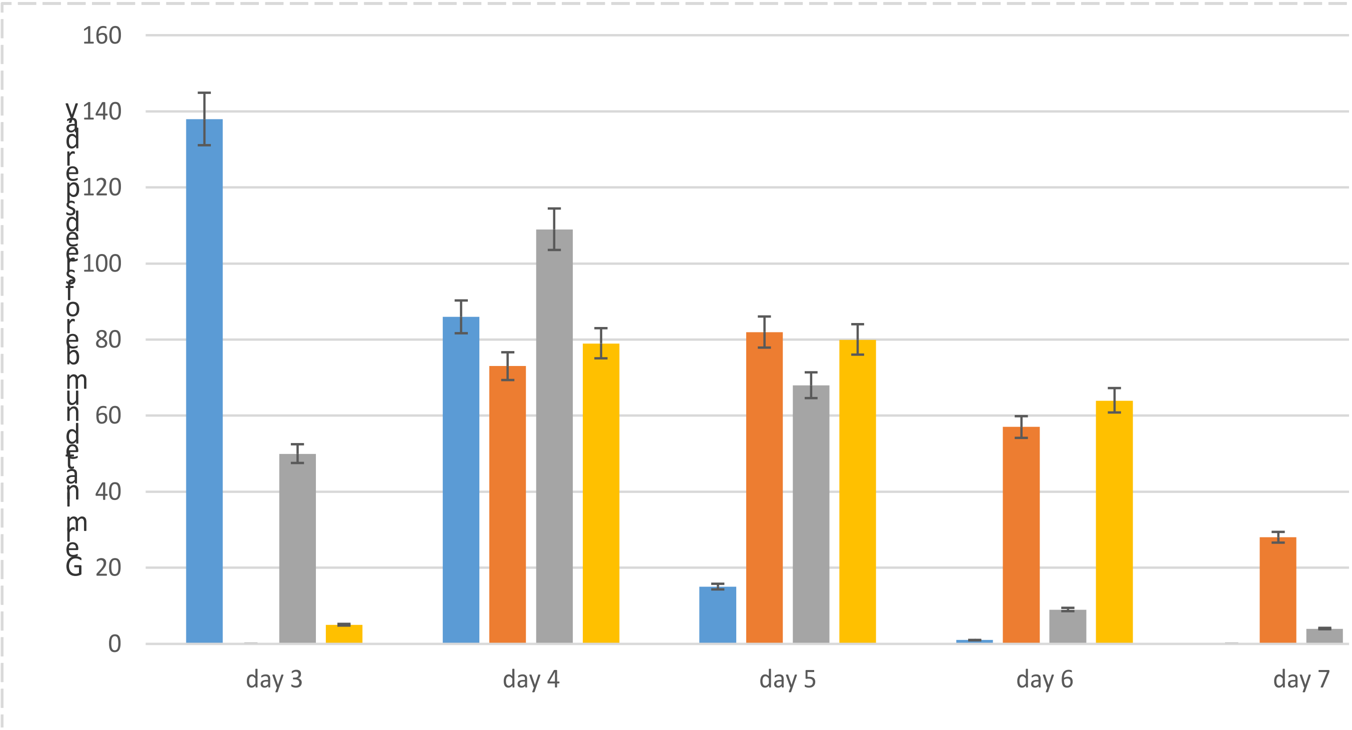

Figure 4.1 Mean germination of seeds in different legumes per day

4.3 Effects of water stress on leaf area index

The genotypes varied significantly (P≤0.05) for the leaf area index (Fig. 4.2 Appendix 1 & 11). Plants that were not stressed had a larger leaf area index compared to the plants which were stressed. Results indicated that beans and Dolichos had the largest leaf area index, followed by cowpeas and green grams in that order.

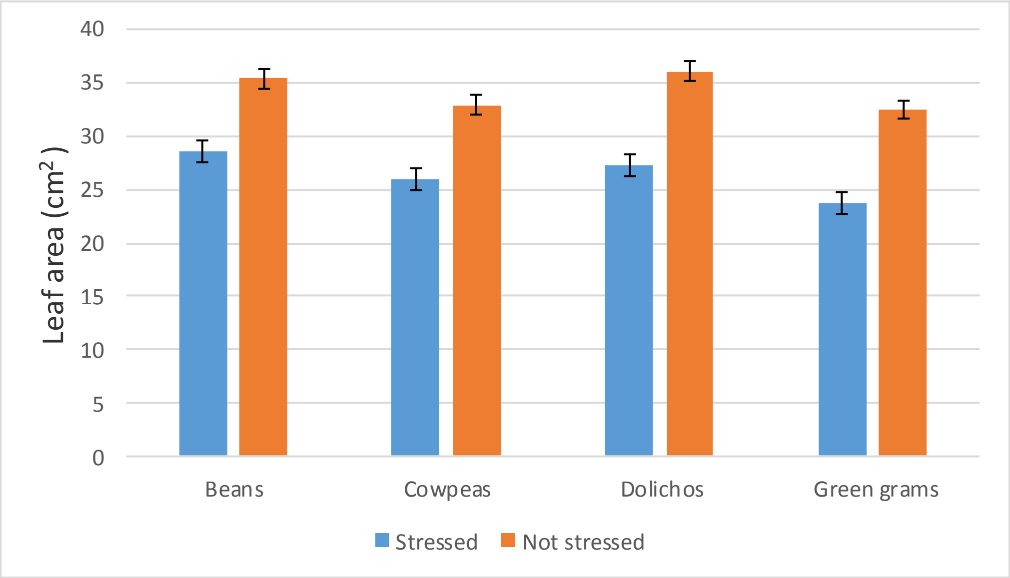

Figure 4.2 Mean leaf area index of stressed and unstressed legumes.

4.4 Effects of water stress on fresh biomass

The genotypes varied significantly (P≤0.05) in fresh biomass production (Fig. 4.3; Appendix 1V). Well-watered legume plants had higher new biomass production compared to water-stressed plants. Results showed that Green grams had the highest biomass production, cowpeas and Dolichos were not significantly (P≤0.05) different from each other. Beans had the lowest biomass production.

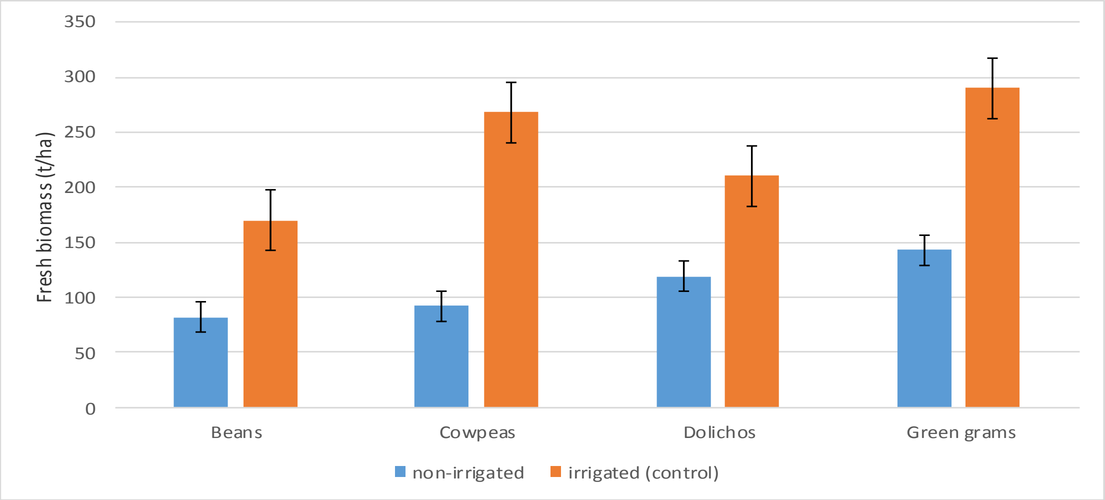

Figure 4.3 mean fresh biomass (t/ha) of legumes under non irrigated and irrigated treatments.

4.5 Effects of water stress on dry biomass production

There was a significant (P≤0.05) difference in the production of dry biomass by the genotypes (Fig. 4.4 Appendix III). Genotypes that were not water stressed produced significantly higher dry biomass than the genotypes, which were not water-stressed. Results indicated that Green grams and Dolichos had the highest dry biomass and were not significantly (P≤0.05) different from each other. Beans and cowpeas were not significantly (P≤0.05) different from each other, and their dry biomass production was lower than Green grams and Dolichos.

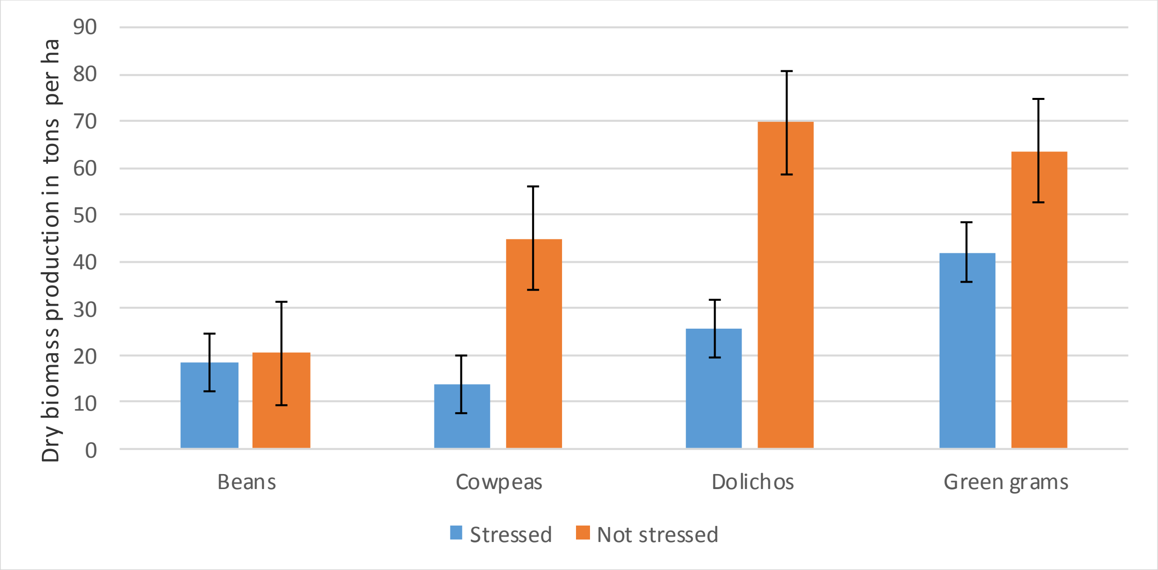

Figure 4.4 Mean dry biomass production per hectare.

4.6 Effects of water stress on number of root nodules

There was a significant (P≤0.05) difference between water-stressed plants versus well-watered plants in the number of root nodules in the genotypes' respective roots (Fig. 4.5; Appendix VI). Unstressed genotypes had significantly more root nodules compared to stressed genotypes. Overall results indicated that Green grams had the highest root nodules than other genotypes, followed by Dolichos, cowpeas, and beans, which were not significantly (P≤0.05) different from each other.

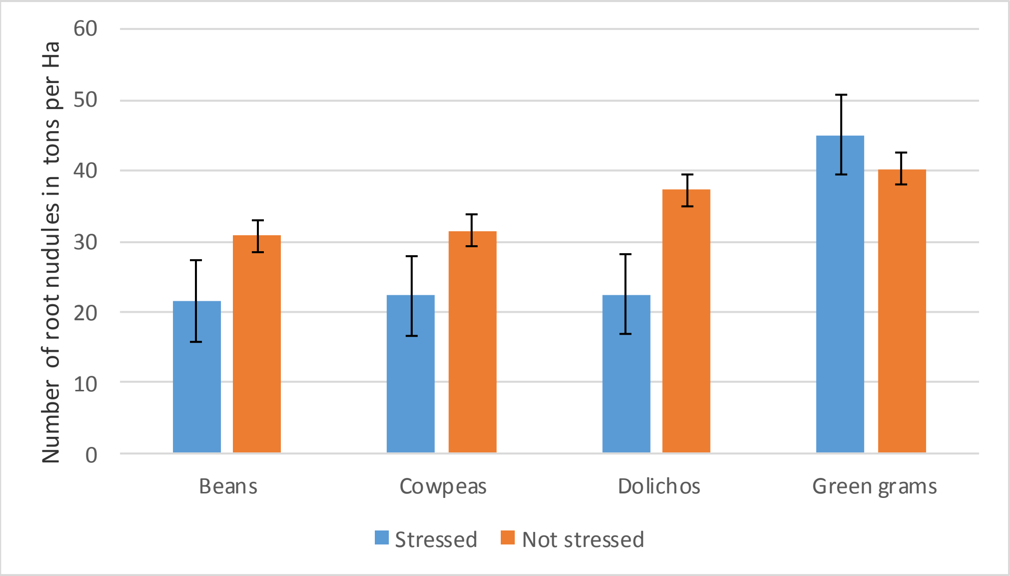

Figure 4.5 Mean number of root nodules in tons per ha

4.7 Effects of water stress on wilting index on legume varieties

Legumes varied significantly (P≤0.05) for leaf wilting. Overall, Beans and Cowpeas had the highest number of wilted leaves and were not significantly (P≤0.05) different from each other, followed by Dolichos and green grams in that order. The irrigated plants showed significant (P≤0.05) differences in the number of wilted leaves, with Beans having a significantly higher (P≤0.05) number of wilted leaves while green grams had the lowest number (Fig. 4.6). Those that were not water stressed showed significant differences in the number of wilted leaves, with cowpeas having the highest number of wilted leaves while green grams had the lowest number of leaves, which wilted (Fig. 4.6).

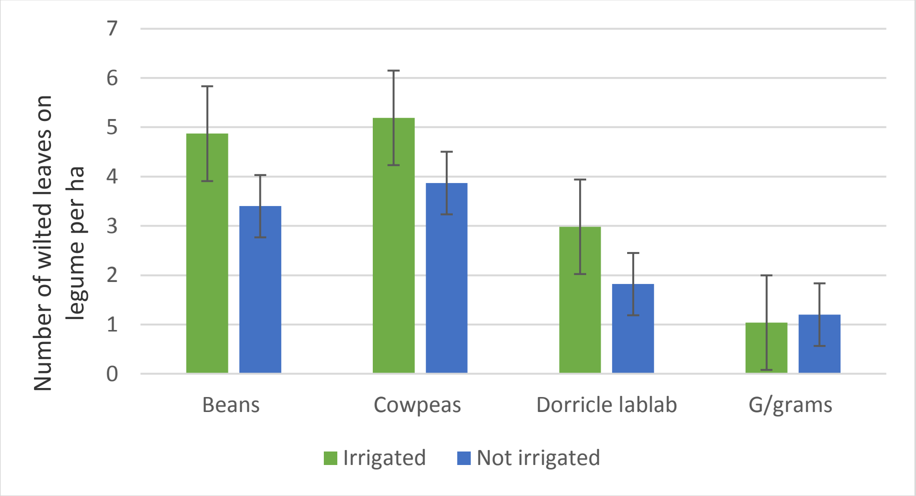

Figure 4.6 Mean number of wilted leaves on legume plants per hectare

4.8 Effects of water stress on number of pods on legume plants

Legumes varied significantly ($P \leq 0.05$) in the number of pods produced per treatment. Overall, Dolichos had the highest number of pods, followed by cowpeas and green grams, which were not significantly different from each other (Fig. 4.7). Beans had the least number of pods. In well-watered plants, Dolichos had the highest number of pods while Beans had the least pods. In the non-irrigated genotypes, Dolichos had the highest number of pods while beans had the least number of pods (Fig. 4.7).

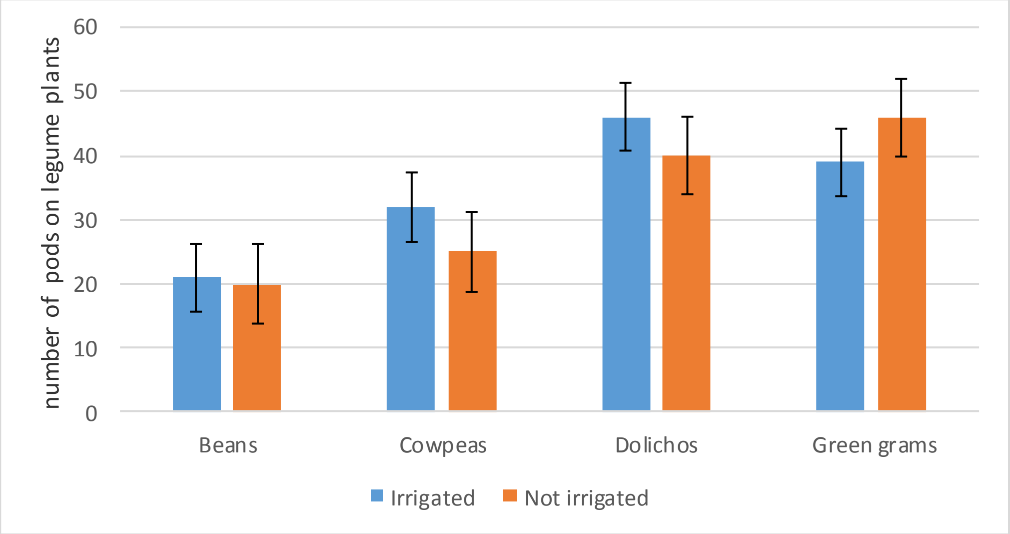

Figure 4.7 Mean number of pods on legume plants

4.9 Effect of water stress on grain yield

Cowpeas (K80) under irrigation produced significantly the highest yield (1.18 tons), followed by G/grams (KS20), Dolichos lablab (1001), and Beans (KAT 56) producing the lowest yields per plot (Fig. 4.8). Underwater stress plot, Green grams had the highest yield (1.2 tons), followed by Cowpeas (K80), Dolichos lablab (1001), and Beans (KAT 56) was significantly affected by water stress to give the lowest grain yield (Fig. 4.8).

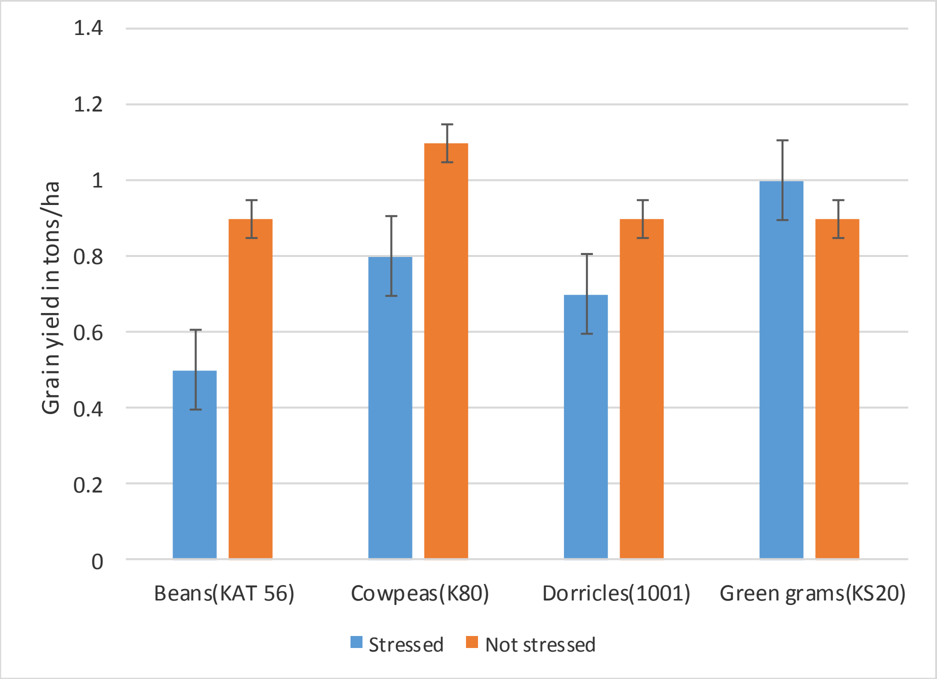

Figure 4.8 Mean grain yield.

4.10 Correlation between traits

Under irrigated treatment, there was a significant (P≤0.01) positive correlation between dry mass and fresh mass (r=0.917) (Table 4.2). Similarly, there was a positive correlation between root nodules and the number of pods per treatment, number of leaves, and pods. For example, DM positively correlated with FM(r=0.917), root nodules and number of pods (r=0.589), RN and WIX (r=0.349), RN and GYD (r=0.592), RN and LA5 (r=0.152) (Table 4.2). There was a negative correlation between leaf area and other variables (dry mass, fresh mass, number of pods, and root nodules. For example, leaf area and dry mass(r=-0.21), fresh mass(r=-0.247), number of pods(r=-0.179), root nodules (0.152) (Table 4.2).

Under non - irrigated treatment, there was a significant (P≤0.01) positive correlation between Dry mass and fresh mass (r=0.907) (Table 4.3). Similarly, there was a positive correlation between root nodules and the number of pods per treatment, number of leaves, and number of pods. For example, DM positively correlated with FM(r=0.907), root nodules and number of pods (r=0.597), RN and WIX (r=0.335), RN and GYD (r=0.587), RN and LA5 (r=0.136) (Table 4.3).

There was a negative correlation between leaf area and other variables(dry mass, fresh mass, number of pods, and root nodules).For example leaf area and dry mass(r=-0.18), fresh mass(r=-0.233), number of pods(r=-0.113), root nodules(0.136) (Table 4.3).

Table 4.2 Correlation between traits in plants under irrigated treatment.

	DM	FM	LA5	WIX	NOL	GYD	Pods	RN
DM	I							
FM	0.917*	1						
LA-5	-0.21	-0.247	1					
WIX	0.543**	0.286	0.154	1				
NOL	0.217	0.371	-0.133	0.253	1			
GYD	0.354	0.481	0.196	0.364	0.087**	1		
Pods	0.403	0.479	0.179	0.353	0.091**	0.532**	1	
RN	0.312	0.327	-0.152	0.349	0.099**	0.592**	0.589**	1

DM-dry mass, FM-fresh mass, LA5-Fifth leaf area, WIX-wilting index, NOL-number of leaves, GYD-grain yield, Pods, RN-Root nodules.

Table 4.3 Correlation between traits in plants under non- irrigated treatment.

	D.M.	FM	LA5	WIX	NOL	GYD	Pods	RN
DM	I							
FM	0.907*	1						
LA-5	-0.18	-0.233	1					
WIX	0.552**	0.302	0.123	1				
NOL	0.186	0.334	-0.113	0.242	1			
GYD	0.338	0.448	0.169	0.321	0.091**	1		
Pods	0.357	0.434	0.166	0.302	0.095**	0.529**	1	
RN	0.274	0.307	-0.136	0.335	0.098**	0.587**	0.597**	1

DM-dry mass, FM-fresh mass, LA5-Fifth leaf area, WIX-wilting index, NOL-number of leaves, GYD-grain yield, Pods, RN-Root nodules

4.11 Rhizobial Specificity and characterization

Table 4.4 Legume rhizobia characteristics

	Cowpeas	Dorricle	Green grams	Beans
SHAPE	Raised	Flat	Raised	Raised
COLOUR	Milky to watery translucent, few sides were pinkish, and big size was Congo red.			White opaque
TEXTURE	Soft	Soft	Soft	Soft
SIZE	Approx. 15 mm	Approx. 16 mm	Approx. 19 mm	Approx. 9 mm

CHAPTER FIVE

DISCUSSION

5.1 Comparison of germination (days) between seeds of different legumes.

The significant differences observed for days to germination in different legume species could be attributed to differences in their seed coats' permeability. This is in agreement with the recent study conducted by Mwami *et al*. (2017) as well as earlier studies by Baskin (2005) and Borji *et a*l. (2007), who attributed the differences in seed germination of different legumes to differences in seed coat permeability. The results deduced that cowpeas' seed coat was more permeable and thus took a shorter period to germinate than those of green grams, lablab, and beans. Fast germinators will take advantage of the available moisture, nutrients, and nitrogen flush besides reaching maturity early to evade moisture stress.

5.2 Effects of water stress on leaf area index.

The observed differences between the leaf area of well-watered plants and water-stressed plants could be due to water's role in the translocation of plants nutrients within the plant. This is in agreement with an earlier study by Gunton and Everson (1980) that attributed the differences in the leaf area of stressed and unstressed legumes to the nature of the environment, concerning access to water, where the genotypes grew. Some studies on the transport of recent assimilate under water stress within the plants from leaves to sink organs (Li *et al*.,2003) found the translocation of newly assimilated carbon from source leave to be delayed under severe water stress. Differences in leaf area among different legumes could be related to the legumes' genotypical differences, as observed by Baskin (2005).In the present study, under water-stressed conditions, dollicles leaves had the largest leaf area index, an indication that it had a larger surface area for photosynthesis and respiration, which lowers the atmospheric temperature around the leaf.

5.3 Effects of water stress on fresh and dry biomass.

Different quantities of biomass produced by legumes could be attributed to genotypical differences among the legumes. This is supported by a previous study by Asfaw (2014) and porch *et al*. (2009), who attributed differences in biomass production among bean varieties to legumes' inherent characteristics. Besides genetic characteristics, as a key factor for different biomass production, water could be cited as a factor determining the differences in biomass production, as it plays a role in nutrients mobilization, especially Nitrogen, which aids in biomass production. A study conducted by Mitova and Stancheva (2013) attributed differences in biomass production to water's role in mobilizing plants' nutrients. In this study, under droughted plants, Green grams had the highest volumes of fresh and dry biomass production, followed by cowpeas, dolichos, and deans had the lowest biomass production. This implies that green gram is likely to give better yields under water-stressed conditions because of high dry biomass as supported by Lee (2018) in his study, which indicated a higher positive correlation between biomass and yield of crops.

5.4 Effects of water stress on the number of leaves.

The different number of leaves between stressed and unstressed legumes could be linked to differences in translocation of organic compounds, which is necessitated by water. A study by Lazana *et al*. (2006) attributes differences between stressed bean genotypes and unstressed bean genotypes to differences in the plant's translocation of organic compounds. Genetic differences could be cited as a factor responsible for differences in the number of leaves produced by bean legumes. A study conducted by porch *et al*. (2009) reported genetic differences as responsible for differences in the number of leaves produced by legumes. Under water-stressed conditions, legumes had a lower number of leaves compared to those in well-watered conditions. Reduction of the number of leaves under water-stressed conditions can be explained as a mechanism for avoiding excessive transpiration or water conservation strategy as supported by Jones's (1992) studies.

5.5 Effects of water stress on the number of wilted leaves.

There was a significant difference in the number of wilted leaves between stressed and unstressed legume species. The observed trend could be because drought or water stress prevents water movement from the root zone to other plant parts. This concurs with previous studies (Hsiao 2000; Rahdari and Hoseini, 2012; Selvakumar *et al*., 2012), which observed that drought or water stress prevents the movement of water-soluble nutrients up the plant, and this leads to wilting of leaves. A similar study carried out by Jaleel *et al.* (2009) reported that wilting of leaves in legumes was due to water unavailability in the root zone. Differences in the number of wilted leaves among the legumes in water deficit environment and well-watered environment could be attributed to differences in some inherent and environmental factors leading to differences in their adaptations. This agrees with the study conducted by White (2005), who attributed the differences in the number of wilted leaves of bean genotypes to both water deficit and well-watered environment to their genetic pool. Under water-stressed conditions, Green grams had the least number of wilted leaves, implying that it had a higher photosynthetic area compared to other legumes and was drought tolerant. Beans had the highest wilted leaves, thus drought susceptible. Cowpeas had the highest number of wilted leaves in well-watered conditions, while green grams had the lowest number of leaves, which wilted, wilting reduces photosynthetic area according to Wang (2018).

5.6 Effects of water stress on root nodules.

The observed differences in the number of nodules among the stressed and unstressed legumes could be due to genotypical differences. This is supported by a previous study conducted by Jaleel *et al*. (2009), which indicated that the differences in root nodules were due to genotypical differences among the bean genotypes. The reduction in root nodules in stressed legumes could be due to reduced nitrogenase activity within the plants. This is in agreement with two studies (Guerin *et al.*, 1990; Kaur *et al.*, 1985) that reduced nodulation in the stressed legumes resulted from low nitrogenase activity, resulting from low leaf water potential in water-stressed legumes. Under drought stress, green grams had the highest root nodules while beans had the least, implying that green grams nodulated the most according to studies conducted by Janet and Sprent (1972), who noted a negative correlation between water stress nitrogen fixation and root nodules. Janet *et al.* (1972) indicated reduced water content in the root nodules reduced nitrogen fixation by legumes. This implies that green grams were drought tolerant due to high root nodules compared to beans susceptible to drought.

5.7 Effect of water stress on grain yield.

The observed differences between water deficit and well-watered bean legumes could be attributed to their genetic and environmental adaptability differences. This agrees with the previous studies conducted by Akcura (2011) and Ali and Shakor (2012), who cited genotype and environmental interaction as a key factor determining differences in yields from water-stressed and well-watered environments. Peymaninia *et al*. (2012) noted variation in yields between water-stressed and well-watered environments due to environmental variables such as soil water, which is essential in plants' nutrients movement. Under water-stressed conditions, green grams had the highest grain yield, while under well-watered conditions, cowpeas had the highest grain yield, with beans having the lowest grain yield. These differences agree with the latest study conducted by Jinpeng Li *et al.* (2018), who found out that irrigation improved grain yield by increasing the uptake and utilization of water and Nitrogen during grain filling.

5.8 Rhizobium specificity.

The trend observed in Rhizobium inoculation of legumes showed that the legumes were specific in picking the inoculant**.** The positive rhizobium specificity observed when inoculated with laboratory isolations could be attributed to legumes sharing genotypic characteristics. This concurs with an earlier study carried out by Michel *et al.* (2016) that reported that legume species that show high specificity in rhizobial symbionts have common genotypic characteristics. However, some legumes could not pick the inoculant when inoculated from a different legume species owing to legume-inoculant specificity. This concurs with Lupwayi *et al. (*2000), who attributed this to a lack of compatibility to genetic differences.

5.9 Correlation between traits.

Under water-stressed conditions, LAI increased with the legume's age, which can be attributed to the increase in nitrogen content in legume plant tissues. This concurs with Werner and Newton's (2005) findings, who found out that LAI increases with the increasing nitrogen absorption. Similar results were reported by Chaillou *et al.* (2003), who found out that LAI increases with the increase in nitrogen fertilizer application.

The differences in the number of pods among legumes under water-stressed conditions could be attributed to individual legume ability to fix Nitrogen even without fertilizer application to genotypical differences among the varieties. Akter *et al.* (2014). The increase in pods could be attributed to efficient absorption of Nitrogen coupled with trace elements, which promoted more photosynthates, leading to the production of more pods required for bean production. The study agrees with the findings of Schon and Blevins (1990); Reinbott and Blevins (1995); El-Abady *et* *al.*,(2008); Yildirim *et al*.,(2008); and Ranđelović (2009) who found out that increase in root nodules increases the number of pods per plant.

There was a reduction in the weight of seeds as legumes' yields increased under water stressed conditions. The relationship was inverse, probably due to substantial diversion of photoassimilates to the pods, which may affect mature seeds' weight. This study concurs with the study conducted by Schmitt *et al*. (2001) and Binford *et al*. (2004), who reported that application of Nitrogen to legumes has no significant effect on the weight of the 100-grain weight.

The significant differences in grain yields in different legumes under water-stressed conditions could be associated with genetic differences in nitrogen fixation in the legumes, which is in agreement with Akter *et al.*'s (2014) findings legumes fixed substantial Nitrogen from the soil. The increase in grain yield with the increasing foliar application rate could be because when legumes are given fertilizer, they respond to it rather than the fixed one. The higher number of pods was positively correlated with higher grain yield, in agreement with Akcura (2011) results. Ali and Shakor (2012) and Peymaninia *et al*. (2012) observed a positive correlation between the number of pods and grain yield.

Root nodules increased with biomass production by the legumes under water-stressed conditions. The increase in biomass production may be attributed to Nitrogen and micro nutrients' ability to stimulate vegetative biomass production. The results were supported by the study carried by Mitova and Stancheva (2013). They reported that biomass production is associated with an increase in Nitrogen concentration in plant tissues leading to increased vegetative growth among the bean varieties as a result of foliar fertilizer application. The results were also in agreement with the study conducted by Kushwaha (2001), Kamithi *et al*. (2009), who reported an increase in nitrogen uptake increases biomass production.

There was a significant difference in harvest index among the legumes. This could be attributed to the legume genotypes' differences in fixing the atmospheric Nitrogen through their root nodules Akter *et al.* (2014) and transferring the assimilates to grain relative to other plant parts. Legumes that had a high harvest index had low biomass and high grain yield. Harvest index was higher in legumes, which put photosynthates to grain rather than vegetative parts.

Under water-stressed conditions, a positive correlation was observed in the wilting index and root nodules. Legumes with higher root nodules had lesser wilted leaves implying they had higher leaf surface area for photosynthesis, therefore coping with strenuous conditions.

A significant positive correlation was observed in pod length and biomass, grain yield and biomass, number of pods and biomass, number of pods, and grain yield. This study agrees with earlier findings of Onder, 1994; Helvacýoglu and Sehirali, 2001, Yorgancilar *et al.*, (2003). These studies indicated that the bean's seed yield per hectare was positively correlated with the number of pods per plant, Helvacýoglu, and Sehirali, (2001); Yorgancilar et al., 2003). A strong positive correlation between grain yield and the number of pods was also reported in other studies conducted by Akcura (2011), Ali and Shakor (2012), and Peymaninia *et al*. (2012).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusions.

The study evaluated four legume species based on their ability to withstand water stress under normal environmental conditions in semi-arid Kitui County. The study showed significant variability among the legumes for most of the traits studied. Beans had a larger leaf area compared to other genotypes. On the biomass production aspect, green grams had the highest fresh and dry biomass compared to other genotypes. Root nodules number was highest in green grams while beans had the highest number of leaves that wilted. Based on the relationship between root nodules and other traits of bean genotypes, the study revealed a significant positive correlation between root nodules and the following selected traits under field conditions: dry and fresh biomass, root nodules, and the number of leaves and number of pods. A negative correlation was found between leaf area and dry mass, fresh mass and leaf area, number of pods and leaf area, root nodules, and leaf area.

Generally, green gram had the highest biomass, root nodules, and significantly high grain yield under water-stressed conditions and can be classified as 'drought tolerant.' In contrast, beans under similar conditions had the lowest biomass and root nodules and consequently lower yield and can be classified as 'drought susceptible.'

6.2 Recommendations.

1. Based on the findings of this study, green grams were identified as high yielding and drought tolerant legume that can be adopted or promoted for sustainable food production in Kitui County.
2. To increase food security in other ASALs of Kenya, rhizobium isolated from this green gram could also be cultured and potentially used as a bio-fertilizer to enhance yield.
3. There is a need to develop legume genotypes through seed breeding, which are ecologically adaptive to ASALs to increase food security in ASALs.

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APPENDICES

Appendix 1-Leaf area index analysis of variance for leaf 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	3	807.0	269.0	0.69	0.557
Treatment	1	842.2	842.2	2.18	0.142
Genotype.Treatment	3	10813.2	3604.4	9.31	<.001
Residual	152	58841.7	387.1		
Total	159	71304.0			

Appendix II-Leaf area index analysis of variance for leaf 5

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	3	25946.5	8648.8	15.26	<.001
Treatment	1	426.1	426.1	0.75	0.387
Genotype.Treatment	3	26660.5	8886.8	15.68	<.001
Residual	152	86173.9	566.9		
Total	159	139207.0			

Appendix III-Dry mass analysis of variance.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	3	1517.31	505.77	7.79	<.001
Treatment	1	112.90	112.90	1.74	0.189
Genotype.Treatment	3	314.70	104.90	1.62	0.188
Residual	152	9863.94	64.89		
Total	159	11808.84			

Appendix IV-Fresh mass analysis of variance.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	3	32771.2	10923.7	13.79	<.001
Treatment	1	6616.5	6616.5	8.35	0.004
Genotype.Treatment	3	9029.7	3009.9	3.80	0.012
Residual	152	120388.2	792.0		
Total	159	168805.5			

Appendix V-Number of leaves analysis of variance.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	3	984.319	328.106	48.36	<.001
Treatment	1	16.256	16.256	2.40	0.124
Genotype.Treatment	3	19.169	6.390	0.94	0.422
Residual	152	1031.350	6.785		
Total	159	2051.094			

Appendix VI-Root nodules analysis of variance.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	3	1696.82	565.61	16.12	<.001
Treatment	1	110.56	110.56	3.15	0.078
Genotype.Treatment	3	179.72	59.91	1.71	0.168
Residual	152	5334.15	35.09		
Total	159	7321.24			

Appendix VII-Pods analysis of variance.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	3	50030.72	16676.91	661.65	<.001
Treatment	1	2287.66	2287.66	90.76	<.001
Genotype. Treatment	3	12154.22	4051.41	160.74	<.001
Residual	152	3831.15	25.20		
Total	159	68303.74			

Appendix VIII-wilting index leaf analysis of variance.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Genotype	3	118.0688	39.3563	47.27	<.001
Treatment	1	31.5063	31.5063	37.84	<.001
Genotype. Treatment	3	20.3688	6.7896	8.16	<.001
Residual	152	126.5500	0.8326		
Total	159	296.4938			