2017-12

Morpho-physiological perfomance of common beans (phaseolus vulgarisl.) cultivars under rhizobial inoculation and water stress condition in Arusha, Tanzania

Vincent, Eutropia

NM-AIST

https://doi.org/10.58694/20.500.12479/266

MORPHO-PHYSIOLOGICAL PERFOMANCE OF COMMON BEANS (*PHASEOLUS VULGARIS* L.) CULTIVARS UNDER RHIZOBIAL INOCULATION AND WATER STRESS CONDITION IN ARUSHA, TANZANIA

Eutropia Vincent

A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Life Sciences of the Nelson Mandela African Institution of Science and Technology

Arusha, Tanzania

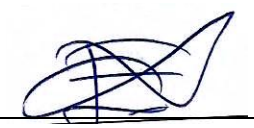
December, 2017

ABSTRACT

The aim of this study was to identify common bean cultivars which can grow/yield better under rhizobial inoculation and at limited water condition. To attain the goal, two seasons field experiment and one season screen house experiment were conducted at Agricultural Seed Agency (ASA) in Arusha Tanzania in the year 2014 /2015 and 2016. The experiment was a split-split plot with three replications, two levels of rhizobia, two stress levels and five cultivars of *P. vulgaris* (L.) (*KAT B9*, *KAT B1*, *F9 Kidney Selection*, *F8 Drought line* and *JESCA*). Stress periods of 10 days were imposed at vegetative and flowering stages of plant growth. Results showed that proline content was high in inoculated and water stressed plants. Concentrations of flavonoids and anthocyanins were higher in non-inoculated water stressed treatments. Leaf chlorophyll content, relative leaf water content and electrolyte leakage were higher in rhizobial inoculated and non-water stressed treatments. The nutrients uptake was higher in rhizobial inoculation and non-water stressed treatments. Rhizobial inoculation significantly increased growth parameters and seed yields while water stress significantly reduced growth parameters at both growth stages. *F8 Drought Line* and *JESCA* varieties significantly recorded higher proline content in field experiment and *KAT B1* in the screen house experiment. Varieties *F8 Drought line*, *JESCA* and *F9 Kidney Selection* significantly recorded higher flavonoids and anthocyanins content in both experiment. Leaf chlorophyll content was significantly higher in *F9 Kidney Selection* and *KAT B1* than in *F8 Drought Line* and *JESCA.* Cultivars *F9 Kidney Selection*, *F8 Drought Line* and *JESCA* had significantly higher relative leaf water content than other cultivars. However, varieties *KAT B9* and *KAT B1* significantly increased percentage in electrolyte leakage.Varieties *F9 Kidney Selection, F8 Drought Line* and *JESCA* significantly recorded higher uptake of N, P, K, Ca and Mg. Varieties *F9 Kidney Selection*, *F8 Drought Line* and *JESCA* had significantly superior measurements reflected in increased plant height, shoot and root dry weight and seed yields. Significant interactions were observed between rhizobial inoculation, water stress and bean varieties. Cultivars *F9 Kidney Selection*, *F8 Drought line, JESCA* and *KAT B1* showed highest level of tolerance against the water stress. With these observations, cultivars *F9 Kidney Selection, F8 Drought line, JESCA* and *KAT B1* can be promoted for production especially in drought prone areas.

DECLARATION

I, **Eutropia Vincent** do hereby declare to the Senate of Nelson Mandela African Institution of Science and Technology (NM-AIST) that this Dissertation is my own original research work and that it has never been submitted for degree award in any other institution.



Eutropia Vincent
The above declaration is confirmed

Supervisors;

19.12.2017

Prof. Patrick A. Ndakidemi **Date**

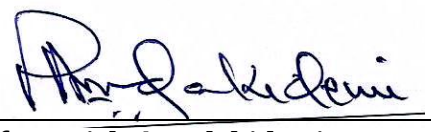
19.12.2017

Dr. Kelvin M. Mtei **Date**

COPYRIGHT

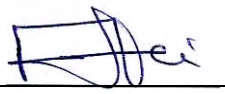
CERTIFICATION

The undersigned certify that they have read the dissertation titled **Morpho-Physiological Performance of Common Beans (P*haseolus vulgaris* L.) Cultivars under Rhizobial Inoculation and Water Stress Condition in Arusha, Tanzania** and recommend for examination in fulfilment of the requirements for the degree of Doctor of Philosophy of Life Sciences of the Nelson Mandela African Institution of Science and Technology.



19.12.2017

Prof. Patrick A. Ndakidemi **Date**



19.12.2017

Dr. Kelvin M. Mtei **Date**

ACKNOWLEDGEMENT

Foremost, I acknowledge the Government of Tanzania through Nelson Mandela African Institution of Science and Technology for supporting my study financially. Secondly, I would like to express my heartfelt gratitude to my supervisor's Prof. P.A. Ndakidemi and Dr. Kelvin M. Mtei for their supervision, direction and assistance stepwise in this academic ladder.

Furthermore, I also extend my sincere gratitude to Agricultural Seed Agency (ASA) farm in Arusha for providing me a research experimental site. My sincere gratitude also goes to Selian Agricultural Research Institute (SARI) for providing breeder seeds for my experiment. I also appreciate and thank field assistant, laboratory technicians and all my colleagues for their support and assistance during the time of my study.

My sincere gratitude goes to my Husband Deogratias, my daughter's Agnes and Daines, my sister Oliva and my young brother Michael for their prayer, patience and love during the time of my study.Words fail me to express my gratefulness to my father and mother for giving me life and become a foundation of my academic excellence.

It is a pleasure to thank those who made this journey possible in any respect during my study period; let wonderful blessings from the Almighty God come in your journey of victory.

DEDICATION

I dedicate this piece of work to my Almighty God in JESUS CHRIST who is the foundation of creature.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND SYMBOLS

ANOVA - Analysis of Variance

ASA-Arusha Seed Agency

BNF - Biological Nitrogen Fixation

Ca - Calcium

cm – Centimeter

CMS – Cell membrane stability

Ch – Chlorophyll

DW - Dry Weight

DNA –Deoxyribonucleic acid

E - East

EC - Electrical conductivity

EL – Electrolyte Leakage

g - Gram

Ha – Hactare

K - Potassium

kg – Kilogram

m.a. s. l – meter above sea level

m - Meter

MC - Moisture content

Mg - Magnesium

mg - Milligram

mm - Millimeter

N - Nitrogen

NM - AIST- Nelson Mandela African Institution of Science and Technology

OAT-ornithine - aminotransferase

PAL – Phenylalanine ammonia-lyase

P – Phosphorus

P5C-pyrroline-5-carboxylate

P5CR-pyrroline-5-carboxylate reductase

Pro – Proline

PDH - Proline dehydrogenase

RLWC- Relative leaf water content

RNA –Ribonucleic acid

ROS – Reactive Oxygen Species

S - South

SARI – Selian Agricultural Research Institute

SSA - Sub Saharan Africa

T - Treatment

CHAPTER ONE

Introduction

1.1 Background Information

Phaseolus vulgaris (L.) is a food legume that is consumed by many people world wide (Ramos *et al*., 1999). It is a fundamental source of minerals, proteins and vitamins, thus it is an alternative source of protein to meat and fish (Beebe *et al*., 2013). The usual intake of common beans has medicinal benefits which add to lower risks of some diseases such as cancer, diabetes and heart diseases (Tryphone *et al*., 2012). Apart from those important benefits of common bean water stress and nutrients limitations have been found to be the next to diseases as major constraints in bean production (Uddin *et al*., 2013).

Nitrogen (N) is among the most abundant elements on earth, however, it is the critical limiting element for growth of most plants due to its unavailability (Graham and Vance, 2000). Plants acquire N from two principal sources, (a) the soil, through commercial fertilizer, manure, and/or mineralization of organic matter; and (b) the atmosphere through symbiotic N_2 fixation. Primarily, N is necessary for the formation of amino acids which are building blocks of protein also aid for plant growth, which is triggered through cell division. It is an essential element in all living systems and needed by all cells and a major component of chlorophyll which converts sunlight into plant energy (Baligar, *et al*., 2001). N is very mobile and normally becomes available to plants in forms of NO_3^- (nitrate) or NH_4^+ (ammonium) ions (Marschner, 1995). It has been reported that nitrate is eagerly mobile in the xylem and can be stored in the vacuoles of roots, shoots and storage organs (Marschner, 1995). Nevertheless, ammonium has to be incorporated into organic compounds in the roots. Normally, leguminous crops meet up their N requirement through BNF which mainly depends on their proper growth, development and also leghemoglobin content of the root nodules (Serraj, 2003). *P. vulgaris* (L.) is a food legume that forms nodules with a range of rhizobial strains (Aguilar, *et al*., 2004). In a symbiotic association, legumes and bacteria contribute to each other and benefit as a result of their relationship (Redmon and Smith, 2004). However, the percentage of N-fixation percentage by *P.vulgaris* is lower than of other legumes (40-50%). Faba beans (*Vicia faba*) had around 75%, peas (*Pisum*

sativum) 70% and 95% with lupines (Werner, 1999). Therefore, rhizobial inoculation is an effective component in improving growth, yields, photosynthesis and plant nutrition in legumes.

On the other hand, water deficit is the most crucial constraint in agriculture (Xoconostle-Cazares *et al*., 2011), and this is attributed by rainfall fluctuation which causes in some part by climate change. Water deficit causes a severe physiological, biochemical and molecular changes in plants (Siddiqui *et al*., 2015). For example, process of photosynthesis in plants is usually inhibited by water stress and this occurs by changing pathway regulation of stomatal closure and lessening flow of CO_2 into mesophyll tissues and also weakening the activity of ribulose 1, 5-bisphosphate carboxylase/oxygenase (Cornic, 2000). It has also being reported that water stress in plant tend to distress some crucial process in plants for instance respiration, translocation, ion uptake, carbohydrates and nutrient assimilation (Farooq *et al*., 2008). During water stress reactive oxygen species (ROS) are normally accelerated, therefore, affects the metabolic response of plants hence death of cells (De Carvalho, 2008). From this condition, oxidation of multicellular components like proteins, lipids and nucleic acids (i.e. DNA & RNA) are greatly accelerated. Plant growth hormones such as abscisic acid, auxins and gibberellins play a critical function in plant growth and development and it mediate various environmental stress responses (Sah *et al*., 2016). Abscisic acid (ABA) is a stress hormone and is the fundamental controller of abiotic stresses (Wani and Kumar, 2015). For example, when environmental conditions are inconsiderate, the intensity of abscisic acid (ABA) in plant tissues is enhanced through abscisic acid (ABA) biosynthesis. The increased abscisic acid (ABA) usually attach to its receptor to commence signal transduction leading to cellular responses to stress (Sah *et al*., 2016). ABA also regulates different physiological processes ranging from stomatal opening towards protein storage and adjustment to several stresses like water stress/drought, salt and cold stress. (Ullah *et al*., 2012). For the plants to cope with water stress and increase the tolerance mechanism, they usually develop a defensive system and cellular pathway by accumulation of osmolytes such as proline, glycinebetaine and proteins together with other bioactive compounds such as phenolic acids (Ndakidemi and Dakora, 2003; Kavikishor *et al*., 2005; Tairo *et al*., 2017). Kumar *et al*. (2006), showed that plants exposed to water stress tend to develop a series of morphological and physiological adaptations, which confer tolerance to these stresses. The overview of physiological and biochemical changes in beans which is attributed by abiotic stress such as

water stress and/or drought together with improved N trough rhizobial inoculation is crucial component to be assessed in legumes particularly *P. vulgaris* (L.).

1.2 Problem Statement and Justification

Phaseolus vulgaris is an important food crop and a useful source of proteins especially in Sub-Saharan Africa. In Tanzania, common bean is an important food and cash crop, which is mostly grown by small-holder farmers. However, common bean production in Tanzania is low and does not meet the increasing demand (Mduruma *et al*., 1998). This is highly attributed to the soil moisture deficiency and nutrients limitation (Mduruma *et al*., 1998). Nutrient limitation results from either the unavailability of costly fertilizers or limited knowledge on their application. Nitrogen (N) is the key component of healthy growing of every plants, however is an expensive input in agriculture costing more than US$45 billion per year globally (Gyaneshwar *et al*., 2002). Supplying nitrogen to plants through biological nitrogen fixation has ecological and economic benefits (Ndakidemi *et al*., 2006) as it can fix up to 300 kg N ha^{-1}per year (Anjum, *et al*., 2006). The need for artificial N fertilizers can be supplemented by N_2 fixation resulting in an economy estimate of US$ 3 billion per crop season (Nicolás *et al*., 2006). In annual basis, the costs of production are usually reduced due to biological nitrogen fixation (BNF). Silva and Uchida (2000) demonstrated that field trials have shown the N captured by crops due to the use of rhizobia inoculants costing about $3.00/ha was equivalent to artificial N fertilizer costing $87.00. On the other hand, drought and/or water stress reduced common bean production in developing world in more than 60% (Ghanbari *et al*., 2013). As a result, the average global yield of beans remained low <900 kg ha^{-1} (Parida *et al*., 2007; Zadehbagheri, 2014). In Tanzania, bean yields are low ranging from 200 to 670 kg ha^{-1} and this is mostly due to unreliability of rainfall during the growing seasons (Mduruma *et al*., 1998). Water stresses may have great economic impacts in these regions, whereby, *P. vulgaris* (L.) is more prone and its poor adaptation to climatic stresses (Mouhouche *et al*., 1998).

Knowing the threat of climate change currently, it is therefore justifiable to find common bean cultivars which will survive better under low soil moisture conditions with improved N through rhizobial inoculants and therefore be promoted to farmers particularly in drought prone areas in relevant agro ecological zones in Tanzania.

1.3 Objectives

1.3.1 General Objective

The proposed study intends to identify *P.vulgaris* cultivars that grow and yield more under water stress and rhizobial inoculation.

1.3.2 Specific Objectives

i. To quantify foliar accumulation of proline in *Phaseolus vulgaris* in response to water stress and rhizobial inoculation

ii. To evaluate the accumulation of flavonoids and anthocyanins in selected common bean (*Phaseolus vulgaris* (L.) cultivars as influenced by water stress and rhizobial inoculation

iii. To determine chlorophyll content in *Phaseolus vulgaris* (L.) cultivars as influenced by water stress and rhizobial inoculation

iv. To examine relative leaf water content and electrolyte leakage in selected common bean cultivars (*Phaseolus vulgaris* (L.) as influenced by water stress and rhizobial inoculation

v. To assess nutrients uptake in *Phaseolus vulgaris* (L.) cultivars as influenced by water stress and rhizobial inoculation

vi. To determine growth components and seed yields in *Phaseolus vulgaris* (L.) as influenced by water stress and rhizobial inoculation

1.3.3 Research Questions

i. Which common bean cultivars will accumulate highest quantities of proline in their leaves as a result of rhizobial inoculation and water stress treatments?

ii. Do rhizobial inoculation and water stress treatments increase or decrease the quantities of flavonoids and anthocyanins in *Phaseolus vulgaris* (L.) cultivars?

iii. Which *P. vulgaris* (L.) cultivars accumulate the highest chlorophyll contents following rhizobial inoculation and water stress?

iv. How are relative leaf water contents and electrolyte leakage affected by water stress and rhizobial inoculation in *P.vulgaris* cultivars assessed?

v. Will the nutrient uptake in *P. vulgaris* (L.) cultivars be affected by rhizobial inoculation and water stress treatments?

vi. How are the growth components and seed yields of the *P. vulgaris* (L.) cultivars are affected by rhizobial inoculation and water stress treatments?

The specific objectives above will enable the identification of *P.vulgaris* cultivars tolerant to water stress at high N levels after rhizobial inoculation for drought prone areas.

1.3.4 Significance of the Study

The study will identify common bean cultivars that have the ability to accumulate high content of proline and secondary metabolites such as flavonoids and anthocyanin which have significant role in drought tolerant studies. Furthermore, the use of rhizobial inoculants will enhance nitrogen fixation and improve the capacity of plants to grow well under water stress.

CHAPTER TWO

Effects of water stress and rhizobial inoculation on physiological growth, mineral nutrition and accumulation of plant metabolites in *Phaseolus vulgaris*

Abstract

Water availability is one of the most essential factors that determine geographical distribution and productivity of plants. Common bean is a warm season crop requiring 90-120 days from planting to maturity. Positive performance of common bean in terms of yields and other growth factors is more dependent on adequate supply of water and in some part plant nutrients than any other single environmental factor. However, inadequate amount of moisture in the soil during the growing season affects the morpho-physiological mechanisms in plants and reduces its growth and development leading to hampered flower production and grain filling resulting to smaller and fewer seeds. Nitrogen fixation is of great deal as it may increase the world food supplies required to feed the rapidly increasing population and perhaps can substitute the expensive nitrogenous fertilizers mainly to smallholder farmers in Sub-Saharan Africa. The potential effects of water stress and rhizobial inoculation in legumes (mainly common beans) with respect to plant secondary compounds, chlorophyll formation and plant nutrition has been given consideration in this review.

Key words; Drought, Common beans, Inoculants, Phytochemicals, varieties

2.1 Introduction

Common bean [*Phaseolus vulgaris* (L.)] is the most important food legume in east and southern Africa (Beebe *et al*., 2011). Common bean has great potential for improving human nutrition due to its high protein content (Manjeru *et al*., 2007), and it is one of the food legumes eaten by many people around the world in different forms (Makunde and Pombi, 2004). In Tanzania, the majority of the smallholder farmers use seeds of the common bean as a major source of protein in cereal-based diets (Peters, 1993). According to Schwarz *et al*. (1996), *P.vulgaris* is one of the best non-meat sources of iron; provide 23-30% of the daily-recommended levels from a single serving. Symbiosis between legumes and nodulating bacteria is the main source of N in most of the cropping systems. Common bean has a tendency to associate with *Rhizobium* and fixes

atmospheric nitrogen in the soil (Manjeru *et al*., 2007). The fixation of atmospheric nitrogen into a usable form improves the soil nitrogen levels and ultimately reducing the costs of production. It has been established that the ability of legumes to form a symbiosis with soil rhizobia by fixing atmospheric nitrogen plays a significant role in a range of agro-ecosystems (Mortier *et al*., 2012). For example, Study by Massawe *et al*. (2017) on legumes and cereals intercrops and inoculated with *Rhizobium* showed the benefit from the nitrogen fixed by the companion leguminous crop. Furthermore, Rahman, (2013) report the improved soil structure and water holding capacity in *Rhizobium* inoculated legumes intercropping system with cereals.

Biologically fixed N_2 is regarded as a renewable resource that should form part of sustainable agro-ecosystems worldwide as it sustains crop productivity (Abd-Alla, 1992). Apart of beneficial response of beans, yet are less adapted to extreme environments of very low rainfall, high temperatures or low fertility acid soils as compared to other legumes such as cowpeas. White and Singh (1991) report that 62% of common bean grown in different regions of the world suffers from water stress at some stage of their growth. Study done by Fairbairn (1993) indicates that 93% of common bean growing areas, the physiological water requirements are not fulfilled and this reduces yields especially when water stress occurs during flowering stage. Common bean is cultivated largely by resource-poor farmers, often on soils that are deficient in important plant nutrients such as nitrogen (N) and phosphorus (P). Climatic and edaphic constraints cause severe yield losses given that heat and drought are widespread events that occur every year (Wortmann *et al*., 1998; Thung and Rao 1999).

Drought is a major factor affecting the growth and development of plants especially in developing world and has caused severe reductions in crop yields in many countries of the world (Beebe *et al*., 2013). It is a serious threat to agriculture as it limits the plants to take up water, which in turn reduces growth rate along with several metabolic changes (Munns, 2002). Its importance is likely to increase in response to the effect of global climate change and increased competition for water. The first signs of drought in plants are visible in leaves, which appear prematurely senescent, although earlier changes, both morphological and metabolic occur in roots (Ramirez-Vallejo and Kelly, 1998). These changes reflect, not merely a progressive reduction of water content in the plant, but qualitative and quantitative changes in its metabolism, suggesting a number of mechanisms by which plants can, within different limits,

tolerate drought and recover from its effects (Ramirez-Vallejo and Kelly, 1998). An essential aspect of the strategy to improve the yield of legumes in stressed environments must involve a combination of stress tolerant cultivars and nutrients (Mabrouk and Belhadj, 2012). On the other hand, plants generate chemicals which are primary and secondary metabolites for their survival and growth. The primary metabolites are substances produced by all plant cells that are directly involved in growth, development or reproduction such as sugars, proteins, amino acids and nucleic acids. The secondary metabolites are not directly involved in growth or reproduction but they are often involved with plant defense (Dixon and Paiva, 1995). These compounds usually belong to one of these three large chemical classes: terpenoids, phenolics and alkaloids and are highly activated as a response to various environmental stresses occurring in plants for instance heat, moisture stress and temperature (Ramakrishna and Ravishankar, 2011). Generally, moisture stress in plants alter 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 of plant water potential and other physiological parameters including plant height and leaf area (Baroowa and Gogoi, 2012). Therefore, this review focuses on the effects of water stress and rhizobial inoculation on physiological growth, mineral nutrition and accumulation of plant metabolites in legumes and other plants, with emphasis on *P vulgaris*.

2.1.1 Response of rhizobial inoculation and moisture deficiency in plant species

Leguminous crops meet up their N requirement through biological nitrogen fixation (BNF) which mainly depends on their proper growth, development and also leghemoglobin content of the root nodules (Serraj, 2003). However, water stress influences all aspects of nodulation and symbiotic N_2 fixation and in some cases reduces rhizobial survival and diversity in soil (Serraj *et al*., 1999). The effects of water stress on N_2 fixation generally have been perceived as a result of physiological responses acting on nitrogenase activity, which involves carbon shortage, oxygen limitation or feedback regulation by N accumulation (Serraj and Adu-Gyamfi, 2004).Water stress affects nodulation process and nodule activity than metabolism of plant shoots and roots (Shirtliffe, *et al*., 1996). However, the adaptability of microsymbiont to water stress varies between rhizobial strains (Shirtliffe, *et al*., 1996). Cell morphology in *Rhizobium meliloti* for example, became irregular and its growth rate slowed down as water potential of the growth media decreased from -0.15 to -1.5 MPa (Busse and Bottomley, 1989). On the other hand,

several species of rhizobia can survive under severe drought conditions by a range of adaptive strategies including production of chaperones and sugars, synthesis of stress enzyme 1-aminocyclopropane 1-carboxylic acid, production of oxopolysaccharides, production of low molecular weight organic compound like trehalose, phosphate solubilization and production of siderophores and phytohormones (Hussain *et al*., 2014). Nevertheless, other studies reveal that rhizobial strains are relatively resistant to soil dehydration and can stay alive in water films nearby soil particles (Serraj *et al*., 1999) as compared with host plants. Zahran *et al*. (1994) showed that subjecting rhizobia to osmotic stress resulted in modification of bacterial membrane lipopolysacharides, which are occupied in the *Rhizobium* host plant recognition process. The process of root hair infection by *Rhizobium* and the formation of infection threads have also been found to be seriously inhibited by water shortage (Graham, 1992). Therefore, characteristics of native rhizobial populations and the selection of strains for inoculation may be essential factor of N_2 fixation in water limited environment (Serraj, 2003).

The environmental conditions may affect the growth, proliferation, symbiotic process and nitrogen fixation by *Rhizobium* in association with leguminous plants. *Rhizobium*-legume response to different environmental stress is complex phenomena that require the intervention of many genetic and biochemical adaptation mechanisms which should be included in future studies. Hence, the better understanding of rhizobial physiological responses to different abiotic and biotic stresses factors is very important to improve crop production by harnessing biological nitrogen fixation process.

2.1.2 Influence of moisture stress and rhizobial inoculation on proline accumulation in legumes

In many developing countries, 20 % of the available protein is provided by beans (Beebe *et al*., 2010). Beans represent also an integral part of dietary protein for 50 % of the world's population (Beebe *et al*., 2010). An adequate N supplement is a key factor for growth and productivity in bean crops (Burns 1992; Mattson *et al*., 1991). Several functions have been proposed for proline accumulation as an adaptive response in plants; for example, proline may function as an organic osmolyte, a sink of energy and reducing power, N-storage compound, a hydroxy-radical scavenger, and a compatible solute that protects enzymes (Saradhi & Saradhi, 1991). Proline is a basic amino acid found in high percentage in basic protein, its non-toxic osmotic solutes which

stabilize the structures of macromolecules and organelles (Gadallah, 1999). In plants proline is synthesized by either glutamate or ornithine pathways in cytoplasm or mitochondria (Delauney *et al*., 1993). The first steps of the proline biosynthesis from glutamate are catalysed by a single bifunctional enzyme, 1-pryrroline-5-carboxylate synthestase (P5CS), which produces glutamic-semialdehyde (GSA). The GSA produced is spontaneously converted into pyrroline-5-carboxylate (P5C), which is then reduced by P5C reductase (P5CR) to proline (Zhang *et al*., 1995). Plants also synthesise proline from ornithine, by omithine - aminotransferase (OAT). If the amino group of ornithine is transaminated, the product would be keto amino valerate, which cyclizes to 1-pryrroline-2-carboxylate (P2C) and is then reduced to proline. Otherwise, trans amination of the amino group yields GSA, which is converted to proline via P5C (Delauney and Verma, 1993). Hare *et al*. (1999) reported that metabolism and accumulation of proline mainly depends on its degradation, which is catalysed primarily by the action of proline dehydrogenase enzyme (PDH). In *Prunus salicina* and *Lagerstroemia indica* Andersen *et al*. (1995) showed that there was a positive relationship between N availability and proline accumulation in these plants. Studies involving French bean showed that there was a decrease in proline contents in their roots and leaves as results of N deficiency (Sánchez, *et al.,* 2002). These were attributed by degradation of proline which was favoured by the stimulation of proline dehydrogenase (PDH). Other results by Dandekar and Uratsu (1988) indicated that under conditions of N deficiency, proline degradation produces glutamate, which is utilised as a nitrogenous source for the synthesis of other amino acids. However, under condition of sufficient N, proline level increase due to the action of ornithine, signifying majority of the ornithine pathway over the glutamine pathway, in addition to the inhibition of proline dehdrogenase activity (Sánchez, *et al.,* 2002). Elboutahiri *et al.,* (2010) reported that *Rhizobium* inoculated alfalfa had the highest leaf proline levels. Generally, N deficiency is characterized by a decrease in proline accumulation in plant tissues, essentially because the degradation of proline is favoured by the stimulation of proline dehdrogenase.

Cellular responses of plants as a result of oxidative and osmotic stresses in plants have been found to act as protection of cellular structures (Hare and Cress, 1997). Oxidative stress is caused by the intracellular accumulation of reactive oxygen species (ROS) and these stress signals may come from the environment, but can also be generated internally and may cause molecular damage to proteins, DNA and membranes of plants (Mager *et al*., 2000; Sharma *et al*., 2012).

Osmotic stress leads to efflux or influx of water from or into the cell resulting to hyper-osmotic stress which causes shrinking and hypo osmotic stress which causes swelling (Kotchoni and Bartels, 2003). The cellular responses to this type of stress deal with the activity of water channels (Mager *et al*., 2000). Biotic and abiotic stress such as water limitation in higher plants result in metabolic irregularities in plants, hence huge accumulation of amino acids for instance proline and glycine betaine content (Tatar and Gevrek, 2008). One of the most remarkable stress characteristics to measure physiological dryness in plant is the tremendous free proline accumulation (Kavikishor *et al*., 2005; Bates *et al*., 1973). Proline, among other amino acids is commonly produced in higher plants and generally accumulates in large extent in response to environmental stresses; for example, exposure of plant to harsh environment such as pathogen attack, heavy metal, salinity, cold and others result in the increment of free proline (Ashraf and Foolad, 2007; Szabados and Savoure, 2009). Proline has been found to act as a vital compatible osmolyte and osmoprotective compound performing as molecular chaperone in osmotic adjustment and protection of cellular structures of the plants (Sharma *et al*., 2012). A part of osmolyte for osmotic adjustment, it stabilizes sub cellular structures such as membrane and proteins and scavenging of free radicals (Matysik *et al*., 2002). It also contribute in alleviating cytoplasmic acidosis and maintaining appropriate $NADP^+$/NADPH ratios compatible with metabolism (Hare and Cress, 1997). According to Stewart (1981), proline does not hamper with normal biochemical reactions but allows the plants to survive under stress. Accumulation of proline might respond to stresses such as temperature, drought and starvation (Sankar *et al*., 2007). High levels of proline enable a plant to maintain low water potentials. By lowering water potentials the accumulation of compatible osmolytes involved in osmoregulation allows additional water to be taken up from the environment, thus buffering the immediate effect of water shortage within the organism (Kumar *et al*., 2006). Once plants accumulate proline in its plant tissues tends to reduce the toxic effects of ions in enzymes activity and also lowers the generation of free radicals formed by drought/water stress (Siddiqui *et al*., 2015). Hayat *et al*. (2012) report that proline is acting as hunter of reactive oxygen species (ROS) and diminish the damage of oxidative stress induced by water stress, heavy metal, salinity and other stresses. Studies reveal that proline perform as solute during stress, where an increase in the proline content would indicate resistance or tolerance to water deficit, serve as parameter for the assortment of highly resistant cultivars (Bates *et al*., 1973; Ashraf and Iram, 2005). In higher

plants, accumulated proline can have many other important functions, prevention of membrane disintegrations and enzyme inactivation in the environment of low water activity. Proline as a solute is widely distributed in plants greater than the other amino acids in the stressed plants; for example proline has found to be accumulated in different legumes, for example, *Glycine max* (L.) and *Phaseolus vulgaris* (L.) as a result of severe water stresses (Kapuya *et al*., 1995). The theory behind proline is therefore very useful to assess the physiological status and more generally to understand stress tolerance in plants species. However, the usefulness of physiological phenomenon is not enough exploited and perhaps not well understood as exceptional amino acids which is highly accumulated in plant cells under stressful environment. Practical understandings of proline as an organic solute accumulated in plant cells will be useful in plant improvement and adaptation in stress conditions.

2.1.3 Effects of moisture stress and rhizobial inoculation on the accumulation of Flavonoids and Anthocyanins in legumes

Plants produce a high diversity of natural products or secondary metabolites with a prominent function in the protection against various environmental stresses. Primary and secondary metabolism is both carried out by plant cell, whereby, primary metabolism entail synthesis of polysaccharides, proteins, lipids, RNA and DNA through utilization of sugars, amino acids, common fatty acids and nucleotides while secondary metabolism is stimulated only during particular stages of growth and development or during period of stress, limitation of nutrients or attack by micro-organisms or pathogens (Ndakidemi and Dakora, 2003; Shilpa *et al*., 2010). Secondary metabolites are more complex than primary metabolites and are generally derived from primary metabolites through modifications, for instance, methylation, hydroxylation and glycosylation (Ravishankar and Rao, 2000). Secondary metabolites are classified based on chemical structure (e.g. aromatic rings, sugar), composition (containing nitrogen or not) and their solubility in various solvents or the pathway by which they are synthesized. These metabolites have been categorized into terpenes (composed entirely of carbon and hydrogen), phenolics (composed of simple sugars, benzene rings, hydrogen and oxygen) and nitrogen and/or sulphur containing compounds (Shilpa *et al*., 2010).Various stresses have made higher plants to produce some bioactive compounds mainly secondary metabolites which facilitate the plant to interact with its environment for adaptation and defense (Ramakrishna and Ravishankar, 2011;

Ndakidemi and Dakora, 2003). Reports by Rao and Ravishankar (2002) and Varisree *et al*. (2004) pointed out that these bioactive compounds can serve important purposes such as acting as food additives, aroma as well as industrially fundamental pharmaceuticals. Besides, they have practical applications in medicinal, nutritive and plant stress physiology adaptation (Gupta *et al*., 2014). The accumulation of secondary metabolites usually depends on the physiological and developmental stage of the plant and usually consists of various signal molecules and they are produced in low quantity less than 1% dry weight (Shilpa *et al*., 2010).

Flavonoids are the most common and widely distributed class of plant phenolics consisting of 15 carbon atoms joined by linear carbon chain (Enyiukwu *et al*., 2014). They are pigments which play an essential function of coloring flowers, fruits and seeds in plants (Veberic *et al*., 2008). This type of secondary metabolites is widely distributed in plants and they are of six groups; flavonols, flavandiols, chalcones, flavones, anthocyanins and tannins (Veberic *et al*., 2008). Phenolic N containing compounds is extensively distributed secondary plant products and is generally derived from L-phenylalanine through nitrogen framework of cinnamate under phenyl propanoid metabolism (Razal *et al*., 1996). Flavonoids are usually synthesized using phenylalanine which may be affected by nitrogen metabolism. Under condition of low N, the level of Phenylalanine ammonia-lyase (PAL) activity increase hence enhances the accumulation of flavonoids (Kondorosi *et al*., 1995; Stewart *et al*., 2001; Mierziak *et al*., 2014). Study by Liu *et al*. (2010) in *C. morifolium* leaves showed that flavonoid concentrations were higher under low nitrogen supply, which implies that the activity of PAL (Phenylalanine ammonia-lyase) was abundant in the leaf of *C. morifolium*. It has been reported that N deficiency results in huge accumulation of secondary compounds mainly phenolics such as flavonols (Stewart *et al*., 2001) and anthocyanins (Chalker-Scott, 1999). For instance, Awad and Jager (2002) reported a decline in the concentration of flavonoids in the skin of apple as a result of nitrogen (N) supply. In *Labisia pumila* (sub- herbaceous plant), studies showed a significantly less production of phenolics under high N level (Ibrahim *et al*., 2011). It can be established that flavonoids metabolism in plants is highly favored in the presence of Phenylalanine ammonia-lyase (PAL) as a result of N deficiency.

Different roles have been proposed for secondary compounds accumulation as an adaptive response towards various stresses such as water stress and/or drought. Water stress is the most

widespread abiotic stress that affects plant growth and development, and usually occurs when the available water in the soil is reduced to a serious level and changing in atmospheric condition adds to the excessive loss of water (Zadehbagheri, 2014). Furthermore, high temperature, solar radiation, cold, drought and salinity increase the loss of water in plant depending on plant species (Adnane, *et al*., 2015). Report by Odjegb and Alokolaro (2013) showed that moisture stress causes oxidative stress and it has been reported to increase the amount of phytochemicals in some plant parts such as flavonoids and phenolic acids. Apart from that, flavonoids participate in stress responses in plant and also play an important role in plant growth and development, defense of plants against insect pests and diseases (Dixon and Steele, 1999; Ndakidemi and Dakora, 2003; Makoi and Ndakidemi, 2007; Kumar and Pandey, 2013). Biosynthesis of flavonoids is noticeable due to oxidative stress formed in plant, which inhibit generation of reactive oxygen species, absorb most of the energetic solar wavelengths and quench reactive oxygen species once they are formed in the plant cells (Kumar and Pandey, 2013). Flavonoids contain antioxidant ability of scavenging reactive oxygen species and, suppressing their formation by either inhibition of enzymes involved in free radical generation or up regulation of antioxidant defenses (Das and Roychoudhury, 2014). Current facts show that flavonoids as antioxidant are situated in the nucleus of mesophyll cells and at the centre of chloroplast where generated H_2O_2, singlet oxygen and hydroxyl radical can be easily quenched (Sharma *et al*., 2012). Flavonoids are recognized for their antioxidant and free radicals scavenging activities and they play a crucial role of preventing oxidative cell damage and also exhibit a high pesticidal activity (Okigbo *et al*., 2009). Anthocyanins are reported to accumulate under various stresses in plants such as drought and cold temperatures (Makoi, *et al*., 2010). Plant tissues having anthocyanins provide a number of functions for example thermoproctection, defense against insect pests and pathogen attack and are relatively resistant to drought (Chalker-Scott, 1999). For the plant to overcome the problem of stress when exceed, they tend to produce secondary metabolites as a defensive mechanism for survive (Ndakidemi and Dakora, 2003). The role of secondary compounds (i.e. flavonoids and anthocyanins) in legume growth in response to water stress and rhizobial inoculation needs further investigation to explore the biochemical and physiology responses into the ecosystems.

2.1.4 Effects of moisture stress and rhizobial inoculation on Chlorophyll contents in legumes

Chlorophyll is the main photosynthetic component of the chloroplast that determines photosynthetic rates (Shobhkhizi *et al*., 2014). Legumes play a fundamental role in agro ecosystems based on their ability to form a symbiosis with soil rhizobia that fix atmospheric nitrogen (Van Rhijn and Vanderleyden, 1995). Nitrogen is a major constituent of chlorophyll, the most essential pigment needed for photosynthesis and amino acids, the building blocks of proteins. It is also found in other bio molecules such as ATP and nucleic acids (Wood, *et al*., 1995; Wagner, 2012). Its deficiency impairs growth and it constitutes one of the major yield limiting factors for crop production decline. Nitrogen is highly needed for all enzymatic reactions in a plant, also is a major part of the chlorophyll molecules and plays a necessary role in photosynthesis and is a major component of several vitamins (Hokmalipour *et al*., 2011). Furthermore, in legumes and other leafy vegetables, N improves the quality and quantity of dry matter and protein (Uchida, 2000). Nitrogen supply has large effect on leaf growth because it increases the leaf area of plants and on that way, its influences on photosynthesis functional (Bojovi ć *et al*., 2009). However, green colour in the leaf is vanished due to nitrogen deficiency and this may cause the decrease in leaf area and intensity of photosynthesis as well (Chu *et al*., 2005). On the other hand, N can be supplied in plants through symbiotic fixing N_2 in legumes and ultimately increase growth and chlorophyll contents in plant leaves. Study done by Anjum *et al*. (2006) in mungbean showed that beneficial rhizobia bacteria influenced the physiological growth conditions of leguminous plants by increasing chlorophyll contents in leaves. It has also been reported that *Bradyrhizobium japonicum* inoculation increased growth and chlorophyll contents in soybean (*Glycine max* L.) (Tairo and Ndakidemi, 2013).

Moisture stress is another detrimental factor which slows down the photosynthesis of plants by damaging the photosynthetic apparatus and cause changes in chlorophyll content (Ommen *et al.* 1999). It has been reported that, drought stress destruct the thylakoid membrane of which photosynthesis and crop yields are being disturbed and this is described by both stomata and non-stomata factors (Anjum *et al*., 2011). Rate of photosynthesis in plants are normally decreased due to moisture stress and at the same time stomatal conductance are lowered in order to conserve water (Santos *et al*., 2006). Under condition of moisture stress in soil, the rate of CO_2

fixation is reduced along with photosynthetic rate resulting in less assimilate production for growth and yields in plants (Mafakheri *et al*., 2010). The decrease in chlorophyll under moisture stress is mainly the result of damage to chloroplasts caused by reactive oxygen species (ROS) (Verbruggen and Hermans, 2008). It has been reported that chlorophyll *a* and *b* are susceptible to soil dryness and results in changes of the ratio of chlorophyll *a* and *b* (Farooq *et al*., 2009; Farhad *et al*., 2011; Tourian *et al*., 2013). From physiological phenomena, leaf chlorophyll content is a unique entity with its own significant interest in plant. Studies revealed that water deficit results in negative impact in plants as majority of chlorophyll are being lost and it is normally occurring in mesophyll cells than in the bundle sheath (Anjum *et al*., 2011). Abu-Muriefah (2013) showed that water stress in common bean (*P.vulgaris* L.) impairs photosynthetic pigments in plant tissues, mainly shoot. Report by Massacci *et al*. (2008) showed reduction in chlorophyll content in drought stressed cotton. Kiani *et al*. (2008) and Farooq *et al*. (2009) observed reduction in tissue concentrations of chlorophylls in sunflower and other plant species under water deficit condition. Santos *et al*. (2006) found that in even moderate water stress condition the net photosynthetic rate decreased in common beans. Both stomatal and non-stomatal limitations are generally accepted to be the main factor of reduced photosynthesis under water stress condition (Chaves *et al*., 2002; Farooq *et al*., 2009). Water stress affects the growth, plant pigments and biomass yields in different plant species, however their tolerance mechanism vary significantly. The decline in photosynthesis observed under water stress could be attributed to stomatal factors, of which the concentration of CO_2 in chloroplasts decreases because of a reduction in stomatal conductance (Daniel *et al*., 2007; Gama *et al*., 2007). However, these can be avoided through several ways for instance stomatal closure, leaf rolling, reductions and consequently decreases in cellular expansion, osmotic adjustments and alterations of various essential physiological and biochemical processes that can affect growth, productivity and yield quality (Lawlor and Cornic, 2002; Abu-Muriefah, 2013). Water stress is a major factor which destructs the photosynthetic apparatus of many plants and cause changes in chlorophyll content. Furthermore, lack of compatible rhizobial strain in a particular legume plants may result into poor plant growth resulting in less chlorophyll formation and photosynthesis.

2.1.5 Relative leaf water content and electrolyte leakage as influenced by moisture stress and rhizobial inoculation in legumes

Relative leaf water content (RLWC) is a measure used to relate cellular water status in a plant (Kramer and Boyer, 1995; Lawlor and Cornic, 2002). RLWC provides an insight of assessing internal plant water status under drought conditions and has successfully been used to identify drought-resistant cultivars (Ghanbari *et al*., 2013). The measurement of RLWC under low soil moisture is of chief importance since high RLWC appears to be a common trait in drought resistant species as species which reveal limited changes in RLWC per unit reduction in water potential are often considered to be relatively drought resistant (Rahaman *et al*., 2000). Study done by Schonfeld *et al*. (1988) indicated that wheat cultivars having high RLWC were more resistant against drought stress. Furthermore, RLWC under well watered treatments in bean leaves was higher than in drought stress treatments (Ramos *et al*., 2003). Report by Lazacano-Ferrat and Lovat (1999) in the stem of bean plant showed that RWC was significantly lower in water stressed treatments as compared with the control supplied with adequate water. Blackman *et al*. (1995) recognized the decline of RLWC of plants subjected to water stress to damages to the cell including cleavage in the membrane and sedimentation of cytoplasm content. Genotypes with high relative water content under stress condition have the ability to retain more water in the leaves under stress. Therefore assessment of water status in crop plants is of fundamental importance under various environmental conditions.

Cell membrane stability is the ability of a plant to resist cellular membrane modification as a result of environmental stress such as drought (Turner *et al*., 2001). Drought stress damages the cell membranes, which leads to increased electrolyte leakage and results in cellular membrane dysfunction (Yordanov *et al*., 2003). Espevig, *et al*. (2012) suggested that increased solute leakage is attributed to the loss of membrane integrity by altering phospholipids and fatty acid composition and to the effect on membrane bound transport proteins, whereby these proteins play a significant role in preventing leakage. Study by Wu and Wallner (1993) showed that CMS is a rapid and sensitive method to evaluate drought tolerance in plants. For example, cell membrane stability was used to determine drought tolerance of 104 rice genotypes (Tripathy *et al*., 2000). It has been reported that cell membrane stability has also been used as a selection method for drought tolerance in grain sorghum (Sullivan *et al*., 1972). A study conducted to

determine the effect of induced drought on different growth and biochemical attributes of black gram (*Vigna mungo*) and green gram (V*igna radiata*) showed a considerable decrease in the membrane stability in the plants grown under drought stress condition as compared with the control plants for both the cultivars (Baroowa and Gogoi, 2012). Therefore, cell membrane stability can be assessed by measuring the cellular electrolyte leakage, in plant cells (Saneoka *et al*., 2004; Farooq and Azam, 2006; Manavalan *et al*., 2009).

Nitrogen plays a crucial important role for the formation of amino acids which is the building blocks of protein. It is also important for cell division and vital for plant growth (Uchida, 2000; Caliskan *et al*., 2008). The soil beneficial bacteria such as *Rhizobium* tend to liberate growth promoting substances which are phytohormones e.g. auxins as secondary compounds in inoculated plants. These phytohormones are known to play a key role in plant growth regulation by promoting root elongation and stimulation of leaf expansion, hence improved plant water relations for better cell membrane stability and relative leaf water content (Werner and Newton, 2005). Determination of water status in response to *Rhizobium* inoculation is very important to maximize yield and economic profitability of common bean production in a particular environment. Biological nitrogen fixation through *Rhizobium* increased the leaf water content in chick pea (Namvar *et al*., 2013), leaf water relations in *Agrostis palustrais* Huds (Saneoka *et al*., 2004), leaf water content in *Sophora davidii* seedling (Fuzhong *et al*., 2008) and in sunflower hybrid (Gholinezhad *et al*., 2009). Therefore, there is a need to assess the effects of water stress and *Rhizobium* inoculation on physiological parameters including cell membrane stability and relative water contents in *P. vulgaris* (L) plants.

2.1.6 Nutrients uptake in legumes as influenced by moisture stress and rhizobial inoculation

Moisture availability plays a major importance in the mineral nutrition of plants since most of the nutrients are dissolved in the water for plant uptake (Lipiec *et al*., 2013). For high rate plant growth and development, crucial nutrients such as carbon, hydrogen and oxygen which are normally supplied from the atmosphere and soil water should be in a required amount (Tairo and Ndakidemi, 2013). Essential elements such as nitrogen (N), phosphorus (P), calcium (Ca), magnesium (Mg), iron (Fe) and zinc (Zn) can either be supplied from soil minerals, organic materials or can be supplied through organic fertilizers (Nyoki and Ndakidemi, 2014). Studies

done by Ndakidemi *et al*. (2011) revealed that rhizobial inoculants considerably enhanced the uptake of Mn, Fe, Cu, Zn, B and Mo in all plant parts of the common bean plant. Another study by Tairo and Ndakidemi, (2014) using strain of *Bradyrhizobium japonicum* showed positive response on the uptake of macronutrients for instance N, P, K, Ca and Mg in roots, shoots, pods and whole plant of the soybean plant.

Water deficit is a major limitation to common bean production in many countries. Hose *et al*. (2001) reported that severity of the water deficit, affects the plant directly by dehydration and indirectly by reducing nutrient uptake. Mostly, common bean are cultivated in regions where water can cause reduction in growth and nutrient uptake, which ultimately reduce yields; therefore approximately, 60 % of common bean has been affected by drought globally (Garg, 2003; Zadehbagher, 2014). Reduced water availability under water stressed conditions inhibits the total nutrient uptake (Farooq *et al*., 2008). Water stress increases root shrinkage that consequently affects nutrient transport to the root surface due to reduced contact between root and soil (Yordanov *et al*., 2003). Reduced absorption of the inorganic nutrients can result from interference in nutrient uptake and lowered transpirational flow due to water stress (Garg, 2003; McWilliams, 2003).

Transpiration in plants is inhibited by drought, but this may not essentially affect nutrient uptake in a related way (Farooq *et al*., 2008). Influence of drought on plant nutrition may also be related to limited availability of energy for assimilation of NO_3^-/NH_4^+, PO_4^{3-} and SO_4^{2-} ions which must be converted through energy dependent processes before these ions can be used for growth and development of plants (Grossman and Takahashi, 2001). Ghanbari *et al*. (2011) noted that water stress normally results in reduced total nutrient uptake and usually reduces the levels of mineral nutrients in crops. It has been reported that water stress reduced the uptake of N, P and K in maize plants (Ali *et al*., 2008). N and K uptake was hampered under drought stress in cotton (McWilliams, 2003). In synopsis, drought stress reduces the availability, uptake, translocation and metabolism of nutrients. A reduced transpiration rate due to water deficit reduces the nutrient absorption and efficiency of their utilization. Although physiological mechanisms of water stress in relation to plant nutrient uptake are relatively understood, further studies are essential to determine the physiological basis of stress in respect to nutrient uptake in different

plant tissues and factors that modulate plant water stress responses for sufficient plant nutrition in plants.

2.1.7 Effects of moisture stress and rhizobial inoculation on growth and seed yields in legumes

Phaseolus vulgaris (L.) is the main essential food legume in east and southern Africa and mostly produced by resource-poor farmers. This crop is very vulnerable abiotic stresses such as water and low soil fertility (Miklas *et al*., 2006). Sufficient supply of nitrogen and water is necessary to attain high and potential yields in these crops (Wood *et al*., 1993). Availability of nitrogen in the soil is of fundamental importance as it increases the leaf area of the plants and as a result influences photosynthesis activity of the plants (Uchida, 2000). It has been reported that plant height in chick pea was increased as a results of nitrogen fertilizer application (Namvar *et al*., 2013). Beneficial soil bacterium (*Rhizobium*) through biological nitrogen fixation can stand as a source of N in plants and reduce the cost of production hence improve crops production (Tairo and Ndakidemi 2013). The inoculation of seeds with sufficient *Rhizobium* is known to enhance nodulation, nitrogen uptake, growth and yield parameters of legume crops (Sogut, 2006; Namvar, *et al*., 2011). Poor soil N is a major limiting factor for crop growth in most areas of the world (Fuzhong *et al*., 2008; Salvagiotti *et al*., 2008). Therefore, determination of growth parameters of common bean crop in response to *Rhizobium* inoculation is very important to maximize yield and economic profitability of common bean production in a particular environment.

Furthermore, of all the environmental factors limiting bean production, water deficit/stress plays a greater role in yield reduction in most of the crop producing areas (Teri *et al*., 1990).

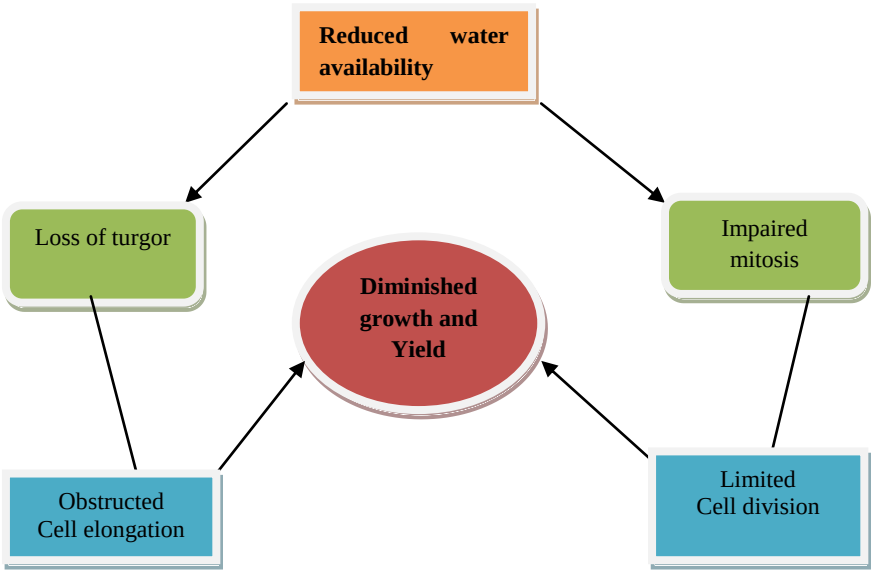


Figure 1: Description of possible mechanisms of growth reduction under water stress

Under water stress conditions, cell elongation in higher plants is inhibited by reduced turgor pressure. Reduced water uptake results in a decrease in tissue water contents; as a result turgor is lost. Likewise, water stress also trims down the photo assimilation and metabolites required for cell division.

As a consequence, impaired mitosis, cell elongation and expansion hence reduced growth (Kuhad *et al*., 1990; Ramirez-Vallejo and Kelly, 1998). Research evidence has established that both quality and quantity of beans are negatively affected by brief periods of water shortage (Beebe *et al*., 2013). Inadequate soil water during the early growth stages in bean plants results in seedling mortality, poor germination and hence reduced plant populations (Rao, 2001). Ghassemi-Golezani *et al*. (2009) reported that water deficit at reproductive stage in plant growth has a great adverse effect on dry matter and biomass yields which have a great implication on seed yields of a particular crop. Water stress occurring during flowering and grain filling periods is the most damaging factor in bean productivity as may cause excessive abortion of flowers and young pods (White *et al*., 1990). Furthermore, study shows that moderate to high water stress levels reduce biomass, days to maturity, number of seeds per pod, harvest index, seed yields and seed weight in common bean (Ramirez-Vallejo and Kelly, 1998). It has been reported that there is a strong relationship between total plant biomass and seed yields under stressed and un-stressed condition (Beebe *et al*., 2013). For instance, stress occurring during vegetative growth stages has little adverse effects on crop development and other yields components as compared

with the anthesis stage (Acosta-Gallegos and Kohashi-Shibata, 1989; Pena-Cabriales and Castellanos, 1993). It has been reported that moisture stress condition in *P.vulgaris* caused a reduction in yields during the reproductive growth stages by 58 - 87 % (Martínez *et al*., 2007). Studies show that in 93% of common bean growing areas, the physiological water requirements are not fulfilled and this affects yields especially when water stress occurs during the flowering stage (Ney *et al*., 1993; Sangakkara, 1994; Manjeru *et al*., 2007). Study done by Kuhad *et al*. (1990) showed that due to water stress, numbers of pods per plant were reduced five to seven fold compared with the change in the mean weight of the seed in mungbean. In the bean, De Malgalhaes *et al*. (1978) attained a 31% decline of number of pods per plant and only 18% for number of seed per plant due to water stress. However, Romic *et al*. (1994) applied supplementary irrigation to beans during the reproductive period and showed pod number to increase from 36 to 105%. Therefore, proper plant growth and development depends on the availability of water and sufficient nutrients in each of the growing stage. Furthermore, inadequate compatible rhizobial strain and water deficit results into poor plant growth and insufficient yields. There is a need to establish the effects of moisture and N stress on growth and yield of *P vulgaris* cultivars growing under these adverse environmental conditions.

Table 1: Economic yield reduction by drought stress in some crops

Crop	Drought imposed at (Growth stage)	Yield reduction
Barley	Seed filling	49-57%
Maize	Grain filling	79-81%
Maize	Reproductive	63-87%
Maize	Reproductive	70-47%
Maize	Vegetative	25-60%
Maize	Reproductive	32-92%
Rice	Reproductive (mild stress)	53-92%
Rice	Reproductive (severe stress)	48-94%
Rice	Grain filling (mild stress)	30-55%
Rice	Grain filling (severe stress)	60%
Rice	Reproductive	24-84%
Chickpea	Reproductive	45-69%
Pigeonpea	Reproductive	40-55%
Common beans	Reproductive	58-87%
Soybean	Reproductive	46-71%
Cowpea	Reproductive	11-60%
Sunflower	Reproductive	60%
Canola	Reproductive	30%
Potato	Flowering	11%

Source; Department of Agronomy, University of Agriculture, Faisalabad-38040, Pakistan, (*Journal of Agronomy*, 2008).

2.1.8 Conclusion

In conclusion, rhizobial inoculation is an effective component factor in improving growth, photosynthesis, yields and plant nutrition in legumes. However, its major role as an alternative to expensive nitrogenous fertilizers is not adequately investigated for improved productivity in various cropping systems involving legumes. On the other hand, water deficit reduces plant growth and development, leading to the production of smaller plant organs and hampered flower

production and grain filling. Plants use several physiological and biochemical processes at cell, tissue, organ and whole-plant levels at different stages of plant development to overcome stress conditions. This includes: accumulation of osmolytes such as proline and secondary compounds such as flavonoids and anthocyanins. The relative leaf water contents and cell membrane stability is another physiological mechanism to cope with stress in a range of plant species. Therefore, maintenance of water status and protection of cell membrane integrity and stability is one of the foremost mechanisms of protecting the plants against the water stress.

CHAPTER THREE

Influence of Water Stress and Rhizobial Inoculation on Accumulation of Proline in Selected Cultivars of *Phaseolus vulgaris* (L.)

Abstract

A two season field experiment and a single season screen house experiment were conducted to assess the effect of water stress periods and rhizobial inoculation in five (5) *P. vulgaris* (L.) cultivars. The experiment consisted of 2 levels of rhizobia (with and without inoculation), two stress levels (With and without water stress) and five cultivars of *P. vulgaris* (L.) (*KAT B9, KAT B1, F9 Kidney Selection, F8 Drought Line and JESCA*). The field experiment was conducted for two consecutive seasons, while the screen house study was done in a season. Results showed that proline content (μmol g^{-1}.FW) was higher in inoculated and water stressed treatments. Variety number 4 (*F8 Drought Line*) and 5 (*JESCA*) significantly recorded higher proline content in field experiment as compared to the rest. However, in the screen house experiment, variety 2 (*KAT B1*) and 4 (*F8 Drought Line*) significantly accumulated more proline than the other tested varieties. Significant interactive effects were also observed between inoculation, water stress periods and the tested *P. vulgaris* varieties.

Key words; Drought, Common bean, Inoculants, Varieties, Water

International Journal of Current Microbiology and Applied Sciences, **(2017) 6(3): 2205-2214**

3.1 Introduction

Proline is an organic osmolyte, N containing compound which stand as osmoprotection agent involved in reducing oxidative damage in plants by reducing free radicals (Matysik *et al.,* 2002; Tatar and Gevrek, 2008). Apart from acting as an osmolyte, proline accumulation has other important cell functions. Proline tends to act as N source in the cell under stress conditions, where the accumulation of this nitrogenous compound could be utilized as a form of stored N (Dandekar and Uratsu, 1988). Under condition of N deficit, proline accumulation in plants declines which implies that the degradation of proline is influenced by the stimulation of the enzyme proline dehydrogenase. However, under conditions of sufficient N, proline level increase due to the action of ornithine, signifying majority of the ornithine pathway over the glutamine

pathway, in addition to the inhibition of proline dehdrogenase activity (Sánchez, *et al.,* 2002). Elboutahiri *et al*. (2010) reported that *Rhizobium* inoculated alfalfa had the highest leaf proline levels. Generally, N deficiency is characterized by a decrease in proline accumulation in plant tissues, essentially because the degradation of proline is favoured by the stimulation of proline dehdrogenase. Proline in plant is synthesized mainly from glutamate (pyrroline-5-carboxylate (P5C), synthetase (P5CS) and P5C reductase (P5CR) and converted back into glutamate by proline dehydrogenase (PDH) and P5C dehydrogenase (Szabados and Savoure, 2009; Delauney and Verma, 1993; Kishor *et al.,* 2008). From the above background, inoculating legumes with appropriate rhizobial strain may result in more accumulation of proline in plant tissues and hence rendering them tolerant to water stress.

Abiotic stress conditions such as water limitation in higher plants result in the accumulation of plant osmolytes mainly proline and glycine betaine (Kavikishor *et al.,* 2005). Majority of plants accumulate compatible osmolytes like proline (Pro), glycine betaine and sugar alcohols, when they are exposed to water stress and/or drought (Tatar and Gevrek, 2008). Proline among other amino acids is commonly produced in higher plants and generally accumulates in large extent in response to environmental stresses (Ashraf and Foolad, 2007). Proline play a very important role in plants, a part of osmolyte for osmotic adjustment, it stabilize sub cellular structures such as membrane and proteins and scavenging free radicals (Matysik *et al.,* 2002; Tatar and Gevrek, 2008; Mafakheri *et al.,* 2010). It also contribute in alleviating cytoplasmic acidosis and maintaining appropriate NADP+/NADPH ratios compatible with metabolism (Hare and Cress, 1997). According to Stewart (1981), proline does not hamper with normal biochemical reactions but allows the plants to survive under stress. Studies have revealed that proline perform as solute during stress, where an increase in the proline content would indicate resistance or tolerance to water deficit, serve as parameter for the assortment of highly resistant cultivars (Bates *et al.,* 1973). For example, the proline content increased under drought stress in pea (Sanchez *et al.,* 1998). In higher plants, accumulated proline can have many other important functions, prevention of membrane disintegrations and enzyme inactivation in the environment of low water activity. Once plants accumulate proline in their tissues, the proline tends to reduce the toxic effects of ions in enzyme activity and also lowers the generation of free radicals formed by abiotic stresses (Siddiqui *et al.,* 2015). The theory behind proline is therefore very useful to assess the physiological status and more generally to understand stress tolerance in plants

species. Therefore the aim of this work is to assess the effects of water stress/drought among the five (5) common bean varieties as influenced by stress phases and rhizobial inoculation respectively.

3.2 Materials and Methods

3.2.1 Description of Site Location

The trial was conducted at Agricultural Seed Agency (ASA) farm in Arusha, located at latitude 3°18′S and longitude 36°38′06.29″E. ASA receives the mean annual rainfall of 819mm, mean temperature of 19.2°C with relative humidity of about 94% and altitude of 1520 m.a.s.l. The field trial was carried out during dry season of January to March 2014 and January to March, 2015 while the screen house experiment was carried out from mid January to March, 2016 under irrigation.

3.2.2 Experimental Design and Treatment Application

The experiment was designed in split split plot with 3 replications. The plot size was 3m x 4m. The field experimental treatments consisted of 2 levels of Rhizobia (with and without inoculation) as the main factor followed by imposing of stress (sub factor) in vegetative and flowering stages of plant growth. Five cultivars of *P. vulgaris* (L.) (*KAT B9, KAT B1, F9 Kidney Selection, F8 Drought Line* and *JESCA*) were assigned to sub-sub plots. The common bean seeds were sown at a spacing of 50 cm x 20 cm, making a plant population density of 200,000 plants per hectare. The BIOFIX legume inoculants were obtained from *MEA* Company Nairobi-Kenya, sold under license from the University of Nairobi. Common bean seeds lines and/or varieties *KAT B9*, *KAT B1*, *F9 Kidney Selection*, *F8 Drought Line* and *JESCA* were obtained from the breeding unit based at Selian Agricultural Research Institute (SARI), Arusha, Tanzania.

Land for field experiment was cleared and all the necessary practices like ploughing and harrowing were done before planting. Moreover, in the screen house experiment, wooden box technique was used to establish the experiment. This was done by collecting the same soil used at field experiment and beans were planted using the protocol developed by Agbicodo *et al.*, (2009) with some modifications. Common bean seeds were thoroughly mixed with *Rhizobium* inoculants to supply 10^9 cells g^{-1} seed, following procedure stipulated by products manufacturer.

To avoid contamination, all non-inoculated seeds were sown first, followed by inoculated seeds. Three seeds were sown and thinned to two plants per hill after full plant establishment. Stress period of 10 days were imposed at vegetative and flowering stages of plant growth by not irrigating.

3.2.3 Plant Harvest and Sample Preparation

Plant leaf samples from field and glasshouse experiments were collected for proline analysis. In the field experiment, 10 plants were randomly sampled from the middle rows of each plot while in the glasshouse experiment two plants from each pot were sampled. The fresh plant leaf samples from each of the growth stages (i.e. vegetative and flowering) were collected from the third young leaf from the top and kept in ice container to maintain their freshness for proline determination.

3.2.4 Determination of Proline Contents in Plant Leaves

Extraction of proline contents in plant leaves was done as described by Bates *et al*. (1973). Extract of 0.5g of plant material were homogenized in 10mL of 3% aqueous sulphosalicylic acid. The homogenate were filtered through Whatman No. 2 filter paper. The 2mL of filtrate were taken in a test tube and 2mL of glacial acetic acid were added followed by 2mL acid ninhydrin. The mixture was then heated in the boiling water bath for 1 hour. The reaction was then terminated by placing the tube in ice bath and 4mL of toluene was added to the reaction mixture and stirred well for 20 - 30 seconds. The toluene layer was separated and warmed to room temperature. The red color intensity was then measured at 520 nm using 2800 UV/Vis Spectrophotometer. A standard curve was developed and the amounts of proline in the test sample were obtained from the standard curve. The proline content on fresh-weight basis was calculated as follows; μmoles/gram tissues = [**(**μg proline/ml) × ml toluene)/115.5μg/μmole]/[(g. sample)/5]

3.2.5 Statistical Analysis

A 3-way ANOVA was used to analyze data collected. The analysis was done using STATISTICA software programe of 2013. Fisher's least significant difference was used to compare treatment means at $p = 0.05$ (Steel and Torrie, 1980).

3.3 Results and Discussion

3.3.1 Effect of inoculation with *Rhizobium* and stress periods on proline content in selected *P. vulgaris* (L.) varieties

Significance increase in proline content (µmol g^{-1}FW) was observed in inoculated compared with non-inoculated treatments (Table 2 & 3). Rhizobial inoculation significantly increased proline content during vegetative stage by 12% and 8% in season one and two respectively (Table 2). In screen house experiment, inoculation with *Rhizobium* strain increased the proline content by 34% in vegetative stage and 31% in flowering stage when compared with un inoculated treatments (Table 3). Water stress treatments significantly increased proline content by 35 and 39% in season one and by 33 and 48% in season two at vegetative and flowering stages respectively (Table 2). In the screen house experiment, water stress treatment increased the proline levels in plants by 36% and 49% during the flowering and vegetative phases (Table 3).

Table 2: Proline content (μmol g^{-1}FW) in *P. vulgaris* (L.) plant leaves as influenced by water stress periods and rhizobial inoculation in field experiment for two consecutive seasons

1st Season			**2nd Season**	
Growth Phases	Vegetative	Flowering	Vegetative	Flowering
Inoculation				
R+	4.39±0.31a	5.65±0.29a	4.96±0.25a	5.70±0.43a
R-	4.36±0.23a	4.95±0.28b	4.57±0.24b	5.65±0.55a
Stress Levels				
StrL1	3.45±0.15b	4.02±0.23b	3.81±0.11b	3.88±0.18b
StrL 2/StrL 3	5.30±0.25a	6.58±0.10a	5.72±0.22a	7.47±0.48a
Varieties				
Vrty 1	3.82±0.32c	4.80±0.53b	4.06±0.35b	4.58±0.57c
Vrty 2	3.63±0.38c	4.87±0.46b	4.42±0.30b	4.55±0.41c
Vrty 3	4.16±0.26bc	4.89±0.47b	4.32±0.25b	3.97±0.45c
Vrty 4	4.58±0.25b	6.12±0.35a	5.69±0.45a	6.43±0.90b
Vrty 5	5.69±0.59a	5.83±0.40a	5.32±0.37a	7.84±0.97a
3-Way Anova (F-Statistics)				
Rhz	0.02ns	17.24***	5.58*	0.02ns
StrL	67.67***	227.86***	135.86***	88.80***
Vrty	10.50***	10.80***	14.83***	11.33***
Rhz*StrL	0.004ns	2.23ns	0.87ns	1.54ns
Rhz*Vrty	1.03ns	0.87ns	0.35ns	0.38ns
StrL*Vrty	1.37ns	3.06*	4.15**	3.48*
Rhz*StrL*Vrty	2.11ns	1.50ns	0.34ns	0.52ns

+R: With *Rhizobium*, −R: Without *Rhizobium.* StrL 1: No water stress, StrL 2: Water stress at Vegetative Stage, StrL 3: Water stress at Flowering Stage. Vrty 1: *KAT B9*. Vrty 2: *KAT B1*. Vrty 3: *F9 Kidney Selection*. Vrty 4: *F8 Drought Line*. Vrty 5: *JESCA*. Values presented are means ± SE. *, **, *** = significant at $p \leq 0.05$, at $p \leq 0.01$ and at $p \leq 0.001$ respectively, ns = Not significant. Means followed by similar letter(s) in a given column are not significantly difference from each other at $p = 0.05$.

Table 3: Proline content (µmol g^{-1}FW) in *P. vulgaris* (L.) plant leaves as influenced by water stress periods and rhizobial inoculation in the screen house

Growth Phases	Vegetative	Flowering
Treatments inoculation		
R+	4.60±0.47a	5.20±0.49a
R-	3.03±0.43b	3.57±0.42b
Stress levels		
StrL 1	2.98±0.43b	2.98±0.43b
StrL 2/StrL 3	4.66±0.46a	5.79±0.41a
Varieties		
Vrty 1	2.62±0.71b	3.72±0.89a
Vrty 2	5.34±0.76a	5.13±0.61a
Vrty 3	2.99±0.67b	4.69±0.74a
Vrty 4	4.08±0.70ab	4.09±0.77a
Vrty 5	4.06±0.71ab	4.27±0.73a
3-Way Anova (F-Statistics)		
Rhz	7.80**	8.29**
StrL	8.97**	24.58***
Vrty	2.87*	0.75ns
Rhz*StrL	0.18ns	0.09ns
Rhz*Vrty	0.70ns	1.07ns
StrL*Vrty	0.69ns	0.48ns
Rhz*StrL*Vrty	3.27*	1.63ns

+R: With *Rhizobium*, −R: Without *Rhizobium*. StrL 1: No water stress, StrL 2: Water stress at Vegetative Stage, StrL 3: Water stress at Flowering Stage. Vrty 1: *KAT B9*, Vrty 2: *KAT B1*, Vrty 3: *F9 Kidney Selection*, Vrty 4: *F8 Drought Line*, Vrty 5: *JESCA*. Values presented are means ± SE. *, **, *** = significant at $p \leq 0.05$ at $p \leq 0.01$ and at $p \leq 0.001$ respectively, ns = Not significant. Means followed by similar letter(s) in a given column are not significantly difference from each other at $p = 0.05$.

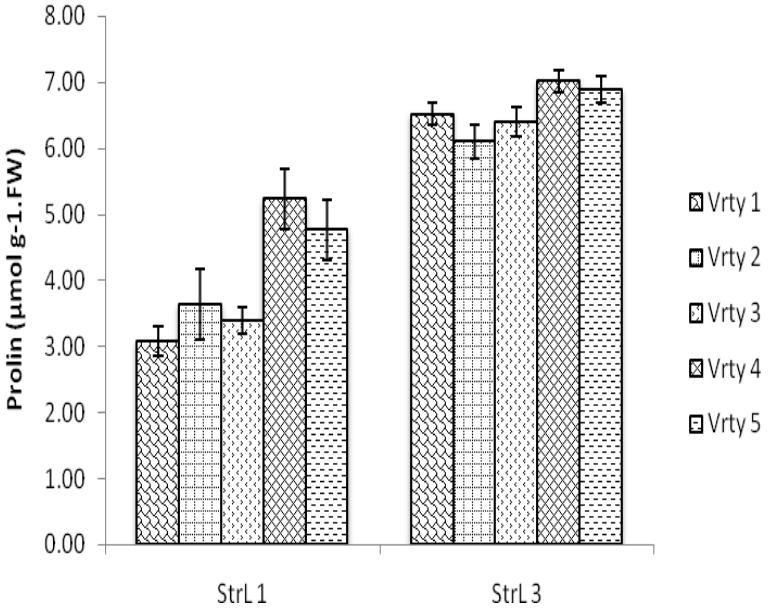


Figure 2: Interactive effects of stress level and five (5) *P. vulgaris* (L.) on proline content (µmol g^{-1}FW) in season (1) field experiment at flowering stage. StrL 1: Control, StrL 3: Water stress at flowering stage. Vrty 1: *KAT B9*, Vrty 2: *KAT B1*, Vrty 3: *F9 Kidney Selection*, Vrty 4: *F8 Drought Line*, Vrty 5: *JESCA*

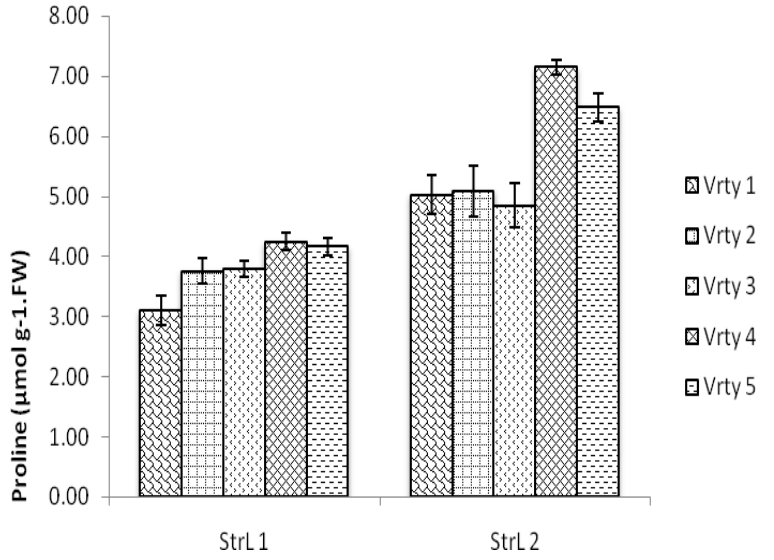


Figure 3: Interactive effects of stress level and five (5) *P. vulgaris* L. on proline content (µmol g^{-1}FW) in season (2) field experiment at vegetative stage. StrL 1: Control, StrL 2: Water

stress at vegetative stage. Vrty 1: *KAT B9*, Vrty 2: *KAT B1*, Vrty 3: *F9 Kidney Selection*, Vrty 4: *F8 Drought Line*, Vrty 5: *JESCA*

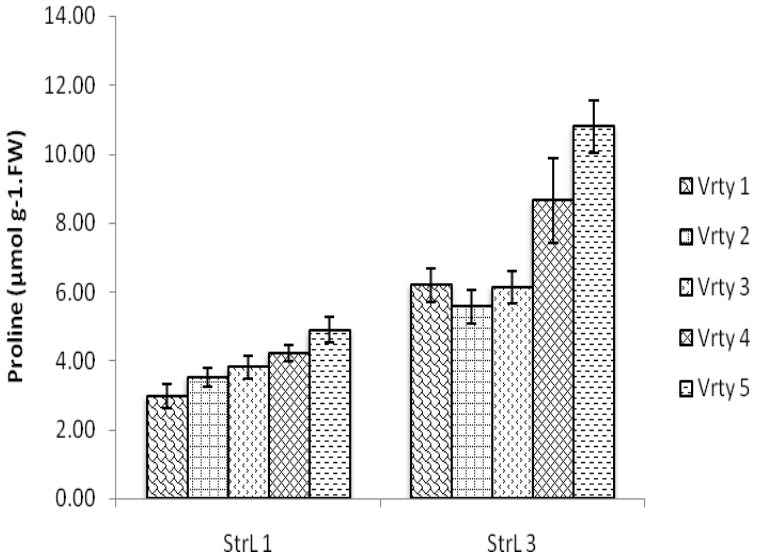


Figure 4: Interactive effects of stress level and five (5) *P. vulgaris* (L.) on proline content (µmol g^{-1}FW) in season (2) field experiment at flowering stage. StrL 1: Control, StrL 3: Water stress at flowering stage. Vrty 1: *KAT B9*, Vrty 2: *KAT B1*, Vrty 3: *F9 Kidney Selection*, Vrty 4: *F8 Drought Line*, Vrty 5: *JESCA*

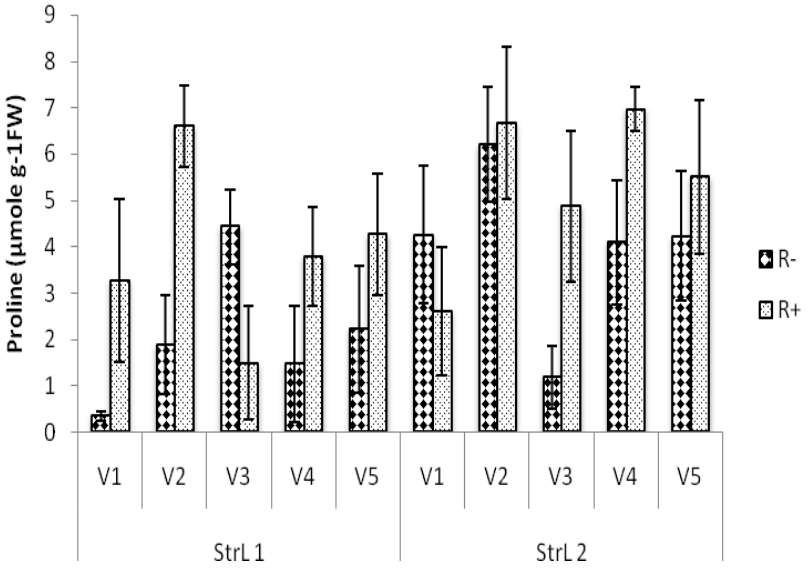


Figure 5: Interactive effects of rhizobial inoculation, stress level and five (5) *P. vulgaris* (L.) on proline content (µmol g^{-1}FW) screen house experiment at vegetative stage. -R: Without rhizobial inoculation, +R: With rhizobial inoculation. StrL 1: Control, StrL: Water stress at vegetative stage. V_1: *KAT B9*, V_2: *KAT B1*, V_3: *F9 Kidney Selection*, V_4: *F8 Drought Line*, V_5: *JESCA*

Significant increase in proline content (µmol g^{-1}FW) was also recorded in variety 4 (*F8 Drought Line*) and 5 (*JESCA*) in field experiment, in season 1 and 2 respectively (Table 2). However, in the screen house experiment the proline content in bean varieties was as follows; *KAT B1*>F8 *Drought Line* >*JESCA* >*F8 Kidney Selection*>*KAT B9* (Table 2).

3.3.2 Interactive effects of inoculation with *Rhizobium* and stress period on proline content in selected *P. vulgaris* (L.) varieties

There were significant interactive effects between stress levels and variety in proline content (µmol g^{-1}FW) at field experiments (Figs. 2, 3 and 4). However, significant interaction was observed in the screen house between rhizobial inoculation, stress and bean varieties during the vegetative stage (Fig. 5). Generally, the water stressed and rhizobial inoculated treatments had increased proline.

Rhizobial inoculation significantly improved proline content (µmol g^{-1}FW) of *P. vulgaris* (L.) as compared with non-inoculated treatment. Studies by other researchers (Kirda *et al.,* 1989; Djibril *et al.,* 2005; Ramos *et al.,* 2005; Daniel *et al.,* 2007; Sassi-Aydi and Abdelly, 2012) have also reported elevated level of proline under condition of sufficient N in which the proline levels increased in the tissues due to the action of ornithine pathway in enhancing proline synthesis, over the glutamine pathway (Sánchez *et al.,* 2002). Elboutahiri *et al*. (2010) reported that *Rhizobium* inoculation in alfalfa resulted in highest leaf proline levels. Another study by Kohl *et al*. (1991) in *Glycine max* plants inoculated with *Bradyrhizobium japonicum* showed higher amounts of proline in their tissues similar to what was found in this study. There was significance increase in proline content (µmol g^{-1}FW) in water stress treatment as compared with un-stressed water treatment. Research evidence has shown that proline is commonly produced in higher plants and generally accumulates in large extent in response to environmental stresses such as water stress and /or drought (Kapuya *et al.,* 1995; Ashraf and Foolad, 2007; Lobato *et*

al., 2008; Tatar and Gevrek, 2008; Siddiqui *et al.,* 2015) and hence serving as a bio indicator of resistance or tolerance to water deficit (Bates *et al.,* 1973). In a closely related study, Sanchez *et al.,* (1998) reported increased proline content in pea plants subjected to drought stress.

Varieties 4 (*F8 Drought line*), 5 (*JESCA*) and 2 (*KAT B1*) significantly increased proline content (µmol g^{-1}FW) of *P. vulgaris* L. in field and screen house experiment as compared with the other studied varieties. It has being established that accumulation of proline in plant tissues has been used as a biomarker and a parameter of choice for water stress tolerance in plants. This is due to the fact that water stressed plants produce proline as an adaptive and survival mechanism under water stress conditions (Ford, 1984; Chiang and Dandekar, 1995; Jaleel *et al.*, 2007; Verbruggen and Hermans, 2008; Farooq *et al.,* 2009; Masoudi-Sadaghiani *et al.,* 2011; Hayat *et al.,* 2012).The significantly higher amount of proline in varieties 4 (*F8 Drought line*), 5 (*JESCA*) and 2 (*KAT B1*) suggests the potential of involving them in more advanced studies related to drought. Furthermore, the interactive effects between rhizobial inoculation, water stress and varieties 4 (*F8 Drought line*), 5 (*JESCA*) and 2 (*KAT B1*) in producing elevated levels of proline is an indication which may warrant further studies.

3.3.3 Conclusion

It can be concluded that rhizobial inoculation and water stress increased proline content in *P. vulgaris* (L.) cultivars. Furthermore, the proline content was higher in varieties number 4 (*F8 Drought line*), 5 (*JESCA*) and 2 (*KAT B1*) and hence indicating their potential to tolerate drought. Interactive effects between rhizobial inoculation, water stress and few identified varieties in enhancing the proline levels in the plants is an indication of various factors which may play a significant role in developing appropriate technology related to water stress tolerance in *P. vulgaris*.

CHAPTER FOUR

Influence of water stress and rhizobial inoculation on accumulation of flavonoids and anthocyanins in selected common bean (*P.vulgaris*) cultivars

Abstract

A two season field experiment and a single season screen house experiment were conducted to assess the effectof water stress periods and rhizobial inoculation in five (5) *P. vulgaris* (L.) cultivars. The experiment consisted of 2 levels of rhizobia (with and without rhizobial inoculation), two stress levels (with and without water stress) and five cultivars of *P. vulgaris* (L.) (*KAT B9, KAT B1, F9 Kidney Selection, F8 Drought line* and *JESCA*). Results showed that flavonoids and anthocyanins (g DM^{-1}) concentrations were higher in non- inoculated and water stressed treatments. Varieties *F8 Drought Line, JESCA* and *F9 Kidney Selection* significantly recorded higher flavonoids and anthocyanins content in both field and screen house experiment as compared with the other tested varieties. Significant interactive effects were also observed between inoculation, water stress periods and the tested *P vulgaris* (L.) varieties.

Key words; Inoculants, Water, Varieties, Phytochemicals, Legumes

International Journal of Biosciences (IJB), **10(3): 333-342.**

4.1 Introduction

Nitrogen (N) is an essential major element for growth and productivity of plants. N is a building block of proteins and important in enzyme biosynthesis and amino acids (Ayoola, 2010). Phenolic compounds containing N are extensively distributed secondary plant products and is generally derived from L-phenylalanine through nitrogen framework of cinnamate under phenyl propanoid metabolism (Razal *et al*., 1996). Flavonoids are usually synthesized using phenylalanine which may be affected by nitrogen metabolism. Under condition of low N, the level of Phenylalanine ammonia-lyase activity increase hence enhances the accumulation of flavonoids (Kondorosi *et al*., 1995; Stewart *et al*., 2001; Mierziak *et al*., 2014). Study by Liu *et al*. (2010) in *C. morifolium* leaves showed that flavonoid concentrations were higher under low nitrogen supply, which implies the activity of PAL (Phenylalanine ammonia-lyase) was abundant in the leaf of *C. morifolium*. It has been reported that N deficiency results in huge accumulation

of secondary compounds mainly phenolics such as flavonols (Stewart *et al*., 2001) and anthocyanins (Chalker-Scott, 1999). For instance, Awad and Jager (2002) reported a decline in the concentration of flavonoids in the skin of apple as a result of nitrogen (N) supply. Other study by Ibrahim *et al*. (2011) on *Labisia pumila* (sub- herbaceous plant) showed a significantly less production of phenolics. It can be concluded that flavonoids metabolism in plants is highly favored in the presence of Phenylalanine ammonia-lyase (PAL) as a result of N deficiency in growth-promoting N availability such as rhizobial inoculation.

Scarcity of water is a severe environmental constraint to plant productivity as it causes severe physiological, biochemical and molecular changes in plants (Siddiqui *et al*., 2015). It tends to distress some crucial process in plants such as respiration, translocation, ion uptake, carbohydrates and nutrient assimilation (Farooq *et al*., 2008). During water stress periods, higher plants are forced to produce some secondary metabolites, which enable the plants to adapt to their environmental conditions (Ramakrishna and Ravishankar, 2011). Secondary metabolites play crucial roles in various biochemical processes in plants (Horbowicz *et al*., 2008; Ramakrishna and Ravishankar, 2011). For example, phenolic compounds such as flavonoids and anthocyanins are known to play key role(s) in plant growth and development, defense of plants against insect pests and diseases, phytopathogens, signaling during nodulation (Chalker-Scott, 1999; Dixon and Steele, 1999; Ndakidemi and Dakora, 2003; Falcone Ferreyra *et al*., 2012). Plant flavonoids are known for, among other roles, to modulate enzymatic activities, protect plant from UV light, oxidant and free radicals, allelopathy, insect attraction or repulsion, nectar guides, probing stimulants, viral, fungal and bacterial protection, nodulation in leguminous plants, pollen germination (Ramchandra and Ravishankar, 2002). Flavonoids and anthocyanins accumulate in plants when subjected to various stress conditions. The production of secondary metabolites in plants can be considered as a strategy of enhancing the defensive mechanism in plant when subjected to nutrient and water stress such as drought. Therefore, this study was conducted to assess the influence of water stress and rhizobial inoculation in accumulation of flavonoids and anthocyanins in selected common bean (*Phaseolus vulgaris* L.) cultivars.

4.2 Material and Methods

4.2.1 Description of Site Location

The trial was conducted at Agricultural Seed Agency (ASA) farm in Arusha, located at Latitude 3°18′S and Longitude 36°38′06.29″E. ASA receives the mean annual rainfall of 819mm, mean temperature of 19.2°C with relative humidity of about 94% and altitude of 1520m.a.s.l. The field trial was carried out during dry season of January, to March 2014 and January, to March, 2015 while the screen house experiment was carried out from mid-January to March, 2016 under irrigation.

4.2.2 Experimental Design and Treatment Application

The experiment was designed in split, split plot with 3 replications. The plot size was 3mx4m. The field experimental treatments consisted of 2 levels of Rhizobia (with and without inoculation) as the main factor followed by imposing of stress (sub factor) in vegetative and flowering stages of plant growth. Five cultivars of *P. vulgaris* (L.) namely *KAT B9, KAT B1, F9 Kidney Selection*, *F8 Drought line* and *JESCA* were assigned to sub-sub plots. The common bean seeds were sown at a spacing of 50 cm x 20cm, making a plant population density of 200,000 plants per hectare. The BIOFIX legume inoculants were obtained from *MEA* Company Nairobi-Kenya, sold under license from the University of Nairobi. *P.vulgaris* (L.) cultivars were obtained from the breeding unit based at Selian Agricultural Research Institute (SARI), Arusha, Tanzania. Land for field experiment was cleared and all the necessary practices like ploughing and harrowing were done before planting. Moreover, in the screen house experiment, wooden box technique was used to establish the experiment using the protocol developed by (Agbicodo *et al*., 2009) with some modifications. This was done by collecting the same soil used for field experiment. The common bean seeds were thoroughly mixed with *Rhizobium* inoculants to supply (10^9 cells g^{-1} seed), following procedure stipulated by products manufacturer. To avoid contamination, all non-inoculated seeds were sown first, followed by inoculated seeds. Three seeds were sown and thinned to two plants per hill after full plant establishment. Stress period of 10 days were imposed at vegetative and flowering stages of plant growth by not irrigating.

4.2.3 Plant Harvest and Sample Preparation

Shoot plant samples from field and glasshouse experiments were collected for flavonoids and anthocyanins analysis. In the field experiment, 10 plants were randomly sampled from the middle rows of each plot while in the glasshouse experiment two plants from each pot were sampled. The shoots of the plants samples were oven dried at 60°C for 48 hours, ground into a fine powder (2 mm sieve) for flavonoids and anthocyanins analysis.

4.2.4 Measurement of Flavonoids (g DM^{-1}) and Anthocyanins (g DM^{-1}) levels in shoots of *P. Vulgaris* (L.)

Flavonoids and anthocyanins concentration in plant parts were measured by the method described by Makoi *et al*. (2010b). In this method, 0.10 g of well-ground (0.85 mm) plant material was weighed and mixed with 10 mLs of acidified methanol prepared at a ratio of 79 : 20 : 1 MeOH : H_2O : HCl. The mixture was incubated for 72 h in darkness for auto-extraction, filtered through Whatman paper Number 2 and absorbance of the clear supernatant measured spectrometrically at 300, 530, and 657nm using acidified methanol as standard. Concentrations of flavonoids was measured using 2800 UV-Vis Spectrophotometer at 300nm and expressed as Abs g^{-1} DM (Mirecki and Teramura, 1984), while anthocyanins concentration in plant shoots was measured as Abs530-1/3Abs657 (Lindoo and Caldwell, 1978) and expressed as Abs g-1 DM. Concentrations of flavonoids compounds were expressed as: Flavonoids (Abs g DM^{-1}) = Abs300. Anthocyanins content was calculated as described in Lindoo and Caldwell, (1978): Anthocyanins (Abs g DM^{-1}) =Abs530 -1/3 Abs 657.

4.2.5 Statistical Analysis

A 3-way ANOVA was used to analyze the data collected. The analysis was done using STATISTICA software programe of 2013. Fisher's least significant difference was used to compare treatment means at $p = 0.05$ (Steel and Torrie, 1980).

4.3 Results

4.3.1 Effect of inoculation with *Rhizobium* and stress periods on flavonoids (g DM^{-1}) in selected *P. vulgaris* (L.) varieties

There were significant increases in flavonoids concentration (g DM^{-1}) in *P. vulgaris* (L.) shoots on non-inoculated treatments as compared with inoculated treatments by 18% in season one at vegetative stage and 28% in season two at flowering stage respectively (Table 4). In screen house experiment, there was a significant increase in flavonoids (g DM^{-1}) on non-inoculated treatments as compared with inoculated treatments at vegetative stage by 3% (Table 6). Flavonoid content significantly increased by 15 % for plants that were stressed at flowering stage and 30% in the second season when plants were water stressed during the vegetative stage (Table 4). For the screen house experiment, water stress significantly increases flavonoids (g DM^{-1}) content by 61% at flowering stage (Table 6). Significant increase in flavonoids (g DM^{-1}) content was recorded in varieties *F8 Drought Line*, *JESCA* and *F9 Kidney Selection* as compared with varieties *KAT B9* and *KAT B1* under field experiments (Table 4). Similarly, significant increase in flavonoid (g DM^{-1}) concentrations was also recorded in varieties *F8 Drought Line*, *JESCA* and *F9 Kidney Selection* in screen house experiments (Table 6).

Table 4: Effects of inoculation with *Rhizobium*, water stress and five *P. vulgaris* (L.) varieties on the accumulation of Flavonoids (g DM^{-1}) in common bean shoots for two consecutive season's field experiment

	1st Season		2nd Season	
Growth Phases	**Vegetative**	**Flowering**	**Vegetative**	**Flowering**
Treatments inoculation				
R+	2.85±0.05b	2.91±0.07a	3.00±0.17a	2.75±0.10b
R-	3.46±0.06a	2.92±0.08a	3.08±0.18a	3.81±0.06a
Stress Levels				
S1	3.13±0.07a	2.69±0.04b	2.51±0.17b	3.27±0.14a
S2/S3	3.18±0.09a	3.15±0.07a	3.57±0.12a	3.29±0.12a
Varieties				
V1	2.97±0.12a	2.58±0.03c	2.46±0.21b	2.87±0.21b
V2	3.06±0.11a	2.72±0.08c	2.20±0.25b	2.98±0.20b
V3	3.24±0.15a	2.95±0.10b	3.42±0.19a	3.58±0.19a
V4	3.26±0.13a	3.19±0.11a	3.72±0.18a	3.62±0.17a
V5	3.25±0.11a	3.16±0.11a	3.40±0.27a	3.34±0.17a
3-Way Anova (F-Statistics)				
Rhz	69.44***	0.01ns	0.30ns	137.22***
StrL	0.39ns	84.01***	51.14***	0.02ns
Vrty	2.58ns	22.37***	16.22***	11.31***
Rhz*StrL	9.04**	2.26ns	0.10ns	2.64ns
Rhz*Vrty	0.65ns	1.32ns	0.25ns	1.27ns
StrL*Vrty	0.20ns	4.60**	1.79ns	0.65ns
Rhz*StrL*Vrty	0.05ns	0.68ns	0.20ns	0.80ns

+R: With *Rhizobium*; −R: Without *Rhizobium*. S1: No water stress, S2: Water stress at Vegetative Stage. S3: Water stress at Flowering Stage. V1: *KAT B9*, V2: *KAT B1*, V3: *F9 Kidney Selection*, V4: *F8 Drought Line*, V5: *JESCA*. Values presented are means ± SE. **, *** = significant at $p \leq 0.01$ and at $p \leq 0.001$ respectively, ns = Not significant. Means followed by similar letter(s) in a given column are not significantly difference from each other at $p = 0.05$.

4.3.2 Effect of inoculation with *Rhizobium* and stress period on anthocyanins (g DM^{-1}) in selected *P. vulgaris* (L.) varieties

Anthocyanins (g DM^{-1}) concentration significantly increased by 71% in non-inoculated treatment in season one at flowering stage and 48% in season two at vegetative stage (Table 5). In screen house experiment, significant increase in anthocyanins (g DM^{-1}) content was 7% and 8% in non-

inoculated as compared with inoculated treatment at vegetative and flowering respectively (Table 6). Water stress significantly increased anthocyanin (g DM^{-1}) concentrations by 46% in season one at vegetative stage and 61% and 59% in season two at vegetative and flowering stage respectively (Table 5). In the screen house experiment, anthocyanins (g DM^{-1}) concentration increased by 91% as a result of stress in flowering stage (Table 6). Anthocyanins (g DM^{-1}) concentrations were significantly more pronounced in varieties *F8 Drought Line*, *F9 Kidney Selection* and *JESCA* in season one under vegetative stage (Table 5). However variety F8 drought line shows significant increase in anthocyanins concentration as compared with the other studied varieties in season one at flowering stage in field experiment (Table 5). Significant increase in anthocyanins (g DM^{-1}) content was recorded in varieties *F9 Kidney Selection*, *F8 Drought Line* and *JESCA* as compared with varieties *KAT B9* and *KAT B1* in season two under field experiment (Table 5). In the screen house experiment, an anthocyanins concentration was higher in variety *F8 Drought Line* in vegetative stage and variety *KAT B1* in flowering stage (Table 5).

Table 5: Effects of inoculation with *Rhizobium*, water stress and five *P. vulgaris* (L.) varieties on the accumulation of anthocyanins (g DM^{-1}) in common bean shoots for two consecutive season's field experiment

	1st Season		2nd Season	
Growth Phases	**Vegetative**	**Flowering**	**Vegetative**	**Flowering**
Treatments inoculation				
R+	0.21±0.01a	0.06±0.007b	0.11±0.009b	0.14±0.01a
R-	0.22±0.02a	0.21±0.02a	0.21±0.04a	0.17±0.02a
Stress Levels				
S1	0.15±0.007b	0.13±0.01a	0.09±0.01b	0.09±0.01b
S2/S3	0.28±0.01a	0.15±0.03a	0.23±0.03a	0.22±0.02a
Varieties				
V1	0.15±0.02d	0.08±0.02c	0.09±0.01b	0.12±0.01a
V2	0.19±0.02cd	0.10±0.02bc	0.10±0.01b	0.12±0.01a
V3	0.24±0.03ab	0.12±0.02bc	0.23±0.06a	0.19±0.05a
V4	0.27±0.03a	0.22±0.06a	0.22±0.06a	0.18±0.03a
V5	0.22±0.02bc	0.17±0.04ab	0.16±0.03ab	0.16±0.02a
3-Way Anova (F-Statistics)				
Rhz	1.55ns	48.03***	9.10**	1.81ns
StrL	92.49***	0.88ns	19.47***	39.63***
Vrty	8.79***	5.33**	3.59*	2.35ns
Rhz*StrL	1.18ns	1.79ns	3.19ns	1.71ns
Rhz*Vrty	0.36ns	1.16ns	1.65ns	0.66ns
StrL*Vrty	0.81ns	1.30ns	1.12ns	1.18ns
Rhz*StrL*Vrty	0.15ns	0.78ns	0.68ns	0.57ns

+R: With *Rhizobium;* −R: Without *Rhizobium*. S1: No water stress. S2: Water stress at Vegetative Stage. S3: Water stress at Flowering Stage. V1: *KAT B9*, V2: *KAT B1*, V3: *F9 Kidney Selection*, V4: *F8 Drought Line*, V5: *JESCA*. Values presented are means ± SE. *, **, *** = significant at $p \leq 0.05$, $p \leq 0.01$ and at $p \leq 0.001$ respectively, ns = Not significant. Means followed by similar letter(s) in a given column are not significantly difference from each other at $p = 0.05$.

Table 6: Effects of inoculation with *Rhizobium*, water stress and five *P. vulgaris* (L.) varieties on the accumulation of Flavonoids (g DM^{-1}) and Anthocyanins (g DM^{-1}) in common bean shoots grown in the screen house

Growth Phases	**Vegetative**		**Flowering**	
Treatments inoculation	**Flavonoids**	**Anthocyanins**	**Flavonoids**	**Anthocyanins**
R+	2.65±0.05b	0.42±0.005b	1.92±0.14a	0.23±0.03b
R-	2.73±0.02a	0.45±0.009a	1.86±0.15a	0.25±0.03a
Stress Levels				
S1	2.68±0.03a	0.44±0.007a	1.06±0.09b	0.04±0.009b
S2/S3	2.71±0.04a	0.44±0.008a	2.72±0.03a	0.44±0.007a
Varieties				
V1	2.37±0.10c	0.39±0.005e	1.52±0.27c	0.22±0.04a
V2	2.69±0.02b	0.41±0.03d	1.71±0.26b	0.26±0.05a
V3	2.73±0.01ab	0.43±0.005c	1.85±0.24b	0.25±0.05a
V4	2.82±0.02a	0.50±0.02a	2.19±0.16a	0.24±0.05a
V5	2.84±0.02a	0.45±0.004b	2.18±0.17a	0.24±0.04a
3-Way Anova (F-Statistics)				
Rhz	4.49*	27.06***	0.98ns	4.83*
StrL	0.58ns	0.05ns	780.26***	1276.32***
Vrty	17.50***	46.20***	20.12***	1.31ns
Rhz*StrL	0.26ns	2.11ns	0.84ns	0.70ns
Rhz*Vrty	4.48**	5.49***	0.21ns	1.03ns
StrL*Vrty	0.66ns	0.72ns	8.45***	1.01ns
Rhz*StrL*Vrty	0.33ns	2.10ns	0.38ns	1.28ns

+R: With *Rhizobium*, −R: Without *Rhizobium.* S1: No water stress, S2: Water stress at Vegetative Stage, S3: Water stress at Flowering Stage. V1: *KAT B9*, V2: *KAT B1*, V3: *F9 Kidney Selection*, V4: *F8 Drought Line*, V5: *JESCA*. Values presented are means ± SE. *, **, *** = significant at $p \leq 0.05$ at $p \leq 0.01$ and at $p \leq 0.001$ respectively, ns = Not significant. Means followed by similar letter(s) in a given column are not significantly difference from each other at $p = 0.05$.

4.3.3 Interactive effects of inoculation with *Rhizobium* and stress period on flavonoids (g DM^{-1}) and anthocyanins (g DM^{-1}) in selected *P. vulgaris* (L.)

There were significant interactions between *Rhizobium*, water stress period and varieties in shoot flavonoids and anthocyanins (g DM^{-1}) concentrations in both fields and screen house experiment (Fig. 6 - 10). Generally, the interactive effects between water stressed, rhizobial inoculated

treatments and varieties had a significant effects in flavonoids and anthocyanins concentrations (Fig. 6 - 10).

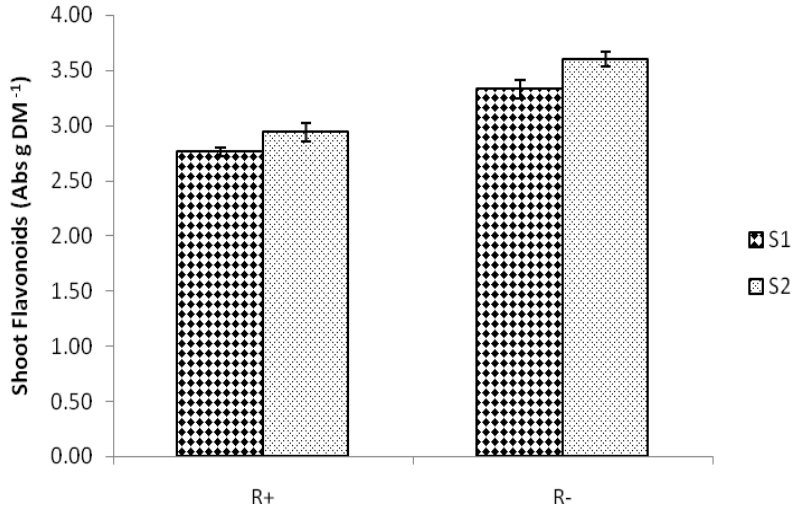


Figure 6: Interactive effects of *Rhizobium* and stress level on shoot flavonoids concentration in season (1) field experiment at vegetative stage. (+R: With *Rhizobium*, -R: Without *Rhizobium*. S1: Control, S2: Water stress at vegetative stage.

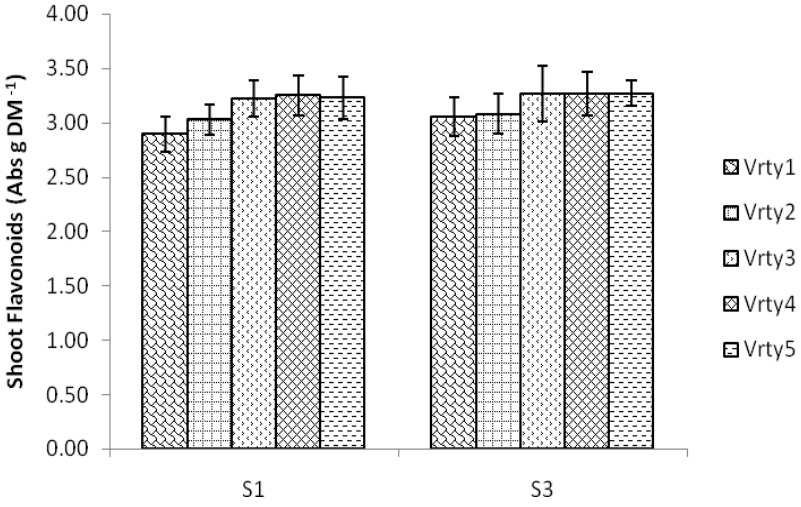


Figure 7: Interactive effects of stress level and five (5) *P. vulgaris* (L.) on shoot flavonoids concentration in season (1) field experiment at flowering stage. S1: Control, S3: Water stress at flowering stage. Vrty1: *KAT B9*, Vrty2: *KAT B1*, Vrty3: *F9 Kidney Selection*, Vrty4: *F8 Drought Line*, Vrty5: *JESCA*

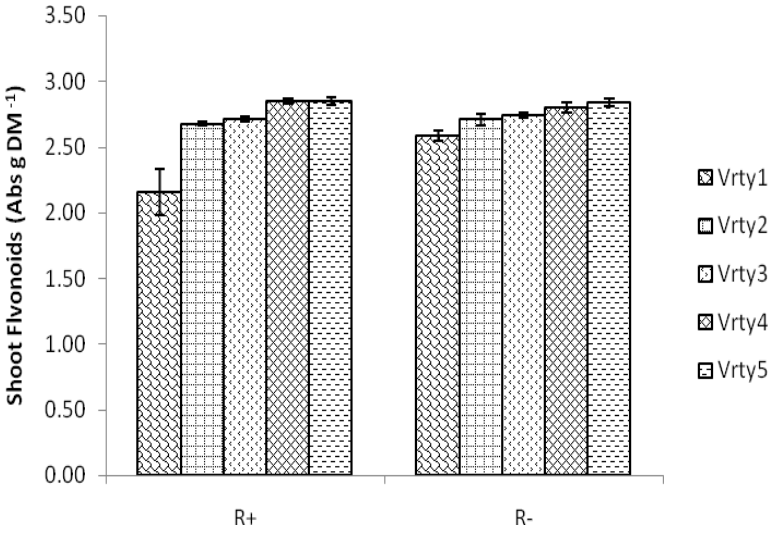


Figure 8: Interactive effects of *Rhizobium* and five (5) *P. vulgaris* (L.) on shoot flavonoids concentration in screen house experiment at vegetative stage. +R: With *Rhizobium*, -R: Without *Rhizobium*. Vrty1: *KAT B9*, Vrty2: *KAT B1*, Vrty3: *F9 Kidney Selection*, Vrty4: *F8 Drought Line*, Vrty5: *JESCA*

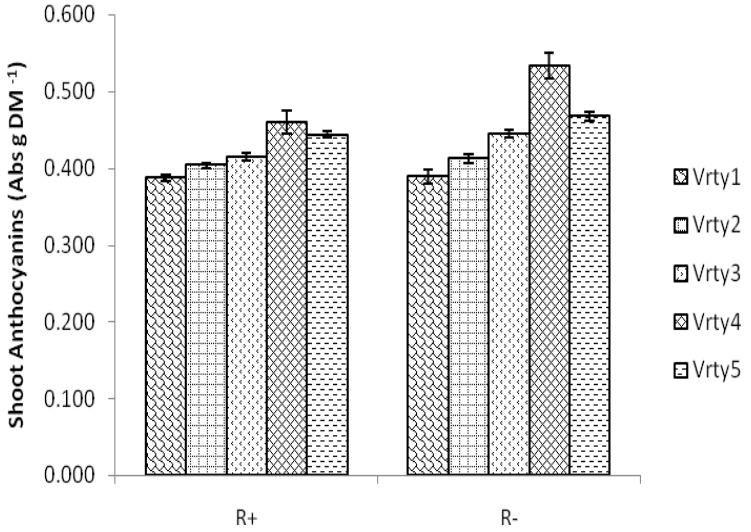


Figure 9: Interactive effects of *Rhizobium* and five (5) *P. vulgaris* (L.) on shoot anthocyanins concentration in screen house experiment at vegetative stage. +R: With *Rhizobium*, -R: Without *Rhizobium*. Vrty1: *KAT B9*, Vrty2: *KAT B1*, Vrty3: *F9 Kidney Selection*, Vrty4: *F8 Drought Line*, Vrty5: *JESCA*

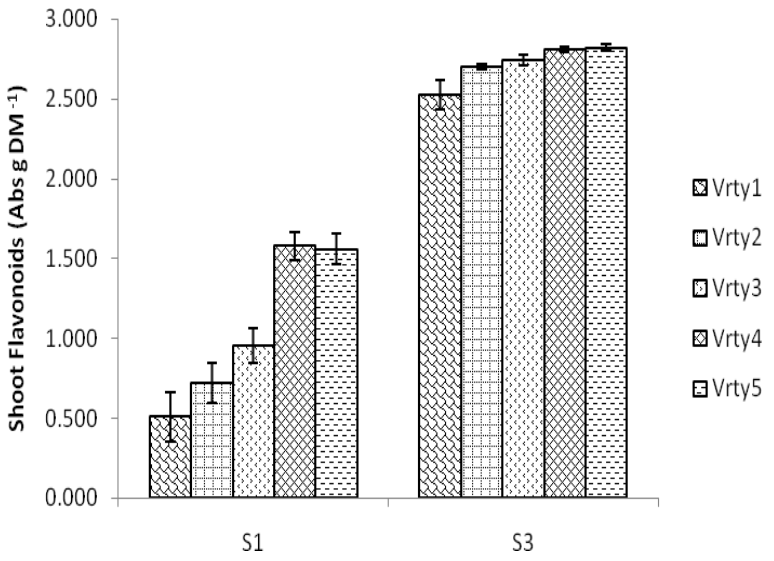


Figure 10: Interactive effects of stress level and five (5) *P. vulgaris* (L.) on shoot flavonoids concentration in screen house experiment under flowering stage. S1: Control, S3: Water stress at flowering stage. Vrty1: *KAT B9*, Vrty2: *KAT B1*, Vrty3: *F9 Kidney Selection*, Vrty4: *F8 Drought Line*, Vrty5: *JESCA*

4.4 Discussion

Rhizobial inoculation significantly reduced the secondary metabolites (i.e. flavonoids and anthocyanins) in bean shoots at all seasons with field and screen house experiments (Tables 1, 2 and 3). The low concentration of these metabolites under rhizobial inoculation suggests that plants were not nutritionally stressed by nitrogen and hence lower accumulation of the secondary metabolites in their tissue. Similar to this study, Makoi *et al*. (2010) showed a decreased level of flavonoids and anthocyanins concentration in *P. vulgaris* (L.) shoots both in fields and screen house experiment inoculated with rhizobia. Studies have revealed that flavonoids are synthesized using phenylalanine pathway which may be affected by nitrogen metabolism (Laurentius *et al*., 2002). Under conditions of low N, the levels of Phenylalanine ammonia-lyase (PAL) activity increase hence increasing accumulation of flavonoids (Stewart *et al*., 2001; Mierziak *et al*., 2014). Study by Liu *et al*. (2010) in *C. morifolium* leaves showed that flavonoids concentrations were low under higher nitrogen supply. Therefore, the reduced levels of flavonoids and anthocyanins` in this study in the inoculated treatments may be due to enhanced nitrogen fixation

and reduced nitrogen stress in the plant. There was significance increase in flavonoids and antocyanins (g^{-1} DM) concentration in water stress treatment as compared with un-stressed water treatment. Several studies have shown that many of secondary compounds are commonly accumulated in plant tissues in response to various environmental stresses such as water stress and/or drought (Balakumar *et al*., 1993; Barnabas *et al*., 2008; Farooq *et al*., 2009; Odjegb *et al*., 2013). Synthesis of these compounds stands as a defensive mechanism of plant metabolites such as sugars, proteins, amino acids, nucleic acids, membrane and lipids against reactive oxygen species (ROS), thus serving as an indicator of tolerance to water deficiency in plants (Larson, 1988; Agati *et al*., 2012). In closely related studies, significant increases in flavonoids and anthocyanins in plant tissues were reported as a result of water stress in various crop plants (Chalker-Scott, 1999; Fini *et al*., 2011). For instance, drought stress significantly increased anthocyanins levels in cowpea seedlings (Balakumar *et al*., 1993) a phenomenon similar to our study.

Generally, the results obtained in this study showed variations in the accumulation of flavonoids and anthocyanins. Varieties *F8 Drought Line, JESCA* and *F9 Kidney Selection* significantly contained more flavonoids and anthocyanins as compared with the other studied varieties. Accumulation of these secondary metabolites in plant tissues has been established as a tolerance mechanism towards several abiotic stresses including water (Larson, 1988; Bergman, 1992; Bongue-Bartelsman and Phillips, 1995; Mazid *et al*., 2011; Di Ferdinando *et al*., 2012; Di Ferdinando *et al*., 2014; Zadehbagheri, 2014). This confirms the previous finding which reported that bean variety *JESCA* was able to withstand moderate salinity in a potted study (Ndakidemi and Makoi, 2009), and varieties *F8 Drought line*, *JESCA* and *KAT B1* accumulated significantly higher amounts of proline in their tissues (Tairo *et al*., 2017) and hence indicating their potential in drought tolerance studies. The significantly higher amounts of flavonoids and anthocyanins concentration in the mentioned varieties provide a room for further detailed studies related to drought and/or water stress in *P. vulgaris* (L.). Significant interaction was also observed between rhizobial inoculation, water stress and varieties. Highest flavonoids values were recorded in water stressed treatments which were not inoculated with rhizobial inoculants, indicating that stress levels were key in controlling the biosynthesis of flavonoids in the *P. vulgaris* (L.) shoots.

4.5 Conclusion

In conclusion, these results showed that flavonoids and athocyanins concentrations (g $^{-1}$ DM) were higher in non rhizobial inoculated treatments as compared with inoculated plots. Furthermore, water stress treatments significantly accumulated more of flavonoids and anthocyanins as compared with unstressed treatments. The accumulation of flavonoids and anthocyanins in plant tissues may be taken as a mechanism used by plants against water deficit. Varieties *F8 Drought Line*, *JESCA* and *F9 Kidney Selection* recorded higher concentrations of flavonoids and anthocyanins as compared with other studied cultivars. These results suggests that flavonoids and anthocyanins are released when plants are subjected to nutritional and water stresses such as those evaluated in this study.

CHAPTER FIVE

Influence of Water Stress and Rhizobial Inoculation on the Accumulation of Chlorophyll in *Phaseolus vulgaris* (L.) Cultivars

Abstracts

Aims: To assess the effect of water stress periods and rhizobial inoculation in five (5) *P. vulgaris* (L.) cultivars.

Study Design: The experiment was designed in split-split plot and replicated 3 (three) times.

Place and Duration of Study: The field experiment was carried out for two consecutive seasons inthe year 2014 and 2015, whereas, the screen house experiment was planted in a single season inthe year 2016 at the Agricultural Seed Agency (ASA) farm in Arusha-Tanzania.

Methodology: The experiment consisted of 2 levels of rhizobia (with and without inoculation), twostress levels (with and without water stress) and five cultivars of *P. vulgaris* (L.) (*KAT B9, KAT B1, F9 Kidney Selection, F8 Drought line* and *JESCA*). The stress period of 10 days were imposed at vegetative and flowering stages of plant growth by not irrigating. Chlorophyll was extracted usingdimethyl sulphoxide (DMSO). Absorbance values were read at 645 nm and 663 nm by 2800 UV/Vis Spectrophotometer.

Results: Results indicated that leaf chlorophyll content was higher in rhizobial inoculated and non-stressed water treatments. Leaf chlorophyll content was significantly higher in varieties 3(*F9 Kidney Selection*) and 2(*KAT B1*) as compared with varieties 1(*KAT B9*), 4(*F8 Drought line*) and 5(*JESCA*). Significant interactions were observed between rhizobial inoculation x water stress and beanvarieties.

Conclusion: Rhizobial inoculation and adequate water supply significantly improved leaf Chlorophyll content in the tested cultivars.

Keywords: *P. vulgaris* (L.), water stress; rhizobial inoculation; chlorophyll

International Journal of Plant & Soil Science, **15(4): 1-13, 2017; Article no. IJPSS.32306**

5.1 Introduction

Light is the environmental factor that has most influence on growth and yield quantity and quality of crops, however low light intensity lowers the rate of photosynthesis (Montanaro *et al*., 2005). Chlorophyll is the main chloroplast component for photosynthesis and substantial

chlorophyll content has a constructive association with photosynthetic rate (Shobhkhizi *et al*., 2014). From physiological phenomena, leaf chlorophyll content is a unique entity with its own significant interest in plant (Mafakheri *et al*., 2010). Water stress is a serious threat to agriculture as it affects growth and plant pigments such as chlorophyll in different plant species. However, water stress tolerance mechanism varies significantly in different plant species. Changes in photosynthetic pigments are of chief importance to water stress and tolerance (Santos *et al*., 2006). Under condition of moisture stress in soil, the rate of CO_2 fixation is reduced along with photosynthetic rate resulting in less assimilate production for growth and yields in plants (Mafakheri *et al*., 2010). A study by Ommen *et al*. (1999) indicated that, moisture stress slow down photosynthesis of plants and cause changes in chlorophyll content by affecting chlorophyll components and by damaging the plant photosynthetic apparatus. The decreases in chlorophyll under this condition are mainly the result of destruction of chloroplasts caused by reactive oxygen species (ROS) (Verbruggen and Hermans, 2008). It has been reported that chlorophyll *a* and *b* are susceptible to soil water deficit (Farooq *et al*., 2009; Farhad *et al*., 2011; Tourian *et al*., 2013). Studies have revealed that water deficit results in negative impact in plants as majority of chlorophyll are lost (Ommen *et al*., 1999; Mafakheri *et al*., 2010; Shobhkhizi *et al*., 2014). Normally, these losses occur in mesophyll cells than in the bundle sheath (Anjum *et al*., 2011). Study by Baroowa and Gogoi (2012) in Black gram and Green gram indicated that chlorophyll content decreased with the increasing water stress and hence confirming that photosynthetic pigments were sensitive to water stress conditions. Report by, Massacci *et al*. (2008) shows reduction in chlorophyll content in drought stressed cotton. Santos *et al*. (2006) found that in moderate water stress conditions, the net photosynthetic rate decreased in common beans. Another study in sunflower plants also shows a significant decrease in chlorophyll content at higher water deficits (Kiani *et al*., 2008). The photosynthetic rate of higher plants is known to be reduced as the relative water content and leaf water potential decreases (Lawlor and Cornic, 2002). Abu-Muriefah, (2013) showed that water stress in common bean (*P. vulgaris* L.) impairs photosynthetic pigments in plant tissues, mainly shoot. It has been further reported that, reduction in leaf chlorophyll content under drought stress might be due to the excessive swelling of chloroplast membranes and distortion of the lamellae vesiculation in the plant tissues (Kaiser *et al*., 1981; Kaiser, 1987). It can be established that the decline in photosynthesis observed under water stress could be attributed by stomatal factors (i.e.stomatal and non-stomatal

limitations); of which the concentration of CO_2 in chloroplasts decreases because of a reduction in stomatal conductance (Chaves *et al*., 2002; Daniel *et al*., 2007; Gama *et al*., 2007; Farooq *et al*., 2009). Apart from water, nitrogen is the major component of the chlorophyll molecules and plays an essential function in photosynthesis process, protein formation and many enzymatic processes in plants (Ahmadi, 1985; Uchida, 2000; Zhou *et al*., 2006; Sara *et al*., 2013). With N_2 deficient soils, the use of nitrogenous fertilizers and/or suitable rhizobial strains might improve legume growth by enhancing photosynthesis and chlorophyll formation. Study by Anjum *et al*. (2006) in Mungbean showed that beneficial rhizobia bacteria influence the physiological growth conditions by providing N through fixation thus increasing chlorophyll contents in leaves. However, N_2 deficiency give a negative responsein plants by showing symptoms of yellowing which demonstrate chlorophyll deterioration hasoccurred in plants and therefore cause reduction in photosynthesis rate (Tairo and Ndakidemi, 2013). It is established that soil moisture deficit has a distinct effect on N_2 fixation as it affects nodule formation, growth and photosynthesis activities. However, appropriate competitive nodulating strains and suitable tolerant host legume varieties may play a significant role in the photosynthesis process and chlorophyll formation under stressed environment (Daniel *et al*., 2007). Study done by Tajini *et al*. (2012) shows reduction in chlorophyll concentration under water deficit in common beans using two strains of rhizobia. Therefore, the objective of this study is to investigate the influence of water stress and rhizobial inoculation on the accumulation of chlorophyll content in selected *P. vulgaris* (L.) cultivars.

5.2 Materials and Methods

5.2.1 Description of Site Location

The trial was conducted at Agricultural Seed Agency (ASA) farm in Arusha, located at Latitude 3°18 ′S and Longitude 36°38 ′06.29″E. ASA receives mean annual rainfall of 819 mm, mean temperature of 19.15°C with relative humidity of about 94% and altitude of 1520 masl. The field trial was carried out during dry season of January, to March 2014 and January, to March, 2015 while the screen house experiment was carried out from mid January to March, 2016 under irrigation.

5.2.2 Experimental Design and Treatment Application

The experiment was designed in split, split plot with 3 replications. The plot size was 3m by 4m. The field experimental treatments consisted of 2 levels of Rhizobia (with and without inoculation) as the main factor followed by imposing of stress (sub factor) in vegetative and flowering stages of plant growth. Five cultivars of *P. vulgaris* (L.) (*KAT B9, KAT B1, F9 Kidney Selection, F8 Drought line and JESCA*) were assigned to subsubplots. These cultivars were selected based on the fact that Varieties *F8 Drought Line, KAT B1* performed well in preliminary screening studies for drought tolerance (Abate, 2012; Mukankusi *et al*., 2015). Bean variety *JESCA* was included because in a potted study, it showed moderate tolerance to salinity (Ndakidemi and Makoi, 2009). Cultivars *F9 Kidney Selection, F8 Drought Line* and *KAT B9* have good adaptability in some production areas in the medium altitude zone of Tanzania. They have earned good approval by beneficiaries and are early maturing, drought tolerant, resistant to major diseases and have sufficient yielding (Abate, 2012; Mukankusi *et al*., 2015). The common bean seeds were sown at a spacing of 50 cm by 20 cm, making a plant population density of 200,000 plants per hectare. The BIOFIX legume inoculants were obtained from *MEA* Company Nairobi-Kenya, sold under license from the University of Nairobi. Common bean seeds lines and/or varieties *KAT B9, KAT B1, F9 Kidney Selection, F8 Drought line and JESCA* were obtained from the breeding unit based at Selian Agricultural Research Institute (SARI), Arusha, Tanzania. Land for field experiment was cleared and all the necessary land preparations like ploughing and harrowing were done before planting. Moreover, in the screen house experiment, wooden box technique was used to establish the experiment. This was done by collecting the same soil used at field experiment and beans were planted using the protocol developed by Agbicodo *et al*. (2009) with some modifications. Common bean seeds were thoroughly mixed with *Rhizobium* inoculants to supply (10^9 cells g^{-1} seed), following procedure stipulated by products manufacturer. To avoid contamination, all non inoculated seeds were sown first, followed by inoculated seeds. Three seeds were sown andthinned to two plants per hill after full plant establishment. Stress period of 10 days were imposed at vegetative and flowering stages of plant growth by not irrigating.

5.2.3 Plant Harvest and Sample Preparation

Plant leaf samples from field and glasshouse eexperiments were collected for chlorophyll analysis. In the field experiment, 10 plants were randomly sampled from the middle rows of each plot while in the glasshouse experiment two plants from each pot were sampled. The fresh plant leaf samples from each of the growth stages (i.e. vegetative and flowering) were collected from the third young leaf from the top and kept in ice container to maintain their freshness for chlorophyll analysis.

5.2.4 Determination of Chlorophyll (Chl) Contents in Plant Leaves

Extraction of chlorophyll concentrations by dimethylsulphoxide (DMSO) was done as described in Hiscox and Israelstam, (1979). A third of the plants leaves from the tip were collected from each plot. A hundred (100 mg) of the middle portion of fresh leaf slices was placed in a 15 ml vial containing 7 ml dimethylsulphoxide (DMSO) and incubated at 4°C for 72 hours. After incubation, the extract was diluted to 10 ml with DMSO. The DMSO technique extracts chlorophyll from shoot tissue without grinding or maceration (Hiscox and Israelstam, 1979). A 3 ml sample of chlorophyll extract was then transferred into curvets for absorbance determination. A spectrophotometer (2800 UV/Vis Spectrophotometer) was used to determine absorbance values at 645 and 663nm, which was then used by Arnon, (1949) to determine Leaf Chlorophyll *'a'*, Leaf Chlorophyll '*b'* and Total Leaf Chlorophyll expressed as mgL^{-1}. The equation is expressed as follows; Chlorophyll '*a'* = [(12.7 * OD at 663) – (2.69 * OD at 645)] Chlorophyll '*b'* = [(22.9 * OD at 645) – (4.68 * OD at 663)] and Chlorophyll Total = [(20.2 * OD at 645) + (8.02 * OD at 663)]. Where by OD = Optical density which present the absorption in 645 and 663 nm.

5.2.5 Statistical Analysis

A 3-way ANOVA was used to analyze the data collected. The analysis was done using STATISTICA software program of 2013. Fisher's least significant difference was used to compare treatment means at $P = 0.05$ (Steel and Torrie, 1980).

5.3 Results

5.3.1 Effect of Inoculation with *Rhizobium* and Stress Period in Chlorophyll '*a*', '*b*' and Total Chlorophyll in Selected *P. vulgaris* (L.) Varieties

Results in Tables 7 and 8 showed that water stress and rhizobial inoculation significantly influenced chlorophyll '*a*', '*b*' and total chlorophyll content in both field and screen house experiment. Rhizobial inoculation significantly increased chlorophyll '*a*' by 17 %, '*b*' by 30 % and total chlorophyll content by 20 % in vegetative stage and 18 % in flowering stage in season one (Table 7). Significant increase in chlorophyll *'a'*, '*b*' and total chlorophyll via rhizobial inoculation was also observed in season two by 47, 70 and 42 % in vegetative and 18 % for chlorophyll '*b*' and 15 % for total chlorophyll in flowering stage respectively (Table 7). In season one, water stress period significantly increased the chlorophyll '*a*' at flowering stage by 14 % over the control (Table 7). In season two, water stress periods significantly influenced chlorophyll '*a*', '*b*' and total chlorophyll content at vegetative stage by 27, 10 and 39 % and at flowering stage by 47, 57 and 38 % respectively (Table 7). However, for screen house experiment, water stress significantly affected chlorophyll '*b*' and total chlorophyll at flowering stage by 5 and10 % respectively (Table 8). In general term, varieties 2 and 3 proved to have significantly greater chlorophyll content under field and screen house experiment in both seasons (Tables 7 and 8).

5.3.2 Interactive effects of inoculation with *Rhizobium* and stress period on chlorophyll '*a*', '*b*' and total chlorophyll in selected *P. vulgaris* (L.) varieties

There was a significant interaction between *Rhizobium* and stress period/levels in chlorophyll '*a*', chlorophyll '*b*' and total chlorophyll content in season one at vegetative and flowering stages together with total chlorophyll in season two at vegetative stage respectively (Figs. 11 - 14 and 16). *Rhizobium* treatment without water stress resulted into increased levels of chlorophyll '*a*', '*b*' and total chlorophyll (mg L^{-1}) content compared with treatments with no *Rhizobium* inoculants with water stress (Figs. 11- 14 and 16). The trend of interaction in chlorophyll '*b*' was also observed between *Rhizobium* and bean varieties at vegetative stage in season two (Fig. 15). Significant interaction in chlorophyll '*a*' content was also observed between water stress and bean varieties in the second season at flowering stage (Fig. 17). Under all the interactions

mentioned, rhizobial inoculation and the control (No stress treatment SI) increased chlorophyll '*a'*, '*b*' and total chlorophyll content in both seasons in this study (Figs. 11-17).

5.4 Discussion

Nitrogen is a primary nutrient which plays most important roles in legumes and is a major constituent of chlorophyll which is the most essential pigment needed for photosynthesis and amino acids in plants (Tairo and Ndakidemi, 2013). In this study, rhizobial inoculation was reported to increase chlorophyll content of *P. vulgaris* (L.) cultivars compared with un-inoculated treatments. The increased chlorophyll in inoculated treatments may be dueto improved plant growth due to enhanced photosynthesis and hence chlorophyll formation. In similar studies, Lalitha and Santhaguru, (2012) showed increased chlorophyll content in inoculated plants with *Rhizobium*. In relation to this study, it has been reported that rhizobial inoculation may influence the physiological growth condition of leguminous plants by increasing leaf photosynthesis (Lippi *et al*., 1999; Zhao *et al*., 2006) and Chl contents in the leaves (Serraj *et al*., 1999; Tajini *et al*., 2008; Bambara and Ndakidemi, 2009; Tairo and Ndakidemi, 2013; Nyoki and Ndakidemi, 2014). Results from this study suggest that the supplied *Rhizobium* promoted the plant growth through a mechanism which increased Chl synthesis and photosynthetic rate in plants.

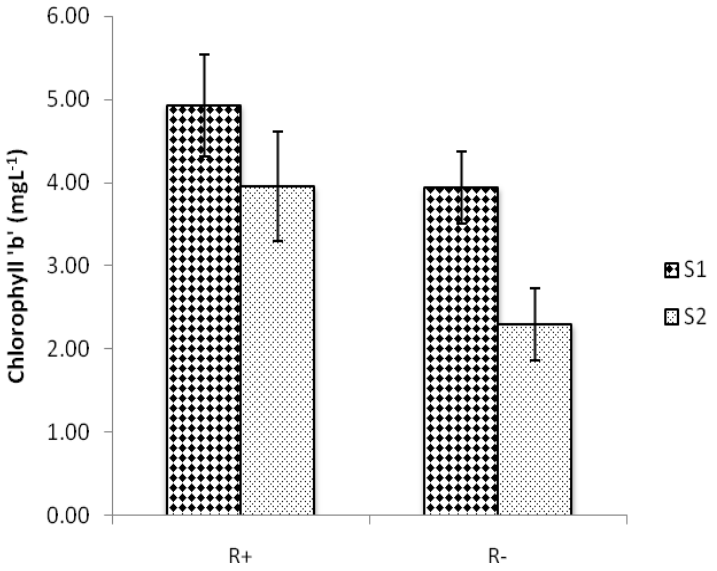


Figure 11: Interactive effects of *Rhizobium* and stress level on chlorophyll '*b*' contents at vegetative stage in season (1) field experiment. +R: With *Rhizobium*, -R: Without *Rhizobium*. S1: Control, S2: Water stress atvegetative stage

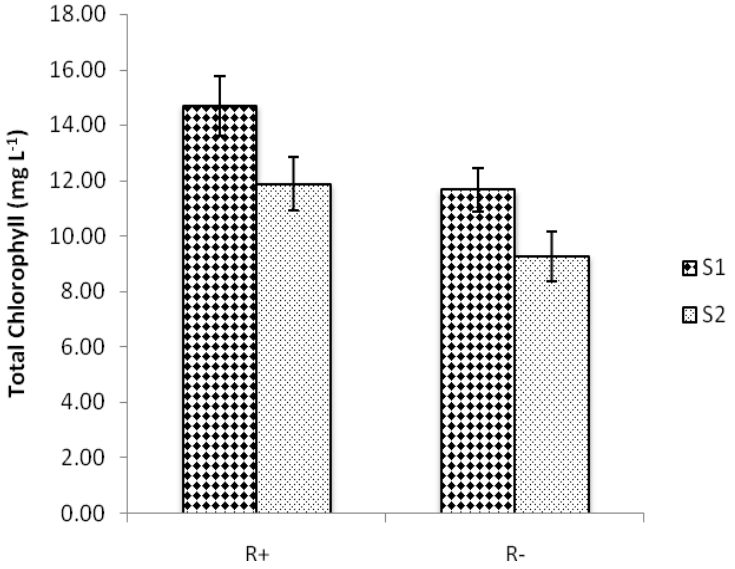


Figure 12: Interactive effects of *Rhizobium* and stress level on total chlorophyll content at vegetative stage in season (1) field experiment. +R: With *Rhizobium*, -R: Without *Rhizobium*. S1: Control, S2: Water stress at vegetative stage

Table 7: Effect of with and without *Rhizobium*, stress period, and five (5) *P. vulgaris* (L.) in the Chlorophyll '*a*', Chlorophyll '*b*' and Total Chlorophyll on plant leaves as measured on field experiment in two consecutive seasons

Growth phases	1st season						2nd season					
	Vegetative			Flowering			Vegetative			Flowering		
Treatments *inoculation*	**Chl a(mgL^{-1})**	**Chl *b*(mgL^{-1})**	**Total Chl (mgL^{-1})**	**Chl a(mgL^{-1})**	**Chl *b*(mgL^{-1})**	**Total Chl (mgL^{-1})**	**Chl a(mgL^{-1})**	**Chl *b*(mgL^{-1})**	**Total Chl (mg^{-1})**	**Chl a(mgL^{-1})**	**Chl b(mgL^{-1})**	**Total Chl (mg^{-1})**
R+	8.34±0.45a	4.43±0.38a	13.18±0.71a	9.99±0.76a	6.06±0.59a	14.81±1.07a	11.29±0.73a	8.38±0.39a	15.98±0.98a	11.65±0.95a	7.28±0.65a	20.20±1.14a
R-	6.91±0.38b	3.12±0.41b	10.58±0.69b	8.24±0.58b	5.70±0.54a	14.07±0.90a	6.04±0.25b	2.54±0.19b	9.21±0.54b	12.94±0.99a	5.94±0.63b	17.25±1.02b
Stress levels												
S_1	7.73±0.43a	3.61±0.44a	11.99±0.86a	9.82±0.80a	6.12±0.62a	14.90±1.08a	10.00±0.83a	5.75±0.68a	15.66±0.93a	16.09±0.90a	9.26±0.47a	23.06±0.93a
S_2/S_3	7.52±0.45a	3.95±0.39a	11.77±0.61a	8.42±0.55b	5.65±0.51a	13.98±0.88a	7.33±0.50b	5.17±0.55b	9.53±0.72b	8.49±0.31b	3.96±0.38b	14.38±0.58b
Varieties												
V_1	7.44±0.44b	3.23±0.34c	12.77±1.08b	8.34±0.31bc	6.44±0.52b	15.04±0.85b	8.67±1.19a	5.43±0.84b	12.80±1.51ab	12.19±1.27bc	6.99±0.98ab	18.51±1.24b
V_2	8.68±0.59b	4.70±0.64b	13.58±0.59ab	9.46±0.41b	7.98±0.50a	16.72±0.72b	9.84±1.36a	6.68±1.09a	14.19±1.81a	16.03±2.05a	8.40±1.03a	23.39±2.13a
V_3	10.41±0.49a	6.48±0.50a	15.31±1.03a	13.92±1.52a	8.92±0.67a	20.39±1.33a	9.53±1.32a	6.81±1.13a	14.33±1.79a	13.62±1.62b	7.51±0.99a	20.46±1.53b
V_4	5.82±0.43c	2.25±0.42c	9.51±0.99c	6.97±0.50c	2.25±0.29d	9.76±1.30c	7.51±0.91a	4.14±0.87c	10.79±1.37b	9.62±0.85d	4.84±0.82c	16.06±1.30c
V_5	5.77±0.36c	2.24±0.38c	8.22±0.76c	6.89±0.70c	3.82±0.57c	10.28±0.95c	7.79±0.90a	4.26±0.73c	10.87±1.30b	10.00±0.94cd	5.32±1.08bc	15.20±1.52c
3-Way Anova (F-Statistics)												
Rhz	12.55**	15.75***	14.36***	6.71*	0.75ns	0.53ns	51.35***	462.41***	89.69***	3.25ns	6.04*	18.96***
StrL	0.26ns	1.04ns	0.10ns	4.27*	1.27ns	0.82ns	13.27***	4.56*	73.69***	113.65***	94.74***	163.32***
Vrty	19.15***	24.19***	14.62***	14.51***	35.96***	15.58***	1.56ns	17.63***	4.63**	11.06***	6.04***	19.23***
Rhz*StrL	0.68ns	16.07***	16.84***	7.03*	9.17**	3.27ns	2.54ns	2.08ns	4.57*	0.40ns	0.82ns	1.41ns
Rhz*Vrty	0.89ns	1.09ns	0.19ns	0.33ns	1.59ns	0.10ns	0.23ns	3.56*	0.36ns	0.16ns	0.33ns	0.15ns
StrL*Vrty	0.40ns	1.44ns	0.87ns	0.29ns	1.04ns	0.37ns	0.13ns	0.87ns	0.38ns	3.42*	0.09ns	2.23ns
Rhz*StrL*Vrty	0.25ns	1.29ns	0.46ns	0.82ns	1.88ns	0.35ns	0.19ns	2.41ns	0.48ns	0.33ns	0.08ns	0.96ns

+R: With *Rhizobium*, −R: Without *Rhizobium*. S_1: No water stress, S_2: Water stress at Vegetative Stage, S3: Water stress at Flowering Stage. V_1: *KAT B9*, V_2: *KAT B1*, V_3: *F9 Kidney Selection*, V_4: *F8 Drought Line*, V_5: *JESCA*. Values presented are means ± SE. *, **, *** = significant at $p \leq 0.05$, at $p \leq 0.01$, and at $p \leq 0.001$ respectively, ns = Not significant. Means followed by similar letter(s) in a given column are not significantly difference from each other at $p = 0.05$

Table 8: Effects of chlorophyll '*a*', chlorophyll '*b*' and total chlorophyll in five (5) *P. vulgaris* (L.) plant leaves as influenced by water stress periods and rhizobial inoculation on screen house experiment in a single season

Growth phases	**Vegetative**			**Flowering**		
Treatments *inoculation*	**Chlorophyll '*a*'(mgL^{-1})**	**Chlorophyll '*b*'(mgL^{-1})**	**Total Chlorophyll(mgL^{-1})**	**Chlorophyll '*a*'(mgL^{-1})**	**Chlorophyll '*b*'(mgL^{-1})**	**Total Chlorophyll(mgL^{-1})**
R+	15.88±0.90a	14.46±0.33a	30.33±1.22a	16.39±0.96a	14.84±0.44a	32.22±1.35a
R-	17.45±0.95a	15.30±0.54a	32.74±1.38a	16.57±0.86a	14.55±0.42a	30.77±1.33a
Stress levels						
S_1	17.81±1.03a	15.00±0.46a	32.81±1.46a	16.59±1.02a	15.04±0.45a	33.19±1.41a
S_2/S_3	15.52±0.78a	14.76±0.44a	30.26±1.11a	16.37±0.79a	14.36±0.41b	29.79±1.21b
Varieties						
V_1	16.67±1.18ab	14.87±0.45a	31.53±1.60ab	13.51±0.34c	14.46±0.15c	30.32±0.68c
V_2	18.62±1.58a	15.99±0.75a	34.60±2.13a	18.63±0.66b	15.89±0.19b	35.81±0.59b
V_3	19.27±1.80a	15.85±0.84a	35.11±2.60a	25.25±0.76a	18.33±0.28a	43.38±1.09a
V_4	14.31±0.86b	13.76±0.29a	28.06±1.13b	13.04±0.93c	12.21±0.57d	24.09±1.37d
V_5	14.46±1.42b	13.93±0.90a	28.38±2.09b	11.96±0.74c	12.59±0.54d	23.87±1.11d
3-Way Anova (F-Statistics)						
Rhz	1.58ns	1.83ns	1.84ns	0.08ns	0.76ns	3.26ns
StrL	3.37ns	0.15ns	2.04ns	0.11ns	4.11*	17.73***
Vrty	2.68*	2.24ns	2.79*	56.99***	45.57***	84.13***
Rhz*StrL	0.12ns	2.33ns	0.60ns	0.13ns	0.98ns	0.19ns
Rhz*Vrty	0.35ns	0.22ns	0.25ns	0.32ns	2.30ns	1.12ns
StrL*Vrty	1.22ns	0.54ns	0.84ns	1.82ns	0.84ns	1.80ns
Rhz*StrL*Vrty	1.12ns	1.40ns	1.29ns	0.52ns	0.61ns	0.45ns

+R: With *Rhizobium*, −R: Without *Rhizobium*. S1: No water stress, S2: Water stress at Vegetative Stage, S3: Water stress at Flowering Stage. V1: *KAT B9*, V2: *KAT B1*, V3: *F9 Kidney Selection*, V4: *F8 Drought Line*, V5: *JESCA*. Values presented are means ± SE. *, *** = significant at $p \leq 0.05$ and at $p \leq 0.001$ respectively, ns = Not significant. Means followed by similar letter(s) in a given column are not significantly difference from each other at $p = 0.05$

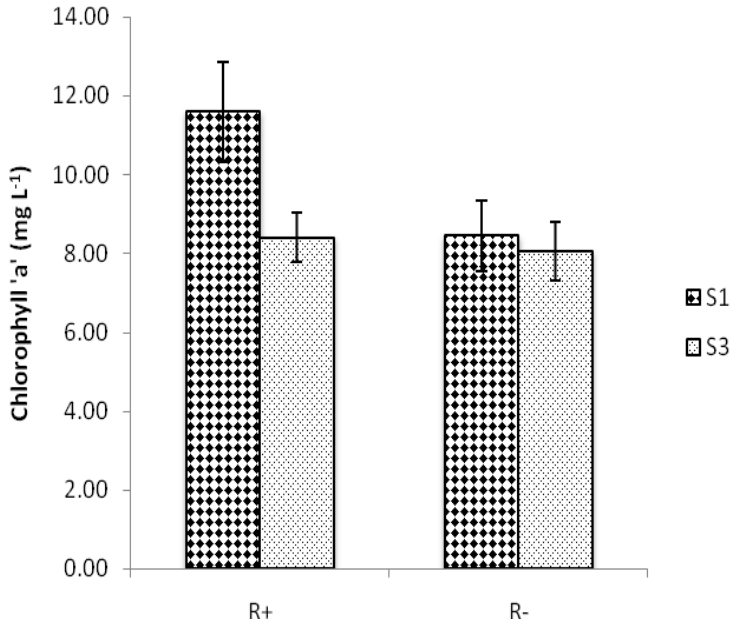


Figure 13: Interactive effects of *Rhizobium* and stress level on chlorophyll '*a*' content in season (1) field experiment at flowering stage. +R: With *Rhizobium*, -R: Without *Rhizobium*. S1: Control, S3: Water stress at flowering stage

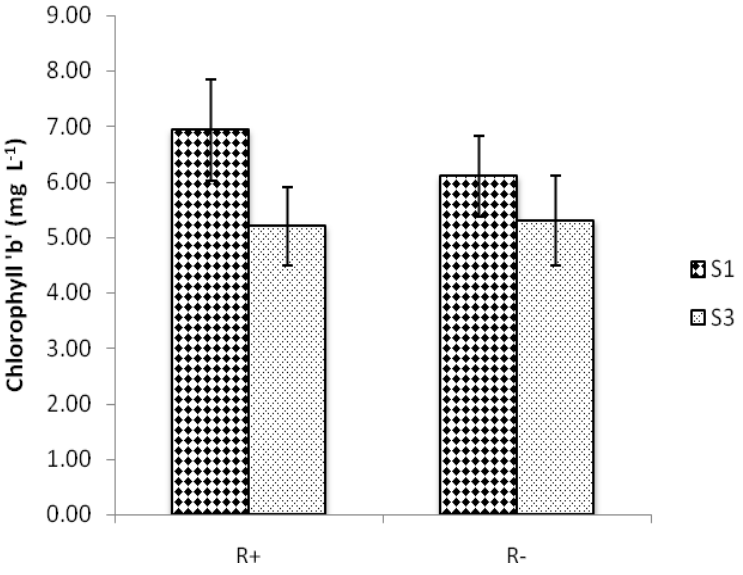


Figure 14: Interactive effects of *Rhizobium* and stress level on chlorophyll 'b'content in season (1) field experiment at flowering stage. +R: With *Rhizobium*, -R: Without *Rhizobium*. S1: Control, S3: Water stress at flowering stage

In the present study, we assessed the effects of water stress in the accumulation of leaf chlorophyll content. Water stress caused a decrease in chlorophyll '*a'*, '*b'* and total chlorophyll content of the common beangrowth in fields and screen house experiments. The decreased or increased chlorophyll level during water stress at particular stages of plant growth has been reported in other plant species depending on the extent and severity of stress (Kpyoarissis *et al*., 1995). The reduction of chlorophyll under water stress condition might be contributed by moisture limitation which affected the photosynthesis process and hence the chlorophyll formation. Cornic, (2000) reported that reduced water content in the plant results in the closure of the stomata and eventually reduces the rate of photosynthesis. Similarly, Foyer *et al*. (1994), Emam *et al*. (2010), Keyvan (2010), Baroowa and Gogoi (2012), Sharma *et al*. (2012), Beebe *et al*. (2013), Uddin *et al*. (2013) and Zadehbagheri (2014) showed that water stress damaged the photosynthetic machinery of the plants and reduced the chlorophyll content.

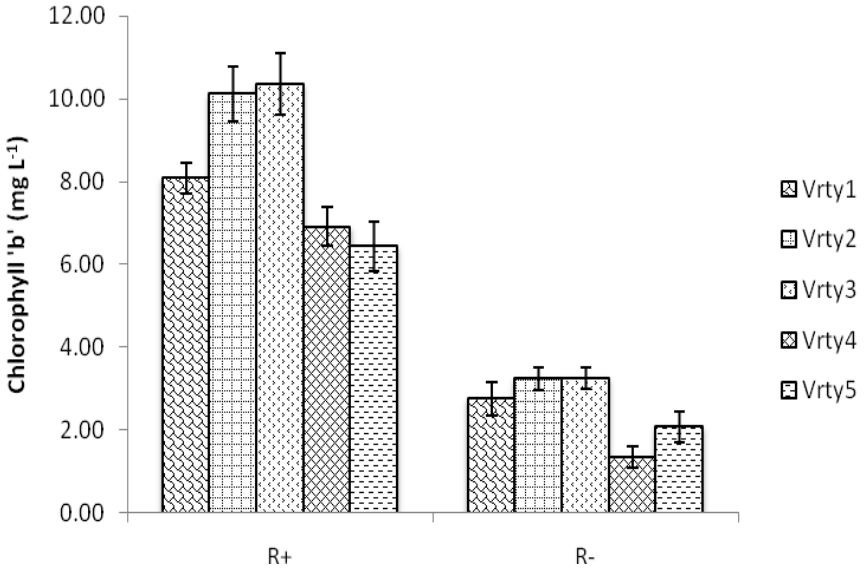


Figure 15: Interactive effects of *Rhizobium* and five (5) *P. vulgaris* (L.) on chlorophyll '*b*' content in season (2) field experiment at vegetative stage. +R: With *Rhizobium*, -R: Without *Rhizobium*. Vrty1: *KAT B9*, Vrty2: *KAT B1*, Vrty3: *F9 Kidney Selection*, Vrty 4: *F8 Drought Line*, Vrty5: *JESCA*

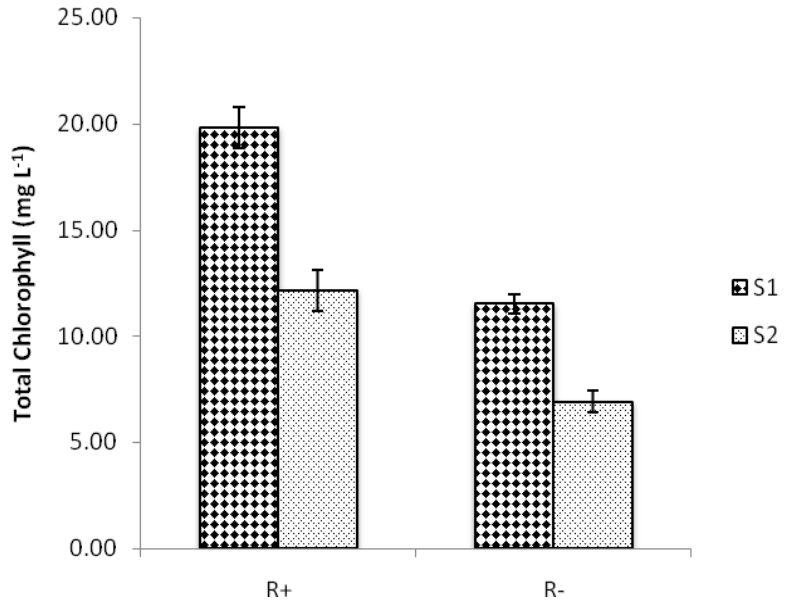


Figure 16: Interactive effects of *Rhizobium* and stress level on chlorophyll total in season (2) field experiment at vegetative stage. +R: With *Rhizobium*, -R: Without *Rhizobium*. S1: Control, S2: Water stress at vegetative stage

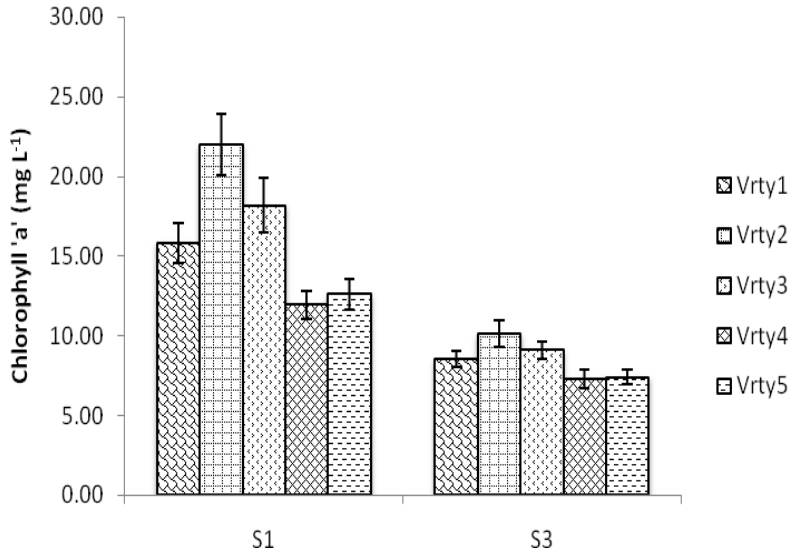


Figure 17: Interactive effects of stress level and five (5) *P. vulgaris* (L.) on chlorophyll '*a*' content inseason (2) field experiment at flowering stage. S1: Control, S3: Water

stress at flowering stage. Vrty1: *KAT B9*, Vrty2: *KAT B1*, Vrty3: *F9 Kidney Selection*, Vrty4: *F8 Drought Line*, Vrty5: *JESCA*

In the present study, significant increase in chlorophyll '*a'*, '*b'* and total chlorophyll content was seen in *F9 Kidney Selection* and *KAT B1* in fields and screen house experiment as compared with varieties *KAT B9*, *F8 Drought line* and *JESCA*. The significance difference among the studied cultivars might be attributed by the genetic makeup in their chlorophyll metabolism. Moreover, the low chlorophyll content in varieties *KAT B9*, *F8 Drought line* and *JESCA* could be attributed by damage to leaf pigments as a result of water deficit. The results of the current study propose that the photosynthesis potential of the tested varieties is different, and hence may affect some of the physiological functions of the plant. These results are in agreement with Nyachiro *et al*. (2001) who reported a significant decrease in chlorophyll '*a'* and '*b'* in six *Triticum aestivum* cultivars. Similar study on common bean showed reduction in net photosynthetic rate and chlorophyll concentration as a result of water stress (Ramos *et al*., 1999; Santos *et al*., 2006). The significant interactive effects observed between Water stress x Rhizobia x Varieties in Chlorophyll '*a'*, Chlorophyll '*b'* and total Chlorophyll is an indication that N from N_2 fixation, enough moisture in the growth media and efficient cultivars are necessary in improving chlorophyll synthesis in *P. vulgaris* (L.).

5.5 Conclusion

In conclusion, rhizobial inoculation and adequate water supply significantly improved total leaf chlorophyll content at vegetative and flowering in season 2 and at flowering in glasshouse and field experiment. Furthermore, the varieties tested also differed significantly in their potential to accumulate chlorophyll in their tissues.

CHAPTER SIX

Influence of Water Stress and Rhizobial Inoculation on Relative Leaf Water content and Electrolyte Leakage in Selected Common Bean cultivars (*Phaseolus vulgaris* L.)

Abstracts

Two seasons' field and one season screen house experiments were conducted to assess the effect of water stress periods and rhizobial inoculation in five *P. vulgaris* cultivars on relative water content and electrolyte leakage. The experiment consisted of 2 levels of rhizobia (with and without inoculation), two stress levels (with and without water stress) and five cultivars of *P. vulgaris* (*KAT B9*, *KAT B1*, *F9 Kidney Selection*, *F8 Drought line* and *JESCA*). Rhizobial inoculated treatments and un-stressed water treatments increased relative leaf water content and electrolyte leakage in field and screen house experiment. Cultivars *F9 Kidney Selection*, *F8 Drought Line* and variety *JESCA* significantly increased relative water content as compared with the other tested cultivars. However, varieties *KAT B9* and *KAT B1* significantly increased electrolyte leakage as compared with the other studied cultivars. Significant interactions were also observed between inoculation x water stress periods and the tested *P vulgaris* cultivars.

Key words; Moisture, Inoculants, Varieties, ions

SAUSSUREA Journal, **7(2): 115-128**

6.1 Introduction

Relative leaf water content (RLWC) is a measure of relative change in cell volume. It sums up the effect of cell turgor and osmotic potential and always depends both on solute concentration and rigidity of cell wall (Kramer and Boyer, 1995). Relative leaf water content (RLWC) is regarded as an essential parameter for quantifying plant water status and a constructive sign of plant water balance as it states the relative water available on the plant tissues (Farooq and Azam, 2006; Manavalan *et al*., 2009). Plant water status is closely related to numerous physiological variables, mainly leaf turgor, growth, stomatal conductance and respiration. Investigation of water status is an essential function of assessing plant growth and predicting potential yields (Waraich *et al*., 2011). Under low soil moisture condition, the quantification of

RLWC is of fundamental role since high RLWC appears to be a common characteristic in drought resistant species (Rahaman *et al*., 2000). Alizade (2002) reported that RLWC is of the best growth/biochemical indicator revealing the stress intensity. Report by Schonfeld *et al*. (1988) showed that wheat cultivars comprised of high RLWC were more resistant against drought stress. Ramos *et al*. (2003) reported that RLWC of bean leaves under drought stress significantly was lesser than control treatments receiving adequate water. Study done on stem of bean plant under water stress condition indicated that there was a significant reduction in RLWC as compared with control (Lazacano-Ferrat and Lovat, 1999). It has been identified that the reduction of RLWC of plants subjected to water stress is associated with the cell damage which consists of cleavage in the membrane and sedimentation of cytoplasm (Blackman *et al*., 1995). Therefore assessment of water status in crop plants is of fundamental importance under various environmental conditions.

Cell membrane stability (CMS) is the ability of a plant to resist cellular membrane modification as a result of environmental stress such as drought (Dhanda *et al*., 2004). Drought stress damages the cell membrane which leads to increased electrolyte leakage. Cell membrane is affected by various detrimental environmental factors. It is generally accepted that maintenance of their integrity and stability is one of the foremost mechanism of achieving high and acceptable yield (Namvar *et al*., 2013). Malfunction of cell membrane as a result of several environmental factors allow and increase more ample space for permeability and leakage of ions, which can be measured by the efflux of electrolytes (Saneoka *et al*., 2004). Study by Wu and Wallner (1993) showed that CMS is a rapid and sensitive method to evaluate drought tolerance in plants. For example, cell membrane stability was used to determine drought tolerance of 104 rice genotypes (Tripathy *et al*., 2000). It has been reported that cell membrane stability has also been used as a selection method for drought tolerance in grain sorghum (Sullivan *et al*., 1979). A study conducted to determine the effect of induced drought on different growth and biochemical attributes of black gram (*Vigna mungo*) and green gram (V*igna radiata*) 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). Therefore, assessment of membrane status can be examined by measuring cellular electrolyte leakage, hence cell membrane stability (CMS) in plant cells (Saneoka *et al*., 2004; Farooq and Azam, 2006; Manavalan *et al*., 2009).

Nitrogen is an essential element for growth and development of plants (Sogut, 2006). It plays vital significant roles in photosynthesis, protein formation, DNA synthesis and many other functions (Caliskan *et al*., 2008; Salvagiotti *et al*., 2008). Supplementation of adequate nitrogen to crops can increase their growth and development and ultimately plants are able to produce higher values of yield components that result in higher seed yields (Caliskan *et al*., 2008). The soil beneficial bacteria such as *Rhizobium* tend to release/synthesize growth promoting substances which are phytohormones like auxins as secondary metabolites in inoculated plants. These phytohormones are known to play a key role in plant growth regulation by promoting root elongation and stimulation of leaf expansion. In addition, great root development and proliferation of plants in response to *Rhizobium* activities improve plant water relations and nutrient uptake that result in a better cell membrane stability and relative leaf water content (Werner and Newton, 2005). Determination of water status in response to *Rhizobium* inoculation is very important to maximize yield and economic profitability of common bean production in a particular environment. N fertilization and/or N as a result of *Rhizobium* inoculation had anticipated to increase the leaf water content in chick pea (Namvar *et al*., 2013), leaf water relation in *Agrostis palustrais* Huds (Saneoka *et al*., 2004), leaf water content in *Sophora davidii* seedling (Fuzhong *et al*., 2008) and in sunflower hybrid (Gholinezhad *et al*., 2009). Therefore, there is a need to assess the effects of water stress and *Rhizobium* inoculation on physiological parameters including cell membrane stability in *P. vulgaris* (L) plant cells.

6.2 Materials and Methods

6.2.1 Narrative of Site Location

The trial was conducted at Agricultural Seed Agency (ASA) farm in Arusha, located at Latitude 3°18′S and Longitude 36°38′06.29″E. ASA receives the mean annual rainfall of 819mm, mean temperature of 19.15°C with relative humidity of about 94% and altitude of 1520 m.a.s.l. The two field trials were carried out under controlled irrigation during dry season of January to March 2014 and January to March, 2015 respectively, while the screen house experiment was carried out from mid January to March, 2016 under irrigation.

6.2.2 Experimental Design and Treatment Application

The experiment was designed in split, split plot with 3 replications. The plot size was 3 m x 4 m. The field experimental treatments consisted of 2 levels of Rhizobia (with and without inoculation) as the main factor followed by imposing of stress (sub factor) in vegetative and flowering stages of plant growth. Five cultivars of *P. vulgaris* (L.) namely, *KAT B9, KAT B1, F9 Kidney Selection, F8 Drought line* and *JESCA* were assigned to sub-sub plots. The common bean seeds were sown at a spacing of 50 cm x 20 cm, making a plant population density of 200,000 plants per hectare. The BIOFIX legume inoculants were obtained from *MEA* Company Nairobi-Kenya, sold under license from the University of Nairobi. Common bean seeds lines and/or varieties *KAT B9*, *KAT B1*, *F9 Kidney Selection*, *F8 Drought line and JESCA* were obtained from the breeding unit based at Selian Agricultural Research Institute (SARI), Arusha, Tanzania. Land for field experiment was cleared and all the necessary practices like ploughing and harrowing were done before planting. Moreover, in the screen house experiment, the wooden box technique was used to establish the experiment. This was done by collecting the same soil used at field experiment and beans were planted using the protocol developed by (Agbicodo et al., 2009) with some modifications. Common bean seeds were thoroughly mixed with *Rhizobium* inoculants to supply (10^9 cells g^{-1} seed), following procedure stipulated by products manufacturer. To avoid contamination, all non-inoculated seeds were sown first, followed by inoculated seeds. Three seeds were sown and thinned to two plants per hill after full plant establishment. Stress period of 10 days were imposed at vegetative and flowering stages of plant growth by not irrigating.

6.2.3 Study of Physiological Parameters in *P. vulgaris* (L.)

(i) Relative Leaf Water Contents (RLWC)

To determine RLWC, 1.5 g of plant leaf were selected and weighed immediately to record fresh weight (FW). In order to determine the turgid weight (TW), which represents fully hydrated weight, the samples were floated in distilled water inside a closed Petri dish for 20 h to regain full turgor. Samples were then placed in an oven at 70 °C for 24 h, so as to obtain dry weight (DW). The RLWC were determined by the equation proposed by Mansouri-far *et al*. (2010) as follows: RLWC = [(fresh weight – dry weight)/ (turgid weight - dry weight)] x 100.

(ii) Measurement of Electrolyte Leakage (EL)

Cell membrane permeability was estimated by electrolyte leakage (EL) according to Valentovic *et al*. (2006) with few modifications. Leaves samples (0.5 g) were excised, washed with deionized water, and placed in test tubes containing 20 mL distilled ionized water and incubated at 25°C after which the electrical conductivity of bathing solution (L1) was determined. The samples were then autoclaved at 120°C for 20 min to release all electrolytes, cooled to 25°C. After cooling, the final electrical conductivity (L2) was determined. The EL was expressed following the formula; EL= (L1/L2) × 100.

6.2.4 Statistical Analysis

A 3-Way ANOVA was used to analyze data collected. The analysis was done using STATISTICA software programe of 2013. Fisher's least significant difference was used to compare treatment means at $p = 0.05$ (Steel and Torrie, 1980).

6.3 Results

6.3.1 Relative leaf water content and electrolyte leakage in *P. vulgaris* (L.) plant leaves as influenced by water stress periods and rhizobial inoculation in field and screen house experiments

The significance increase in leaf relative water content was observed in inoculated compared with non-inoculated treatments (Tables 9 - 11). Rhizobial inoculation significantly increased leaf relative water content during vegetative and flowering growth stages by 9 % and 7 % in season one (Table 9). In screen house experiment, inoculation with *Rhizobium* strain increased the leaf relative water content by 15 % in flowering as compared with un-inoculated treatments (Table 11). Water stress treatments significantly increased leaf relative water content by 7 % in season two at vegetative stage in field experiment and by 13 % in screen house experiment at vegetative stage (Tables 9 & 11). In field experiment, significant increase in leaf relative water content (%) was also recorded in varieties *F8 Drought Line*, *JESCA* and *F9 Kidney Selection* as compared with varieties *KAT B9* and *KAT B1* respectively (Table 9). Varieties *JESCA*, *F8 Drought Line*, *F9 Kidney Selection* and *KAT B1* significantly increased leaf relative water content in screen house experiment at flowering stage as compared with variety *KAT B9* (Table 11). The cell

membrane stability was significantly ($P \leq 0.05$) affected by rhizobial inoculation. Inoculated treatment had statistically more electrolyte leakage than non-inoculated treatment. Rhizobial inoculation increased electrolyte leakage by 6 % and 7 % in season one at flowering stage and season two in vegetative stage respectively (Table 9). Water stress treatments significantly increased electrolyte leakage by 8 % in season one at vegetative stage (Table 9). Varieties *KAT B9* and *KAT B1* significantly increased electrolyte leakage percentage in season one and two at vegetative and flowering growth stages as compared with the other varieties (Table 9). In screen house experiment, significant increase in electrolyte leakage percentage was also recorded in varieties *KAT B9* and *KAT B1* as compared with varieties *F9 Kidney Selection*, *F8 Drought Line* and *JESCA* respectively (Table 11).

6.3.2 Interactive effect of inoculation with *Rhizobium* and stress periods on relative leaf water content and electrolyte leakage in selected *P. vulgaris* (L.) cultivars

There were significant interactive effects between rhizobial inoculation and water stress treatments in Relative Leaf water content in fields and screen house experiments (Figs. 18 & 20). Rhizobial inoculated treatments and un- stressed water treatments increased relative leaf water content in the studied *P.vulgaris* (L.) cultivars. Significant interaction in leaf relative water content (%) was also observed between rhizobial inoculation and varieties whereby varieties *F8 Drought Line, JESCA* and *F9 Kidney Selection* significantly increased relative leaf water content at inoculated and non-inoculated treatments as compared with *KAT B9* and *KAT* B1 in field experiment at flowering stage (Fig. 19). However, significant interaction in Electrolyte leakage (%) was observed in the screen house experiment between rhizobial inoculation, stress level and bean varieties during flowering stage whereby varieties *KAT B9* and *KAT B1* did well at non-stressed water treatments and at rhizobial inoculated treatments in screen house experiments (Fig. 21). Generally, non- stressed water treatments and rhizobial inoculated treatments had increased relative leaf water content and Electrolyte leakage in the selected *P. vulgaris* cultivars.

Table 9: Effect of *Rhizobium*, stress period and five (5) *P. vulgaris* in Relative Leaf Water Content as measured on field experiment in two consecutive seasons

	1st Season		2nd Season	
	Vegetative Stage	**Flowering Stage**	**Vegetative Stage**	**Flowering Stage**
Treatments				
R+	55.70±2.30a	51.67±2.39a	52.77±2.19a	51.00±2.63a
R-	50.67±2.21b	48.27±2.14b	51.76±1.81a	47.20±2.50a
Stress Levels				
S_1	54.90±2.42a	50.70±2.32a	54.10±2.02a	50.33±2.70a
S_2/S_3	51.47±2.15a	49.23±2.26a	50.43±1.93b	47.87±2.45a
Varieties				
V_1	41.67±1.88c	35.75±1.46c	40.75±1.49c	38.42±1.91c
V_2	44.00±4.06c	40.50±2.07c	41.75±1.62c	32.75±1.45c
V_3	55.17±2.64b	53.83±2.66b	55.42±1.94b	51.75±3.23b
V_4	65.00±1.81a	60.25±1.78a	61.75±1.19a	60.33±2.41a
V_5	60.08±1.62ab	59.50±2.60a	61.67±1.97a	62.25±1.97a
3-Way Anova (F-Statistics)				
Rhz	5.31*	4.45*	0.44ns	3.28ns
StrL	2.47ns	0.83ns	5.98*	1.38ns
Vrty	17.12**	38.73***	38.37***	31.20***
Rhz*StrL	0.20ns	13.24***	3.83ns	2.83ns
Rhz*Vrty	1.29ns	2.97*	0.26ns	0.74ns
StrL*Vrty	0.89ns	0.41ns	0.35ns	0.05ns
Rhz*StrL*Vrty	1.02ns	1.78ns	0.48ns	0.28ns

+R: With *Rhizobium*, −R: Without *Rhizobium.* S_1: No water stress, S_2: Water stress at Vegetative Stage, S_3: Water stress at Flowering Stage. V_1: *KAT B9*, V_2: *KAT B1*, V_3: *F9 Kidney Selection*, V_4: *F8 Drought Line*, V_5: *JESCA*. Values presented are means ± SE. *, **, *** = significant at $p \leq 0.05$, at $p \leq 0.01$, and at $p \leq 0.001$ respectively, ns = Not significant. Means followed by similar letter(s) in a given column are not significantly difference from each other at $p = 0.05$.

Table 10: Effect of *Rhizobium*, stress period and five (5) *P. vulgaris* in Electrolyte Leakage as measured on field experiment in two consecutive seasons

	1st Season		2nd Season	
	Vegetative Stage	**Flowering Stage**	**Vegetative Stage**	**Flowering Stage**
Treatments				
R+	58.03±2.37a	64.40±1.98a	65.87±2.75a	66.20±2.56a
R-	60.90±2.76a	60.33±2.01b	61.20±3.03b	65.80±2.51a
Stress Levels				
S_1	62.10±2.66a	63.63±1.89a	63.63±2.72a	66.40±2.32a
S_2/S_3	56.83±2.41b	61.10±2.14a	63.43±3.11a	65.60±2.73a
Varieties				
V_1	72.25±2.20a	73.83±1.54a	81.00±1.93a	81.08±1.94a
V_2	73.50±2.80a	70.42±1.82a	79.33±2.23a	79.25±1.95a
V_3	53.58±2.72b	53.42±2.69b	54.08±2.34b	52.92±1.67b
V_4	45.58±1.97c	56.33±2.25b	52.25±1.88b	58.17±2.38b
V_5	52.42±2.52bc	57.83±2.49b	51.00±3.22b	58.58±2.40b
3-Way Anova (F-Statistics)				
Rhz	1.67ns	4.80*	5.09*	0.04ns
StrL	5.63*	1.86ns	0.01ns	0.16ns
Vrty	25.83***	19.33***	43.38***	33.58***
Rhz*StrL	0.23ns	0.05ns	0.13ns	0.85ns
Rhz*Vrty	0.81ns	2.30ns	0.49ns	0.44ns
StrL*Vrty	0.36ns	1.22ns	1.74ns	0.36ns
Rhz*StrL*Vrty	0.48ns	0.23ns	0.92ns	0.60ns

+R: With *Rhizobium*, −R: Without *Rhizobium*. S_1: No water stress, S_2: Water stress at Vegetative Stage, S_3: Water stress at Flowering Stage. V_1: *KAT B9*, V_2: *KAT B1*, V_3: *F9 Kidney Selection*, V_4: *F8 Drought Line*, V_5: *JESCA*. Values presented are means ± SE. * and *** = significant at $p \leq 0.05$ and at $p \leq 0.001$ respectively, ns = Not significant. Means followed by similar letter(s) in a given column are not significantly difference from each other at $p = 0.05$.

Table 11: Effect of *Rhizobium*, stress period, and five (5) *P. vulgaris* in Relative Leaf water Content (%) and Electrolyte leakage (%) as measured on Screen house experiment in a single season

	Relative Leaf water Content (%)		**Electrolyte leakage (%)**	
	Vegetative	**Flowering**	**Vegetative**	**Flowering**
Treatments				
R+	77.28±2.51a	74.23±2.23a	60.90±1.99a	70.90±2.96a
R-	73.73±2.71a	63.11±2.58b	63.47±1.93a	71.67±2.54a
Stress Levels				
S_1	80.76±2.30a	68.85±2.64a	62.30±1.92a	71.20±2.52a
S_2/S_3	70.25±2.66b	68.50±2.50a	62.07±2.03a	71.37±2.98a
Varieties				
V_1	74.96±3.33a	57.51±3.10b	76.25±1.28a	78.58±4.45a
V_2	73.93±5.09a	69.75±3.17a	69.50±1.70b	77.50±3.95a
V_3	78.50±4.07a	75.65±4.09a	51.58±2.05d	72.17±3.15ab
V_4	75.12±4.50a	69.61±4.05a	57.25±1.47c	63.50±3.70b
V_5	75.00±3.88a	70.84±4.59a	56.33±1.45cd	64.67±4.85b
3-Way Anova (F-Statistics)				
Rhz	1.05ns	11.34**	2.94ns	0.05ns
StrL	9.22**	0.01ns	0.02ns	0.002ns
Vrty	0.20ns	3.30*	37.69***	3.12*
Rhz*StrL	4.34*	1.27ns	0.04ns	1.46ns
Rhz*Vrty	0.87ns	0.69ns	0.05ns	0.83ns
StrL*Vrty	1.83ns	0.59ns	0.36ns	0.25ns
Rhz*StrL*Vrty	0.91ns	0.88ns	1.60ns	2.91*

+R: With *Rhizobium*, −R: Without *Rhizobium*. S_1: No water stress, S_2: Water stress at Vegetative Stage, S_3: Water stress at Flowering Stage. V_1: *KAT B9*, V_2: *KAT B1*, V_3: *F9 Kidney Selection*, V_4: *F8 Drought Line*, V_5: *JESCA*. Values presented are means ± SE. *, **, *** = significant at $p \leq 0.05$, at $p \leq 0.01$, and at $p \leq 0.001$ respectively, ns = Not significant. Means followed by similar letter(s) in a given column are not significantly difference from each other at $p = 0.05$.

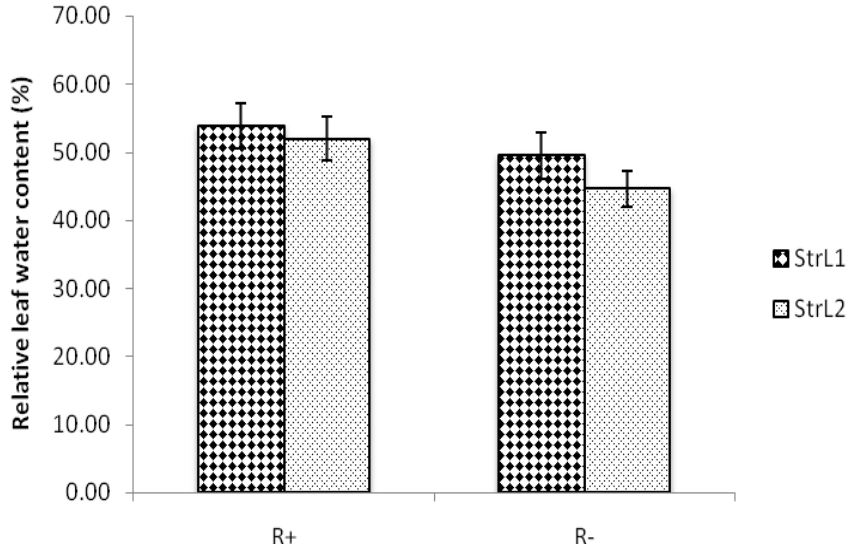


Figure 18: Interactive effects of *Rhizobium* and stress level on Relative leaf water content (%) in season (1) field experiment at flowering stage. +R: With *Rhizobium*, -R: Without *Rhizobium*. S1: Control, S3: Water stress at flowering stage

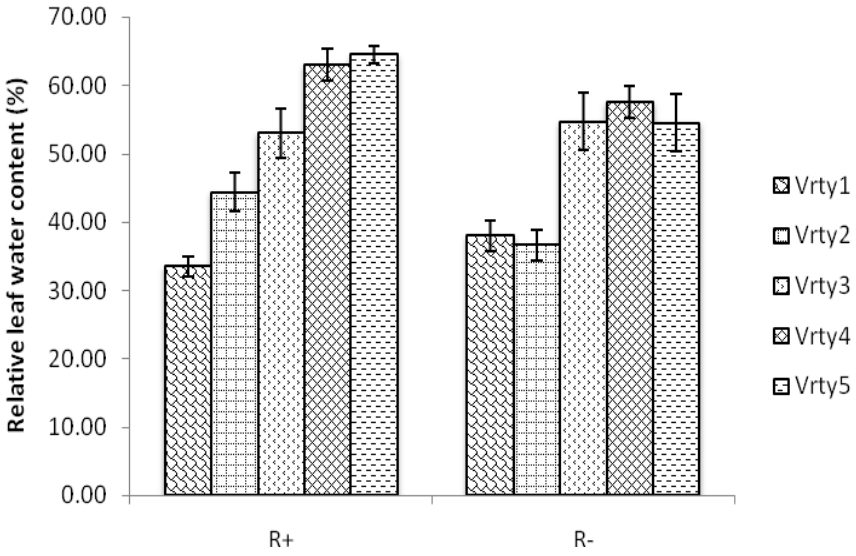


Figure 19: Interactive effects of *Rhizobium* and (5) *P.vulgaris* L. on Relative leaf water content (%) in season (1) field experiment at flowering stage. +R: With *Rhizobium*, -R: Without *Rhizobium*. V_1: *KAT B9*, V_2: *KAT B1*, V_3: *F9 Kidney Selection*, V_4: *F8 Drought Line*, V_5: *JESCA*

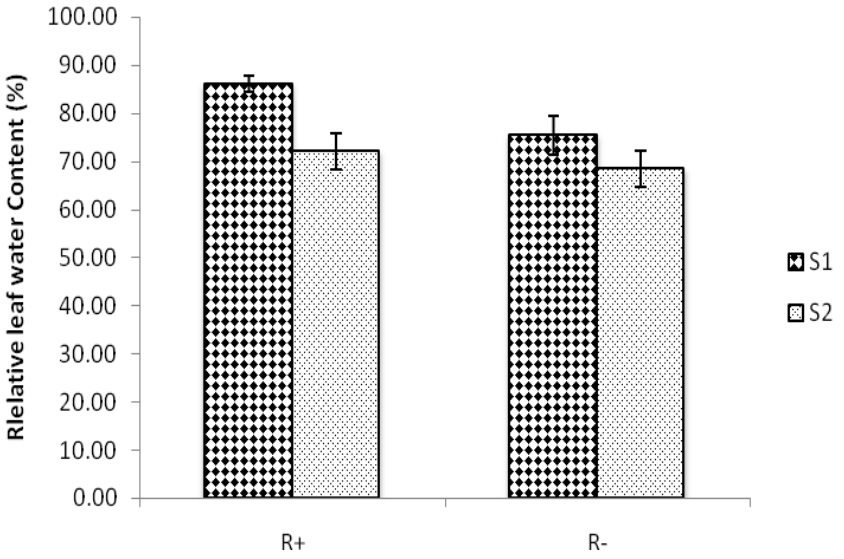


Figure 20: Interactive effects of *Rhizobium* and stress level on Relative leaf water content (%) screen house experiment at vegetative stage. +R: With *Rhizobium*, -R: Without *Rhizobium*. S1: Control, S2: Water stress at vegetative stage

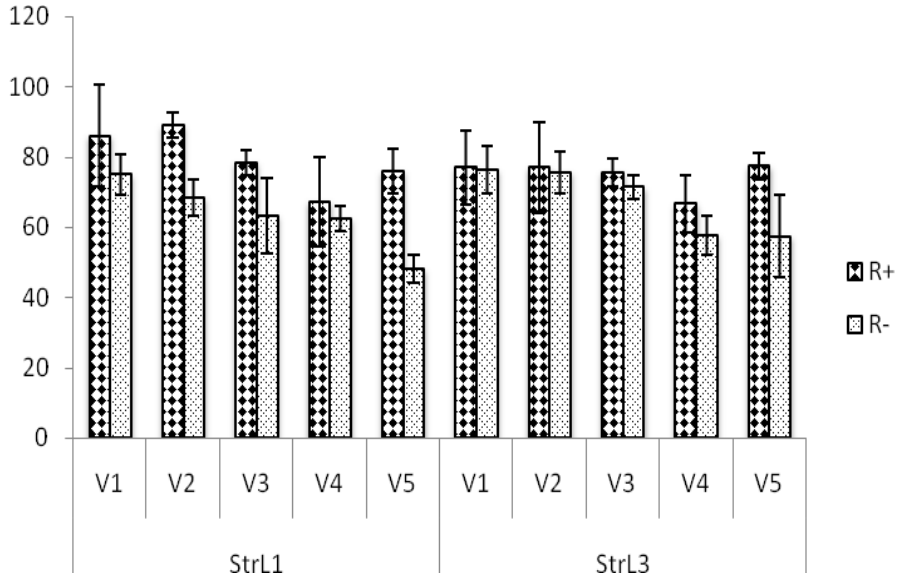


Figure 21: Interactive effects of rhizobial inoculation, stress level and five (5) *P. vulgaris* (L.) on Electrolyte Leakage (%) in screen house experiment at flowering stage. -R: Without rhizobial inoculation, +R: With rhizobial inoculation. StrL1: Control, StrL3: Water

stress at flowering stage, V_1: *KAT B9*, V_2: *KAT B1*, V_3: *F9 Kidney Selection*, V_4: *F8 Drought Line*, V_5: *JESCA*

6.4 Discussion

In the present study, we assessed the effects of *Rhizobium* inoculation and water stress periods on agronomic parameters in common bean (*P. vulgaris* L). *Rhizobium* inoculation had significantly positive response in relative leaf water content (RLWC) and electrolyte leakage (EL) both in field and screen house experiment (Table 9 - 11). These improvements in inoculated treatments could be attributed to improved BIOFIX legume inoculants which increased nitrogen supply to the plants and consequently improved the parameters examined. Relative leaf water content of the common bean showed significant response to *Rhizobium* inoculation. *Rhizobium* inoculation increased the leaf RWC compared with non-inoculated plants. Study done by Saneoka *et al*. (2004) and Fuzhong *et al*. (2008) on *Agrostis palustrais* Huds and *Sophora davidii* seedling showed similar findings as a result of inoculation. It has been reported that adequate level of nitrogen tend to increase the protein synthesis, cell wall thickness and cause absorption of additional water by protoplasm and improve the relative leaf water content (Saneoka *et al*., 2004). Study by Namvar *et al*. (2013) on chick pea showed a significant increase in RLWC as a result of *Rhizobium* inoculation.

Measuring plant water status is an important physiological index in identification of plant response to drought stress. Results showed that water stress significantly affected relative leaf water content (Tables 9 & 11). This decrease under moisture stress may be due to decreased leaf water potential and decreased availability/absorption and translocation of water from soil to roots and ultimately to leaves. These results are in line with the results of Khadem *et al*. (2010) who reported the reduction of RLWC as a result of water stress in corn leaf. It has been reported that RLWC in lentil was decreased by drought stress as compared with non-stressed conditions (Saneoka *et al*., 2004). Decline in RLWC of the leaf due to water stress is related to the reduction of soil moisture. Under such conditions, the stomata tend to be closed to avoid more water loss. The closure of stomata is due to abscisic acid made in the root of the stressed plants which is accumulated in stomata cells (Chaves *et al*., 2002). Furthermore, the decrease in relative leaf water content under water stress treatment could be associated with their ability of water

absorption from soil. Report by Ghanbari *et al*. (2013) showed that relative leaf water content is an essential indicator of assessing internal plant water status under drought conditions and has effectively been used to recognize drought-resistant cultivars of common bean. Cultivars and/or genotypes contained higher amount of relative water content condition have the ability to retain more water in their tissues. Report by Siddiqui *et al*. (2015) on different genotypes of faba bean showed that drought tolerance genotypes reveal high leaf RLWC as compared with sensitive genotypes. Study done by Khanna-Chopra and Selote (2007) showed that drought-resistant wheat plants exhibit better leaf water relations in terms of turgor potential and RLWC as compared to sensitive genotypes. Sanchez- Rodriguez *et al*. (2010) showed that RLWC was accepted for separating resistant and sensitive cultivars in tomato plants as well.

The cell membrane stability was significantly affected by N through inoculation with *Rhizobium* (Tables 10 and 11). Inoculated treatments contained significantly more stable cell membrane than non-inoculated plants. *Rhizobium* inoculation increased the cell membrane stability (CMS) as compared with non-inoculated one. Similar results were also reported by Saneoka *et al*. (2004) in *Agrostis palustrais* Huds. These researchers suggested that higher N levels helped to increase cell membrane stability which suggests that N nutrition may play an important role in maintaining cell compartmentalization and hence preventing the efflux of electrolyte under different condition in the cell. Under various environmental conditions, plant membranes are often associated with permeability and loss of its integrity (Blokhina *et al*., 2003). Increased leakage of solutes is an indication of damage caused to membrane (Surendar *et al*., 2013). Therefore, the ability of cell membranes to control the rate of ion movement in and out of the cells is used as a test of damage to a great range of tissues. Study done by Daniells and Watson, (1984) in wheat showed that cell membrane stability decreased under moisture stress and temperature stress at anthesis stage. Cell membrane stability was reduced in upland rice as a result of moisture stress condition (Konwar, 2009). It has been shown that the reduction in cell membrane stability (CMS) estimated by taking relative ion leakage, is an indicator of membrane damage as a result of membrane and/or lipid peroxidation caused by reactive oxygen species (ROS) (Upadhaya *et al*., 1989). The reduction in CMS is related to production of ROS which causes damage to membrane, lipids and protein. However, in some other studies indicated higher cell membrane stability under water stress condition.

Varieties *F9 Kidney Selection*, *F8 Drought line* and *JESCA* significantly increased relative leaf water content as compared with the other tested varieties. It has being established that leaf relative water content was introduced as a best criterion for plant water status, thus an indicator of choice for water stress tolerance in plants. There were significantly higher amount of electrolyte leakage in varieties *KAT B9* and *KAT B1* as compared with the other tested varieties.The movement of ions across the membrane is mainly associated with various adverse environmental conditions hence test of damage to plant tissues. It has been reported that the electrolyte leakage of the sensitive maize cultivar was seemed to be higher as compared with the resistant cultivar (Valentovic *et al*., 2006). Furthermore, the interactive effect between rhizobial inoculation, water stress and the tested varieties in the assessed physiological parameters is an indication which may justifies the genetic potentiality of some of the tested cultivars and hence a need for further studies under adverse environmental conditions such as drought and nutritional requirements.

6.5 Conclusion

In conclusion, rhizobial inoculation and non- stressed water treatments increased the relative water content and electrolyte leakage among the studied *P.vulgaris* cultivars. Furthermore, percentage relative leaf water content was higher in varieties *F9 Kidney Selection, F8 Drought Line* and *JESCA* hence indicating their potential to tolerate drought. The percentage electrolyte leakage was recorded in varieties *KAT B9* and *KAT B1*. The interactive effects between rhizobial inoculation, water stress and some identified cultivars provide insights for further studies in addressing the drought problem in *P. vulgaris* (L.).

CHAPTER SEVEN

Nutrient uptake in *Phaseolus vulgaris* (L.) cultivars as influenced by water stress and Rhizobial Inoculation

Abstract

Two season's field experiments were conducted to assess the effect of water stress periods and rhizobial inoculation in five *P. vulgaris* (L.) cultivars. The experiment consisted of with and without rhizobial inoculation, with and without water stress and five cultivars of *P. vulgaris* (L.) namely, *KAT B9*, *KAT B1*, *F9 Kidney Selection*, *F8 Drought line* and *JESCA*. Results showed that nutrients uptake (mg plant^{-1}) were higher in rhizobial inoculation and non-stressed water treatments. Varieties *F9 Kidney Selection*, *F8 Drought Line* and *JESCA* significantly recorded higher uptake of N, P, K, Ca and Mg in each growth stage of both seasons as compared with the other tested varieties. Additionally, significant interactive effects were observed between rhizobial and varieties on K and N uptake in both seasons, rhizobial and stress periods on P, Mg and K uptake in all seasons. Furthermore, significant interactive effects were also observed between inoculation, water stress periods and the tested *P. vulgaris* (L.) cultivars on N in season one and two respectively. Therefore, inoculated, non-stressed water treatments contain positive significant effects in the uptake of mineral nutrients. However, non-inoculated water stressed treatments had a negative effects on mineral nutrients uptake in the studied cultivars of *P.vulgaris* (L.).

Key words; Nutrients, *P.vulgaris*, inoculants, lines

Manuscript ready for submission to a journal

7.1 Introduction

Plant nutrition is of crucial role for plant growth and development. Plants require essential and non-essential elements of which every element and/or nutrients plays different functions which allow the plant to grow and reproduce (Uchida, 2000). Every plant nutrient is needed in different amounts by the plant and varies in how mobile it is within the plant. Most of the mineral elements for example nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium

(Mg), sulfur (S), iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), boron (B), molybdenum (Mo) and chlorine (CI) are supplied either from soil minerals and soil organic matter or by organic or inorganic fertilizers (Roy *et al*., 2006). However, carbon (C), hydrogen (H) and oxygen (O) are supplied by atmospheric carbon dioxide and water (Roy *et al*., 2006) and they represent 90 - 96 % of the dry matter of all plants (Samarah *et al*., 2004). The plant obtains the remaining 4 - 10 % from the soil and/or fertilizer inputs (Uchida, 2000). Uptake of the major elements N, P, K, Ca and Mg in various plant organs highly depends on the action of fauna activities available in the rhizosphere soils and the capability of the soil to replenish in the particular soil solutions (Makoi *et al*., 2013). For plant to grow and reproduce properly, mineral nutrients are essential chemical elements which are normally acquired in the form of inorganic ions from the soil (Taiz and Zeiger, 2006). All macronutrients are incorporated into important organic compounds such as amino acids and proteins (N and S), nucleic acids (N and P), phospholipids (P) and chlorophyll (Mg) apart from K and Ca (Amtmann and Blatt, 2009).

Mineral nutrients uptake by plants is a very effective process due to the large surface area of the roots and their ability to absorb inorganic ions at low concentrations in the soil solution. Therefore, availability of moisture in the soil is the great determinant of mineral nutrients to move through the soil matrix and be taken up by plants (Taiz and Zeiger, 2006); however, drought is an environmental factor which may result in nutrient deficiency.The physiochemical characteristics of the soil can lead to a reduced mobility and absorbance of individual nutrients (Amtmann and Blatt, 2009). Water stress inhibits the root to take plentiful nutrients from the soil because of reduced root activity, slow down of ion diffusion and reduction rates of water movement in the soil solution (Silva *et al*., 2011).

N is necessary for the formation of amino acids which are building blocks of protein also aid for plant growth which stimulated through cell division. It is an essential element in all living systems and needed by all cells and a major component of chlorophyll which converts sunlight into plant energy (Baligar, *et al*., 2001). N is very mobile and is normally available to plants in forms of NO_3^-(nitrate) or NH_4^+ (ammonium) ions (Marschner, 1995). It has been reported that nitrate is eagerly mobile in the xylem and can be stored in the vacuoles of roots, shoots and storage organs (Marschner, 1995); however ammonium has to be incorporated into organic compounds in the roots. Specifically, the demand for N can be fulfilled by mineral N fertilizers

or by other alternative means of using beneficial bacteria such as *Rhizobium* (Uchida, 2000). However, water stress reduces N fixation and growth in nodulated legumes (Zahran, 1999). The nitrogen status of a plant has a significant influence over its water relation as nitrogen and water often interact. When the soil faces a prolonged period of drought, nitrogen mobility is severely restricted by the dehydrated soil. Therefore moisture is directly proportional to mineral nutrients (N) absorption by plants and translocation from the roots to the shoots. Therefore, N is a constituent of plant proteins and is required for vegetative growth of plants.

Furthermore, phosphorus (P) is an essential mineral nutrient required in relatively large amount in order to maintain growth. P plays a significant fundamental role in conserving and transporting energy in the cell metabolism (Amtmann and Blatt, 2009). P occur in the soil in the form of orthophosphate and plants absorb in the form of $H_2PO_4^-$ and HPO_4^{2-} according to the pH of the growing medium (Mengel and Kirkby, 2001). P is essential for plant growth and function including symbiotic N_2-fixation processes. Microorganisms such as *Bradyrhizobium* inoculants may have effects on the chemistry of nutrients in soils by enhancing nutrients uptake by plants. For example, Makoi *et al*. (2013) reported improved uptake of macronutrients following inoculation with efficient strains of *Rhizobium*. Additionally supply of water is required for phosphate availability and absorption by plants. Phosphate ions move through diffusion and if the water content in the soil decreases, the amount of water-filled pores decrease hence P mobility decreases (Faye *et al*., 2006). Drought causes a reduction in P absorption and transport within the plant, therefore a decrease in available P in the soil, reduces P uptake by plants (Sardans and Penuela, 2004). It has been reported that water stress and/or dry soil decreases P content in the roots and shoots of *Spartina alterniflora* (Brown *et al*., 2006). Therefore, a strong regulation effect which is brought about by P in various plant functions such as carbohydrate synthesis during reproductive phase and root formation indicate its significance effects in this study.

Not only that but also K is another macronutrients which is taken up in great quantity by plants and is of essential role in the regulation of water status (Mengel and Kirkby, 2001). K is highly mobile in within individual cells and tissues as well as in extensive distance through xylem and phloem tissues of the plants (Marschner, 1995); it is the most abundant cation in the cytoplasm. Taiz and Zeiger (2006) reported that K is accumulated passively by both the cytosol and vacuole

however when extracellular K^+ concentrations are in small amount then it can be taken up actively. It has been reported that K ion plays a crucial physiological processes in plants for instance stomatal movement, protein synthesis, enzyme activation, osmoregulation, photosynthesis, cell extension (Marschener, 1995; Mengel and Kirkby, 2001; Mengel, 2007; Farooq *et al*., 2009). Potassium ion (K^+) accumulation in plant tissues are normally influenced by water condition in plants (Restrepo-Diaz *et al*., 2008). Studies showed that stomata opening mechanism is normally governing by K^+ concentration (Mengel and Kirkby 2001; Larcher, 2006; Taiz and Zeiger, 2006; Mengel, 2007). Plants tend to reduce stomata aperture beneath minimal water stress (Silva *et al*., 2003) however, when water stress becomes severe the stomata normally close (Larcher, 2006). Study by Mahouachi (2007) in banana plants found reduced levels of K^+ under drought condition. Restrepo-Diaz *et al*. (2008) also showed similar results on K^+ in the stressed leaves of olive plants.

Magnesium (Mg) is small, strongly electropositive divalent, whereby its major function is in chlorophyll molecules (Mengel and Kirkby, 2001; Amtmann and Blatt, 2009). Mg uptake is affected by the conditions of the soil and rhizosphere such as drought or irregular water availability. As Mg is not physically or physiologically available under conditions of water deficit, the plant roots are not capable of absorbing adequate Mg to sustain normal plant growth. It has been reported that Mg uptake in both the roots and shoots of *Spartina alterniflora* were reduced under drought conditions (Brown *et al*., 2006).

Besides, calcium is an alkaline earth metal that plays essential role in living organisms, it plays a fundamental functions as a signal for many cell processes for instance synthesis of new cell walls in the mitotic spindle during cell division (Taiz and Zeiger, 2006; Shao *et al*., 2008). Ca is abundant element in the soil and is readily available macroelements for plant uptake (Utrillas *et al*., 1995). Calcium desorption in the soil solution is normally attributed by the release of protons from the roots which eventually promotes the exchange reaction of the Ca bond in the organic and mineral soil phases (Van Praag *et al*., 2000). Study done on Bermuda grass (*Cynodon dactylon* (L.)) showed that Ca content increased during drought when grown in Mediterranean field conditions. However, contradictory results claim that drought tended to decrease Ca concentrations in the above ground biomass and this effect was attributed to the reduction in transpiration flux (Sardans *et al*., 2008). Therefore, there is a need to assess the effects of water

stress and *Rhizobium* inoculation on nutrient uptake in selected *P. Vulgaris* (L) cultivars and identify the potential ones which can perform well in water stressed environment.

7.2 Material and Methods

7.2.1 Description of Site Location

The trial was conducted at Agricultural Seed Agency (ASA) farm in Arusha, located at Latitude 3°18′S and Longitude 36°38′06.29″E. ASA receives the mean annual rainfall of 819mm, mean temperature of 19.15°C with relative humidity of about 94% and altitude of 1520 m.a.s.l. The trial was carried out during dry season of January, to March 2014 and January, to March, 2015 under irrigation.

7.2.2 Experimental Design and Treatment Application

The experiment was designed in split-split plot with 3 replications in plots of 3m x 4m size. The treatments consisted of 2 levels of Rhizobia (with and without inoculation) as the main factor, imposing stress in vegetative and flowering stages of plant growth as sub factor plot and five cultivars of *P. vulgaris* (L.) namely, *KAT B9, KAT B1, F9 Kidney Selection, F8 Drought line* and *JESCA* were assigned to sub-sub plots. The common bean seeds lines / varieties were obtained from the breeding unit based at Selian Agricultural Research Institute (SARI), Arusha, Tanzania. BIOFIX legume inoculants were obtained from *MEA* Company Nairobi-Kenya, sold under license from the University of Nairobi. Land for field experiment was cleared and all the necessary practices like ploughing and harrowing were done before planting. The bean seeds were thoroughly mixed with *Rhizobium* inoculants to supply (10^9 cells g^{-1} seed) as prescribed by products manufacturer. The seeds were sown at a spacing of 50 cm x 20 cm, making a plant population density of 200,000 plants per hectare and to avoid contamination, all non-inoculated seeds were sown first, followed by inoculated seeds. Three seeds were sown and thinned to two plants per hill after full plant establishment. Stress period of 10 days were imposed at vegetative (between 20 days when third trifoliolate leaf unfolded secondary branching begins to show from branch up to 30 days) and at flowering stages (around 40 days when plants begin to exhibit blossom and one blossom opens at any node in the given plants) by not irrigating. Plant samples were taken immediately after imposing of stress in each of the growth stages of vegetative and flowering respectively.

7.2.3 Plant Harvest and Sample Preparation

Shoot plant samples were collected for nutrients analysis. The common bean cultivars were sampled for dry matter and nutrient determination. 10 plants were randomly sampled from the middle rows of each plot. The shoots of the plant samples were oven dried at 60˚C for 48 hours, ground into a fine powder (2 mm sieve) for nutrients analysis.

7.2.4 Determination of Nutrients in the Shoots of *P.vulgaris* Cultivars

The dried shoot samples were taken to determine mineral nutrient content by appropriate methods for each nutrient element: Kjeldahl digestion method for N determination and wet oxidation method using spectrophotometer and flame photometer for P and K determination as described by Kaewpradit *et al*. (2009). Ca and Mg content were determined through atomic absorption spectrophotometer as decribed by Broekaert (2002). Nutrient uptake (mg plant^{-1}) was then calculated as the product of nutrient concentration (mg g^{-1}) and the weight of the plant dry weight (g plant^{-1}). The nutrients uptake was calculated following standard method; Nutrient Uptake (mg plant^{-1}) = Concentration of nutrient (mg g^{-1}) x plant dry weight (g plant^{-1})

7.2.5 Statistical Analysis

A 3-Way ANOVA was used to analyze the data collected. The analysis was done using STATISTICA software 2013. Fisher's least significant difference was used to compare treatment means at $p = 0.05$ (Steel and Torrie, 1980).

7.3 Results

The study presents results that were obtained from field experiment conducted twice (season 1 and 2).

7.3.1 Nutrients uptake (mg plant^{-1}) as influenced by water stress periods and rhizobial inoculation in selected *P.vulgaris* cultivars

The nutrient uptake in the two seasons is presented in Tables 12 and 13. Significance increase in nutrients uptake (mg plant^{-1}) was observed in inoculated compared with non-inoculated treatments (Tables 12 and 13). Rhizobial inoculation significantly increased Ca uptake by 3 % in season one at vegetative stage and P by 13% as well as Mg by 23 % in season one during flowering stage (Table 12). Furthermore rhizobial inoculation showed significant increase in N

uptake by 20 %, Mg by 32 % in season two at vegetative stage and K by 36 % and Ca by 18 % in season two at flowering stage respectively (Table 13). For plants imposed with stress at flowering stage in season one the water stress treatments significantly decreased Ca uptake (Table 1) and K uptake in season two at flowering stage (Table 13). For varieties, significant increase in nutrients uptake (mg plant^{-1}) was recorded in varieties *F9 Kidney Selection*, *F8 Drought Line* and *JESCA* as compared with varieties *KAT B9* and *KAT B1* at season 1 and 2 respectively (Tables 12 and 13).

7.3.2 Interactive effect of inoculation with *Rhizobium* and stress periods on nutrients uptake (mg plant^{-1}) in selected *P. vulgaris* (L.) cultivars

The interaction effects are presented in Figs. 22-28. There was a significant interaction between *Rhizobium inoculation* and stress period/levels in P uptake and Mg uptake both in season one and two at flowering stage (Figs. 24 and 25) as well as K uptake at flowering stage in season two (Fig. 28). Interactive effects of *Rhizobium* treatment and varieties increased K uptake where by varieties *F9 Kidney Selection* and *F8 Drought Line* significantly showed increased K uptake at inoculated treatments in season one at vegetative stage (Fig. 22); and N uptake in season two at vegetative stage (Fig. 26). Furthermore significant interaction was also observed between rhizobial inoculations, stress periods and bean cultivars during flowering stage in season one and at vegetative stage in season two (Figs. 23 and 27).

Table 12: Effect of *Rhizobium*, stress period and five (5) *P. vulgaris* on nutrients uptake (mg plant^{-1}) as measured on fields experiments in two consecutive seasons

	1st Season									
Growth Phases	Vegetative					Flowering				
Treatments	N	P	K	Ca	Mg	N	P	K	Ca	Mg
R+	3.22±0.05a	0.18±0.003a	1.78±0.03a	1.49±0.02a	0.40±0.02a	3.07±0.21a	0.23±0.009a	1.36±0.03a	1.44±0.04a	0.39±0.01a
R-	3.21±0.04a	0.19±0.007a	1.74±0.02a	1.45±0.01b	0.36±0.01a	2.72±0.18a	0.20±0.008b	1.43±0.04a	1.39±0.03a	0.30±0.02b
Stress Levels										
S_1	3.24±0.04a	0.18±0.005a	1.77±0.03a	1.46±0.02a	0.38±0.02a	2.79±0.19a	0.22±0.011a	1.38±0.04a	1.48±0.04a	0.34±0.02a
S_2/S_3	3.20±0.05a	0.19±0.006a	1.75±0.03a	1.47±0.02a	0.37±0.02a	3.00±0.21a	0.21±0.009a	1.41±0.03a	1.35±0.04b	0.35±0.01a
Varieties										
V_1	3.04±0.07b	0.18±0.004bc	1.82±0.04a	1.47±0.01a	0.32±0.02c	3.23±0.30a	0.23±0.017a	1.17±0.04b	1.24±0.05b	0.32±0.01b
V_2	3.02±0.06b	0.17±0.005c	1.70±0.05b	1.48±0.03a	0.33±0.03bc	2.74±0.32ab	0.21±0.014a	1.26±0.04b	1.23±0.05b	0.33±0.01b
V_3	3.37±0.05a	0.20±0.008ab	1.71±003b	1.46±0.02a	0.44±0.03a	2.34±0.17b	0.19±0.011a	1.52±0.03a	1.54±0.03a	0.35±0.02a
V_4	3.34±0.04a	0.21±0.014a	1.74±0.06ab	1.45±0.02a	0.41±0.04ab	3.12±0.37ab	0.22±0.015a	1.56±0.04a	1.55±0.02a	0.36±0.02a
V_5	3.33±0.07a	0.19±0.002ab	1.84±0.03a	1.50±0.03a	0.39±0.02abc	3.04±0.35ab	0.22±0.018a	1.46±0.06a	1.50±0.05a	0.35±0.01ab
3-Way Anova (F-Statistics)										
Rhz	0.01ns	0.59ns	1.25ns	4.34*	2.86ns	1.83ns	5.74*	3.89ns	1.38ns	201.30***
StrL	0.57ns	1.27ns	0.61ns	0.07ns	0.12ns	0.67ns	0.04ns	0.82ns	11.65**	0.04ns
Vrty	7.27***	4.29**	2.68*	0.83ns	3.12*	1.52ns	0.74ns	14.09***	13.55***	4.69**
Rhz*StrL	2.78ns	0.01ns	0.09ns	2.51ns	5.99ns	1.93ns	0.57ns	0.06ns	1.38ns	0.26ns
Rhz*Vrty	0.56ns	0.69ns	4.60**	1.75ns	0.34ns	1.17ns	0.71ns	0.26ns	0.72ns	0.12ns
StrL*Vrty	0.31ns	0.54ns	0.81ns	1.47ns	1.33ns	0.27ns	0.24ns	1.17ns	0.50ns	0.30ns
Rhz*StrL*Vrty	0.40ns	0.75ns	0.53ns	1.01ns	0.20ns	2.72*	0.27ns	0.58ns	0.15ns	0.35ns

+R: inoculation, −R: no inoculation. S_1: No water stress, S_2: Water stress at Vegetative Stage, S_3: Water stress at Flowering Stage. V_1: *KAT B9*, V_2: *KAT B1*, V_3: *F9 Kidney Selection*, V_4: *F8 Drought Line*, V_5: *JESCA*. Values presented are means ± SE. **, *** = significant at $p \leq 0.01$ and at $p \leq 0.001$ respectively, ns = Not significant. Means followed by similar letter(s) in a given column are not significantly difference from each other at $p = 0.05$.

Table 13: Effect of *Rhizobium*, stress period, and five (5) *P. vulgaris* on nutrients uptake (mg plant^{-1}) as measured on field's experiments in two consecutive seasons

2nd Season										
Growth Phases	Vegetative					Flowering				
Treatments	N	P	K	Ca	Mg	N	P	K	Ca	Mg
R+	4.86±0.03a	0.26±0.006a	2.12±0.04a	1.62±0.05a	0.60±0.02a	4.36±0.14a	0.24±0.017a	2.54±0.06a	1.73±0.05a	0.49±0.02a
R-	3.88±0.11b	0.25±0.009a	2.05±0.05a	1.54±0.04a	0.41±0.01b	4.24±0.12a	0.22±0.013a	1.63±0.02b	1.42±0.03b	0.48±0.03a
Stress Levels										
S_1	4.40±0.12a	0.26±0.007a	2.09±0.04a	1.62±0.04a	0.51±0.02a	4.32±0.13a	0.23±0.017a	2.14±0.11a	1.60±0.05a	0.51±0.02a
S_2/S_3	4.34±0.13a	0.25±0.009a	2.08±0.05a	1.54±0.05a	0.50±0.02a	4.28±0.11a	0.22±0.014a	2.02±0.08b	1.55±0.04a	0.46±0.03a
Varieties										
V_1	3.98±0.23c	0.22±0.011c	1.81±0.04d	1.40±0.06c	0.43±0.02c	3.78±0.12bc	0.19±0.026a	1.90±0.13b	1.57±0.08a	0.46±0.04a
V_2	4.19±0.22bc	0.21±0.012c	1.95±0.05c	1.50±0.06bc	0.46±0.02c	3.69±0.13c	0.24±0.023a	1.89±0.14b	1.56±0.07a	0.54±0.03a
V_3	4.75±0.09a	0.29±0.002a	2.32±0.03a	1.62±0.08ab	0.59±0.04a	5.09±0.09a	0.25±0.022a	2.26±0.16a	1.58±0.07a	0.46±0.03a
V_4	4.60±0.12a	0.28±0.003ab	2.20±0.04ab	1.71±0.07a	0.56±0.03a	4.75±0.14a	0.20±0.010a	2.19±0.15a	1.59±0.08a	0.48±0.04a
V_5	4.33±0.19b	0.27±0.001b	2.13±0.05b	1.68±0.06ab	0.50±0.03b	4.17±0.14b	0.24±0.036a	2.17±0.17a	1.58±0.09a	0.50±0.05a
3-Way Anova (F-Statistics)										
Rhz	180.71***	2.81ns	2.70ns	1.82ns	215.75***	0.80ns	0.74ns	457.45***	21.90***	0.09ns
StrL	0.73ns	0.65ns	0.05ns	1.59ns	0.06ns	0.10ns	0.01ns	8.17**	0.46ns	2.06ns
Vrty	14.54***	15.91***	20.21***	3.49*	21.88***	18.91***	1.18ns	13.12***	0.02ns	0.63ns
Rhz*StrL	2.98ns	1.94ns	1.01ns	0.19ns	0.02ns	0.71ns	8.77**	12.47**	0.56ns	4.15*
Rhz*Vrty	5.84***	0.47ns	0.38ns	0.58ns	0.68ns	0.45ns	1.17ns	2.13ns	0.35ns	0.99ns
StrL*Vrty	2.11ns	0.86ns	0.45ns	0.81ns	1.02ns	0.11ns	0.89ns	1.38ns	0.23ns	1.32ns
Rhz*StrL*Vrty	3.16*	0.68ns	0.15ns	1.90ns	0.09ns	0.34ns	0.25ns	1.34ns	0.22ns	0.06ns

+R: inoculation, −R: no inoculation. S_1: No water stress, S_2: Water stress at Vegetative Stage, S_3: Water stress at Flowering Stage. V_1: *KAT B9*, V_2: *KAT B1*, V_3: *F9 Kidney Selection*, V_4: *F8 Drought Line*, V_5: *JESCA*. Values presented are means ± SE. **, *** = significant at $p \leq 0.01$ and at $p \leq 0.001$ respectively, ns = Not significant. Means followed by similar letter(s) in a given column are not significantly difference from each other at $p = 0.05$.

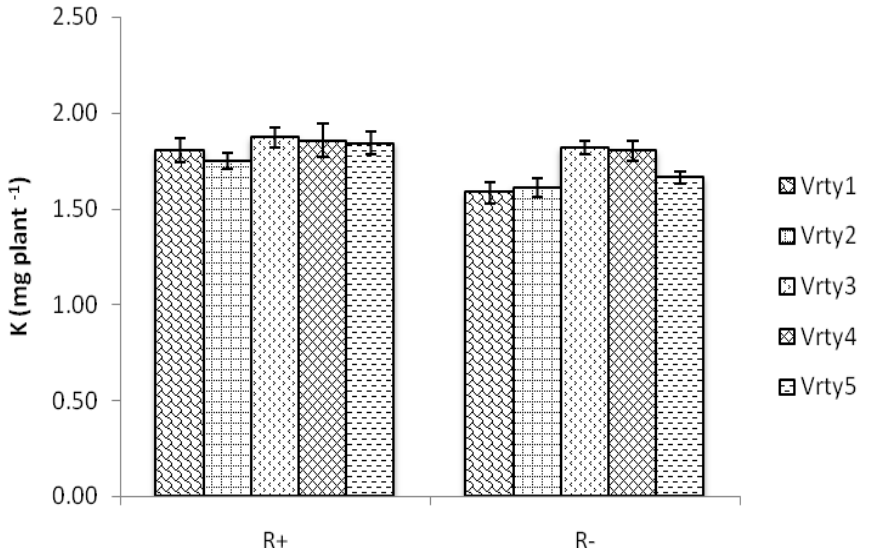


Figure 22: Interactive effects of *Rhizobium* inoculation and five *P. vulgaris* (L.) cultivars in K uptake (mg plant^{-1}) on field experiment at vegetative stage in season one. +R: inoculation, -R: no inoculation. V_1: *KAT B9*, V_2: *KAT B1*, V_3: *F9 Kidney Selection*, V_4: *F8 Drought Line*, V_5: *JESCA*

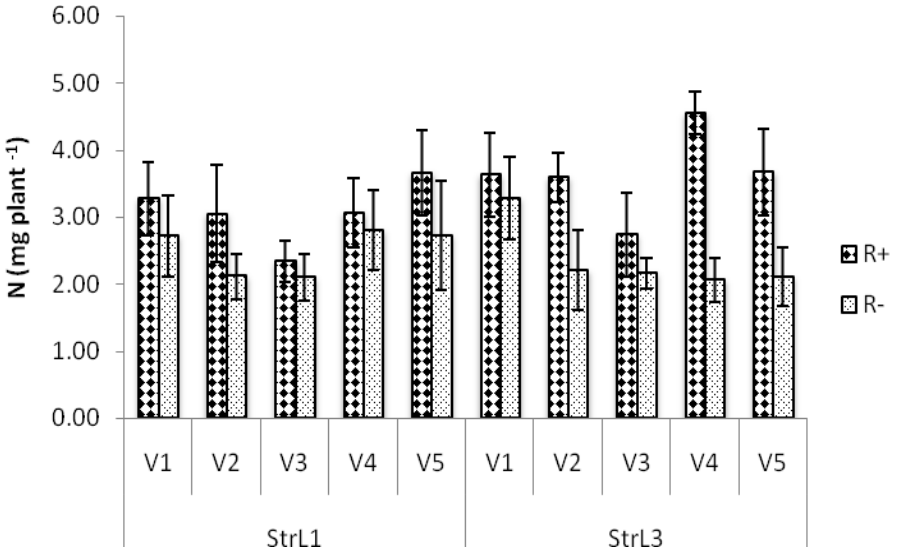


Figure 23: Interactive effects of rhizobial inoculation, stress level and five (5) *P. vulgaris* (L.) on N uptake (mg plant^{-1}) at flowering stage in season one. +R: inoculation, -R: no

inoculation. StrL1: Control, StrL3: Water stress at flowering stage. V_1: *KAT B9*, V_2: *KAT B1*, V_3: *F9 Kidney Selection*, V_4: *F8 Drought Line*, V_5: *JESCA*

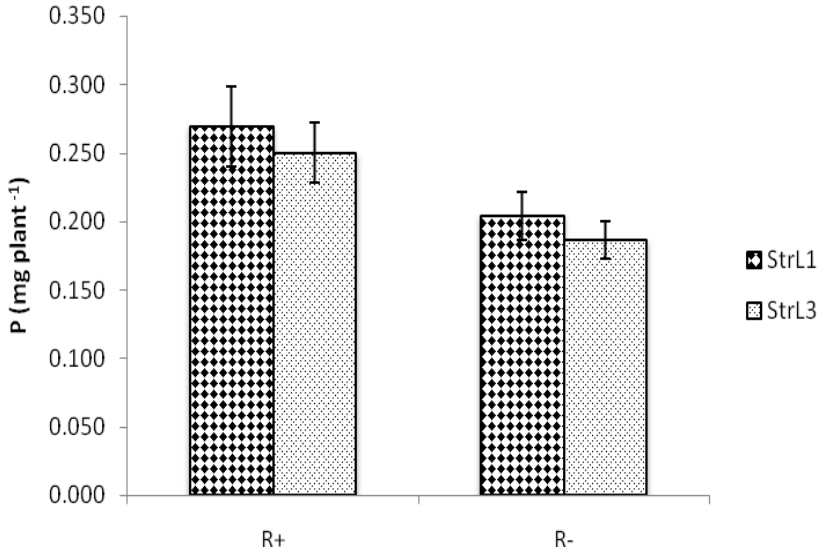


Figure 24: Interactive effects of *Rhizobium* and Stress level on P uptake (mg plant^{-1}) in field experiment at flowering stage in season two. +R: inoculation, -R: no inoculation. StrL1: Control StrL3: Water stress at flowering stage

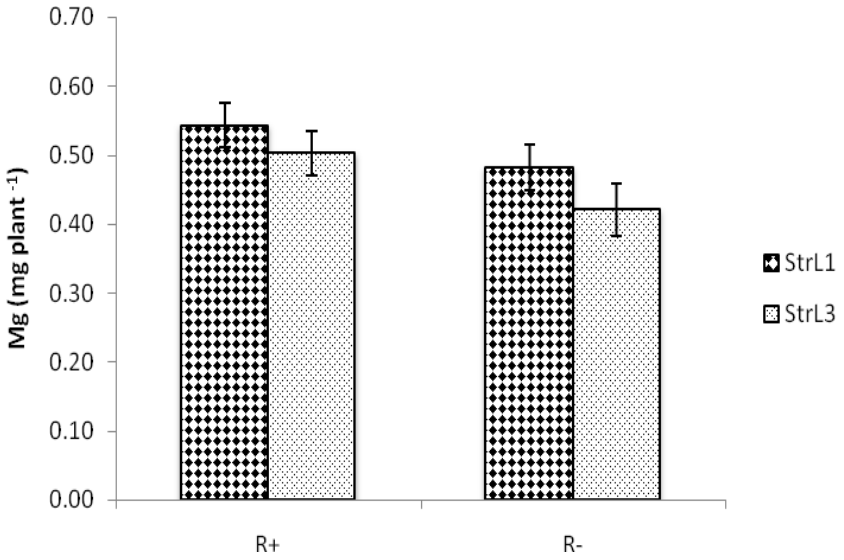


Figure 25: Interactive effects of *Rhizobium* and Stress level on Mg uptake (mg plant^{-1}) in field experiment at flowering stage in season two. +R: inoculation, -R: no inoculation. StrL1: Control StrL3: Water stress at flowering stage

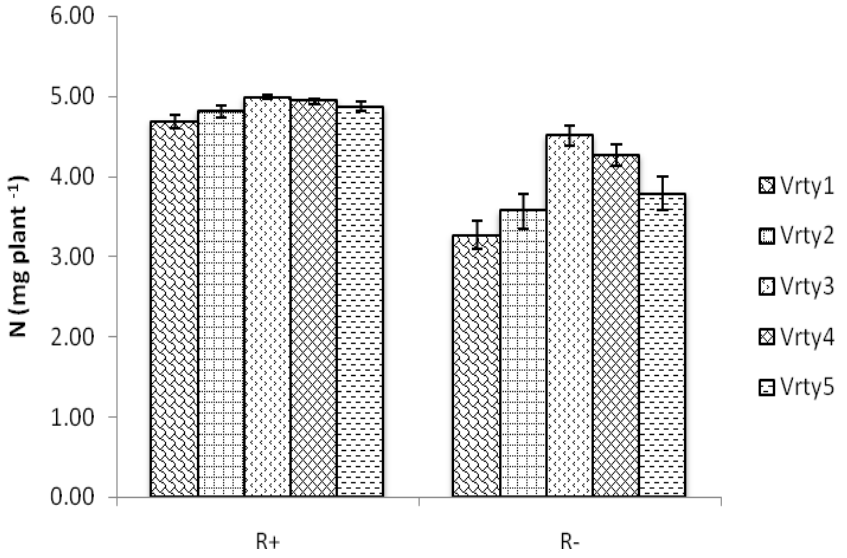


Figure 26: Interactive effects of *Rhizobium* and five (5) *P. vulgaris* (L.) on N uptake (mg plant^{-1}) in field experiment at vegetative stage in season two. +R: inoculation, -R: no inoculation, Vrty1: *KAT B9*, Vrty2: *KAT B1*, Vrty3: *F9 Kidney Selection*, Vrty4: *F8 Drought Line*, Vrty5: *JESCA*

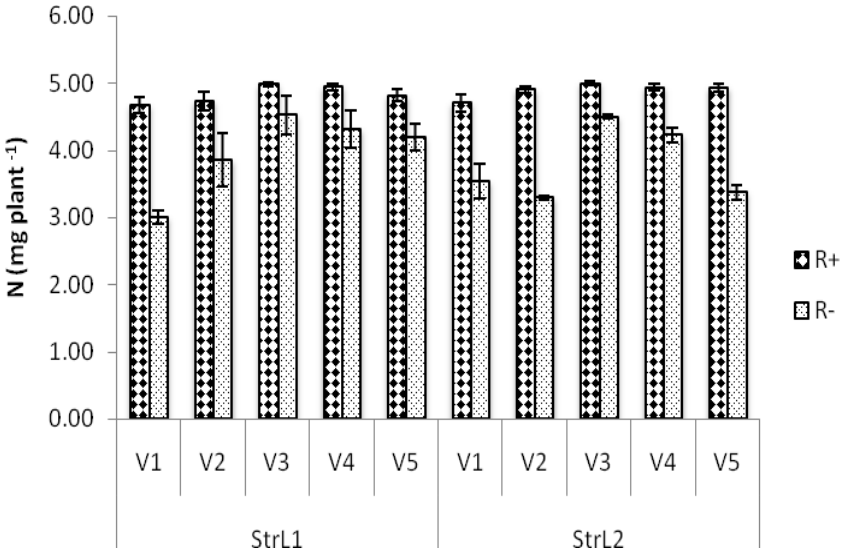


Figure 27: Interactive effects of *Rhizobium* and Stress level and five (5) *P. vulgaris* (L.) on N uptake (mg plant^{-1}) in field experiment at vegetative stage in season two. +R: inoculation, -R: no inoculation. StrL1: Control, StrL2: Water stress at vegetative stage, V1: *KAT B9*, V2: *KAT B1*, V3: *F9 Kidney Selection*, V4: *F8 Drought Line*, V5: *JESCA*

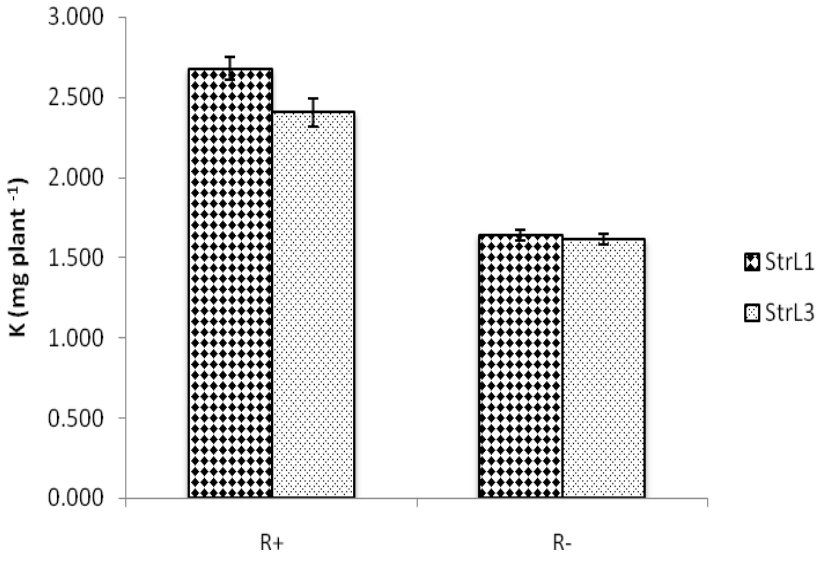


Figure 28: Interactive effects of *Rhizobium* and Stress level on K uptake (mg plant^{-1}) in field experiment at flowering stage in season two. +R: inoculation, -R: no inoculation. StrL1: Control StrL3: Water stress at flowering stage

7.4 Discussion

The results revealed a significant increase in the uptake of mineral elements as a result of rhizobial inoculation. Secretion of chemical substances by rhizobia bacteria and production of plant growth hormones might have stimulated plant growth and eventually increased the uptake of nutrients in plant tissues (Perveen *et al*., 2002; Khan and Zaidi, 2007). Studies (Noel *et al*., 1996; Ahmad *et al*., 2008; Wani *et al*., 2008a) indicated that rhizobia bacteria tend to synthesize phytohormones, siderophores, indole 3-acetic acid (IAA) and cytokinins which stimulate plant growth and eventually increase the uptake of these nutrients by plants. Report by Mausumi and Raychaudhuri (2008) indicated significant increase of P and Ca uptake in groundnuts as a result of *Rhizobium* inoculation. The increase in mineral nutrients uptake might have been influenced

by enough soil volume thus increased root length which facilitated the plants to capture nutrients in the nearby plant roots and allow for more nutrients uptake. The rhizosphere tends to be modified by the released dead cells of rhizobial inoculants. The dead cells of rhizobial inoculants may contain mineral nutrients that can solubilise unavailable soil nutrients to become available and utilized by plants (Halder and Chakrabartty 1993; Abd-Alla, 1994; Phillips and Dakora, 2002). Microorganisms such as rhizobial inoculants may have effects on the chemistry of nutrients in soils by enhancing nutrients uptake by plants. Study done by Makoi *et al*. (2013) in common bean showed improvement of macronutrients using strain of *Rhizobium*. It has also been reported that rhizobial inoculation significantly increased the uptake of macronutrients (N, P, K, Ca and Mg) in roots, shoots, pods and the whole plant of soybean grown in glasshouse and field experiment respectively (Tairo and Ndakidemi, 2014).

Generally, the decrease in nutrient uptake during water stress was observed throughout the experiments. Water stress causes decrease in moisture availability in soils which strongly influences nutrient absorption and uptake by plants (Garg, 2003). Decreasing water availability under drought results in limited total nutrient uptake and their diminished tissue concentrations in crop plants. An important effect of water deficit is on the acquisition of nutrients by the root and their transport to shoots. Furthermore, in this study, water stress in some of the tested bean lines increased their nutrient uptake. It is reported that plant species and genotypes may vary in their response to mineral elements uptake under water stress (Garg, 2003). Most nutrients are absorbed by plant roots as ions and water is the medium of transport. Under fully irrigated conditions, when soil water potential is high, the absorption and transport of water and nutrients are also high. During the soil dryness roots are not capable of taking up nutrients from the soil because of the lack of activity of fine roots, water movement and ionic diffusion of nutrients (Prasad *et al*., 2008). Therefore, limited soil moisture due to drought results in a reduction of total nutrients uptake in crop plants (Baligar *et al*., 2001; Gunes *et al*., 2006). Non- stressed water treatments significantly increased the uptake of Ca in season one at flowering stage and K in season two at flowering stage respectively. The significance difference in response to nutrient uptakes could possibly be due to root responses to water stress as roots play an important role in drought adaptation in different soil types (Vadez *et al*., 2007). Baligar *et al*. (2001) noted that drought stress generally results in reduced total nutrients uptake and frequently reduces the levels of mineral nutrients in crops. Ali *et al*. (2008) reported that water stress decreased uptake of N, P

and K in corn plants. Study done by Ghanbari *et al*. (2011) in Pearl Millet showed a decreased level of N, P and K under drought stress condition as well. Likewise, N and K uptake was hindered under drought stress in cotton (McWilliams, 2003). On the other hand, moisture stress stimulates an increase in N, a decline in P, however, no ultimate effects on K (Garg, 2003). Therefore water is one of the main factors determining the availability of mineral nutrients in the soil as well as absorption by plants and translocation from the roots to the shoots.

In this experiment, variety *F8 Drought line*, *JESCA* and *F9 Kidney Selection* significantly increased mineral nutrients uptake of *P. vulgaris* (L.) in season one and two in either of the growth stages as compared with the other studied varieties (i.e. *KAT B9* and *KAT B1*). Study done by Nyoki and Ndakidemi (2014) showed that *B. japonicum* inoculation significantly improved the uptake of macro elements such as N, P, K, Ca, Mg and Na in different tissues of cowpea in both screen house and field experiments as compared with the control treatments. In a closely related studies by Tairo and Ndakidemi (2014) indicated that *Rhizobium* inoculation significantly increased the uptake of macronutrients in various organs of the soybean plants. Report by Baqual and Das (2006) showed that *Rhizobium* inoculation significantly improved the uptake of N, P, and K in the leaves of mulberry plant. However, water stress reduced the uptake of mineral nutrients in varieties *KAT B9* and *KAT B1* in this study. Study done by Nahar and Gretzmacher (2002) showed diminishing concentrations of N, P, K, S, Na, Ca and Mg with increasing water stress in tomato plants. Report by Bharambe and Joshi (1993) in Sorghum indicate the uptake of N, P, K, Ca and Mg was negatively affected under irrigation treatments of decreasing soil water potential below -33 Kpa. Furthermore, the interactive effects between rhizobial inoculation, water stress and few identified cultivars in mineral nutrients uptake in the shoots of *P.vulgaris* is an indication which may justify additional studies under adverse environmental conditions of water and nutritional status.

7.5 Conclusion

Rhizobial inoculation and non- stressed water treatments increased mineral nutrients in *P. vulgaris* (L.) cultivars. Furthermore, mineral nutrients were higher in cultivars *F8 Drought line*, *JESCA*, and *F9 Kidney Selection* as compared with *KAT B9* and *KAT B1* and hence indicating their potential to tolerate drought and accumulate mineral nutrients in their tissues/organs. Therefore, cultivars *F9 Kidney Selection*, *F8 Drought line* and variety *JESCA* can be promoted

for production especially in drought prone areas; however *KAT B9* and *KAT B1* might be preferred for further examination. Interactive effects between rhizobial inoculation, water stress and few identified varieties in enhancing the mineral nutrients in the plants is an indication of various factors which may play a significant role in developing appropriate technology related to water stress tolerance in *P. vulgaris*.

CHAPTER EIGHT

Influence of Water Stress and Rhizobial Inoculation on Growth and Yield of Selected Common Bean cultivars (*Phaseolus vulgaris* L.)

Abstract

Two season's field experiment and a single season screen house experiment were conducted to assess the effect of water stress periods and rhizobial inoculation in five *P. vulgaris* cultivars. The experiment consisted of two levels of rhizobia (with and without inoculation), two water stress levels (with and without water stress) and five cultivars of *P. vulgaris* (*KAT B9, KAT B1*, *F9 Kidney Selection*, *F8 Drought line* and *JESCA*). Results showed that rhizobial inoculation significantly increased plant height (cm), leaf area (cm^2), shoot and root dry weight (g $plant^{-1}$) and seed yields (kg ha^{-1}) at vegetative and flowering in field experiment. Furthermore, water stress treatments significantly reduced plant height (cm), stem diameter (mm), shoot and root dry weight (g $plant^{-1}$) and seed yields (kg ha^{-1}) in both growth stages at field experiment. For screen house experiment rhizobial inoculation significantly increased leaf area (cm^2), number of leaves, stem girth (mm), shoot and root dry weight (g $plant^{-1}$) at both growth stages. Additionally, water stress treatments significantly reduced number of leaves, stem diameter (mm), shoot and root dry weight (g $plant^{-1}$) in both growth stages. Varieties *F9 Kidney Selection, F8 Drought Line* and *JESCA* had significantly superior measurements reflected in increased plant height (cm), shoot and root dry weight (g $plant^{-1}$) and seed yields (kg ha^{-1}) as compared with *KAT B9* and *KAT B1*. Furthermore, significant interactive effects were also seen between rhizobial inoculation x stress level and the tested bean cultivars on plant height, number of leaves per plant, stem diameter, shoot dry weight and seed yields.

Key words; Moisture, Inoculants, Varieties, Growth

Journal of Biodiversity and Environmental Sciences, 11 (2):164 - 178

8.1 Introduction

Nitrogen is the major element in all plants and constitutes a constructive effect on growth of legumes as it improves the quality and quantity of dry matter yields and proteins (Wood *et al*.,

1993; Caliskan, *et al*., 2008). The most important role of nitrogen in the plant is its presence in the structure of protein and nucleic acids, which are the most important building and information substances of every cell. For that reason, sufficient supply of nitrogen is necessary to attain high potential yields in crops. Nitrogen availability in the soil plays a positive significance functions on plant growth as it increases the leaf area of the plants and as a result influences photosynthesis activity of the plants (Uchida, 2000). Report by Namvar *et al*. (2013) on chick pea showed that plant height was increased with application of nitrogen fertilizer. However, inadequate N in the growth media/soil is the major limiting factor for crop growth in most areas of the world (Fuzhong *et al*., 2008; Salvagiotti *et al*., 2008). On the other hand, the source of N through fixation using beneficial soil bacterium (*Rhizobium*) can efficiently reduce the cost of production and improve crops production (Tairo and Ndakidemi, 2013). Common bean can acquire its N_2 requirement through N fixation when grown in association with effective and compatible *Rhizobium* strain (Makoi *et al*., 2010). Inoculation of seeds with sufficient *Rhizobium* is known to enhance nodulation, nitrogen fixation, growth rate and yield parameters of legume crops (Sogut, 2006; Namvar *et al*., 2011). Therefore, determination of growth parameters of common bean crop in response to *Rhizobium* inoculation is very important to maximize yield and economic profitability of common bean production in a particular environment.

Plants experience water stress either when the water supply to their roots becomes limiting or when the transpiration rate becomes intense (Nielsen and Nelson, 1998). Water stress is one of the most restrictive features in crop growth which mainly decrease growth and finally the dry matter production. Water stress has been found to impair plant growth and development, whereby, the foremost effect of water stress in plants impairs germination and poor stand establishment (Harris *et al*., 2002). Cell growth is the one which is highly affected during water deficit in plants due to reduction in turgor pressure. Expansion of young cells is given by growth which is brought about by daughter-cell production by meristematic cell division. Nonami (1998) reported that in severe water shortage, cell elongation of higher plants can be inhibited by disruption of water flow from xylem to the surrounding elongating cells. In general terms, water deficit diminish cell division, cell elongation and cell enlargement as a result reduce growth of the crop (Hussain *et al*., 2008; Farooq, *et al*., 2009). At the same time, plant height, number of leaves per plant, leaf area, leaf longevity and soil water potential as well as fresh and dry biomass production are also reduced due to adverse effect of water deficit (Zhao *et al*., 2006;

Emam *et al*., 2010; Baroowa and Gogoi, 2012). It has been reportedthat growth, development and performance of common bean isadversely affected if the quantity of water supplied is insufficient to meet the basic needs of plants (Seki *et al*., 2002). Therefore, there is a need to assess the effects of water stress and *Rhizobium* inoculation on growth and yield of selected *P. Vulgaris* (L) cultivars and identify the potential ones which can perform well in water stressed environment.

8.2 Materials and Methods

8.2.1 Narrative of Site Location

The trial was conducted at Agricultural Seed Agency (ASA) farm in Arusha, located at Latitude 3°18′S and Longitude 36°38′06.29″E. ASA receives the mean annual rainfall of 819mm, mean temperature of 19.15°C with relative humidity of about 94% and altitude of 1520 m.a.s.l. The field trial was carried out during dry season of January to March 2014 and January to March, 2015 while the screen house experiment was carried out from mid-January to March, 2016 under irrigation.

8.2.2 Experimental Design and Treatment Application

The experiment was designed in a split, split plot arrangement with 3 replications. The plot size was 3m x 4m. The field experimental treatments consisted of 2 levels of Rhizobia (with and without inoculation) as the main factor followed by imposing water stress (sub factor) in vegetative and flowering stages of plant growth. Five cultivars of *P. vulgaris*: (*KAT B9, KAT B1, F9 Kidney Selection, F8 Drought line* and *JESCA*) were assigned to sub-sub plots. The common bean seeds were sown at a spacing of 50 cm x 20 cm, making a plant population density of 200,000 plants per hectare. The BIOFIX legume inoculants were obtained from *MEA* Company Nairobi - Kenya, sold under license from the University of Nairobi. Common bean seeds were obtained from the breeding unit based at the Selian Agricultural Research Institute (SARI), Arusha, Tanzania. Land for field experiment was cleared and all the necessary practices like ploughing and harrowing were done before planting. Moreover, in the screen house experiment, the wooden box technique was used to establish the experiment. This was done by collecting the same soil used at field experiment and beans were planted using the protocol developed by

(Agbicodo *et al*., 2009) with some modifications. Common bean seeds were thoroughly mixed with *Rhizobium* inoculants to supply (10^9 cells g^{-1} seed), following procedure stipulated by products manufacturer. To avoid contamination, all non-inoculated seeds were sown first, followed by inoculated seeds. Three seeds were sown and thinned to two plants per hill after full plant establishment. Water stress period of 10 days were imposed at vegetative and flowering stages of plant growth by not irrigating.

8.2.3 Study of Growth Parameters and Yield in *P. vulgaris* (L.)

Growth parameters in field and screen house experiment were collected in vegetative and flowering growth stages upon stress periods correspondingly. Plant height (cm) was taken using a meter rule. Plant height was measured from the base to the growing tip of the shoot in (cm) in each of the season at each growth stages. In field experiment, 10 plants were randomly selected in the two middle rows from each field plot for measuring the height of the plant at two stress periods. The same procedure was applied to the screen house experiment, whereby only two plants in each row were measured for height. After recording the data, the average was worked out to get a representative plant height from each of the experimental units. Number of leaves per plant was recorded in each of the growth stages of the *P. vulgaris* (L.). This was conducted in the same interval to the height of the plant at each stages of the common bean growth. The same exercise was also conducted for the glasshouse experiment and the average worked out as well. Stem girth (mm) was measured and measured at each of the growth stages using a veneer caliper in both glasshouse and field experiments. Leaf area (LA) was estimated according to Peksen, (2007), i.e. LA = 0.919 + 0.682LW, Where by LA = Leaf area (cm^2), L = Leaflet length (cm), W = Maximum width of the leaflet (cm). Shoot and root dry weight (g $plants^{-1}$) was also measured after oven drying the plant samples at 65°C for 48 hours and average worked out. Seed yields (g $plot^{-1}$) were evaluated by randomly sampling two middle rows from each stressed stages of the net plot threshed and then adjusted to constant moisture by air drying and weighs them. The plot yield was then converted to kg ha^{-1}.

8.2.4 Statistical Analysis

A 3-Way ANOVA was used to analyze data collected. The analysis was done using STATISTICA software programe of 2013. Fisher's least significant difference was used to compare treatment means at $p = 0.05$ (Steel and Torrie, 1980).

8.3 Results

8.3.1 Effect of inoculation with *Rhizobium* and stress periods on plant height, number of leaves per plant, stem girth, leaf area, shoot dry weight root dry weight and seed yields

Rhizobial inoculation increased plant height (cm) by 12 and 9 % at vegetative and flowering growth stages respectively in field experiment (Table 14). Furthermore, rhizobial inoculation significantly increased leaf area by 17 % in the second seasonat vegetative growth stage (Table 17). Shoot and root dry weight (g plant^{-1}) were significantly increased through rhizobial inoculation by 32 % in season one atvegetative stage and 31 % and 20 % in season two atvegetative and flowering stage both in field experiments respectively (Table 18). Rhizobial inoculation showed significant increase in seed yields (kg ha^{-1}) by 53 %, 59 % and 33 %, 31 % in season one and two both at vegetative and flowering growth stages under field experiment (Table 19). For screen house experiment, rhizobial inoculation significantly increased the number of leaves by 39 % at vegetative stage and 30 % at flowering stage (Table 21). Stem diameter were also increased as a result of rhizobial inoculation by 29 % and 20 % at vegetative and flowering growth stages respectively (Table 21). Rhizobial inoculation significantly increased the shoot and root dry weight (g plant^{-1}) by 28 % at vegetative stage and 32 % and 28 % at vegetative and flowering stages respectively (Table 22).

For plants exposed to water stress at vegetative stage, the water stress treatments significantly decreased plant height (Table 14), stem girth (Table 16) shoot and root dry weight (Table 18) in season one. Water stress imposed at vegetative stage also significantly decreased shoot and root dry weight (Table 18) and seed yield in season 2 (Table 19). Imposing water stress at flowering stage significantly reduced plant height (Table 14), shoot and root dry weight (Table 18) and seed yield (Table 19) for measurements taken in season 2. In the screen house experiment, water

stress treatment imposed at vegetative stage significantly reduced number of leaves per plant, stem girth (Table 21) shoot and root dry weight (Table 22).

For the measured parameters, the performance of the varieties was as follows; Varieties *F9 Kidney Selection*, *F8 Drought line* and *JESCA* had superior measurements for girth (Table 16), shoot and root dry weight (Table 18) and seed yield (Table 19) in plants imposed with stress at vegetative stage in season one as compared with other tested varieties. Imposing stress at flowering stage in season one significantly reduced number of leaves (Table 15), shoot and root dry weight (Table 18) and seed yield (Table 19) in varieties *KATB9* and *KATB1* as compared with varieties *F9 Kidney Selection*, *F8 Drought line* and *JESCA* which had better performances.

In season two, measurements taken from plants imposed with stress at vegetative stage indicated the superiority in varieties *F9 Kidney Selection*, *F8 Drought line* and *JESCA* with respect to number of leaves per plant (Table 15), shoot and root dry weight (Table 18) and seed yield (Table 19). Water stress imposed at flowering stage also significantly decreased plant height (Table 1), number of leaves per plant (Table 15), stem girth (Table 16), leaf area (Table 17), shoot and root dry weight (Table 18) and seed yield in season 2 (Table 19) in varieties *KATB9* and *KATB1* as compared with varieties *F9 Kidney Selection*, *F8 Drought line* and *JESCA*. In the screen house experiment, varieties *F9 Kidney Selection* and *JESCA* had superior root dry weight (g plant^{-1}) as compared to other varieties in plants imposed with stress at vegetative stage (Table 22).

8.3.2 Interactive effect of inoculation with *Rhizobium* and stress periods on plant height, number of leaves per plant, stem girth, leaf area, shoot dry weight, root dry weight, and seed yields

There was significant interaction between *Rhizobium* and water stress in plant height (cm), shoot dry weight (g plant^{-1}) and seed yields (kg ha^{-1}) (Fig. 29, 33-36). Applying *Rhizobium* inoculants and stressing plants with water enhanced the growth parameters of plant height, shoot dry weight and seed yields in vegetative and flowering growth stages respectively compared with the un-inoculated treatments. Furthermore, significant interactive effects was also seen between stress level and varieties on plant height (cm) during flowering stage, number of leaves and stem girth (mm) (Fig. 30 - 32). Even under water stress treatments at vegetative and flowering stages of

growth, varieties *JESCA*, *F9 Kidney Selection* and *F8 Drought Line* performed well in the above measured parameters as compared with varieties *KAT B9* and *KAT B1* respectively.

Table 14: Plant height (cm) in *P. Vulgaris* as influenced by rhizobial inoculation and water stress periods in field experiments for two consecutive seasons

	1st Season		2nd Season	
	Vegetative Stage	**Flowering Stage**	**Vegetative Stage**	**Flowering Stage**
Treatments				
R+	10.83±0.23a	17.63±0.46a	13.01±0.22a	19.95±0.45a
R-	9.57±0.32b	16.05±0.50b	12.90±0.23a	19.36±0.46a
Stress Levels				
S_1	10.62±0.27a	17.14±0.48a	13.05±0.22a	20.82±0.38a
S_2/S_3	9.78±0.32b	16.53±0.51a	12.87±0.22a	18.49±0.42b
Varieties				
V_1	10.39±0.30a	16.68±0.89a	13.32±0.37a	18.76±0.96b
V_2	10.25±0.48a	17.03±0.66a	13.24±0.23a	18.73±0.76b
V_3	10.82±0.41a	16.04±0.49a	12.56±0.21a	19.32±0.45b
V_4	10.14±0.49a	17.78±0.69a	13.17±0.41a	20.19±0.62ab
V_5	9.40±0.61a	16.68±1.10a	12.50±0.44a	21.26±0.48a
3-Way Anova (F-Statistics)				
Rhz	13.06***	5.06*	0.10ns	1.44ns
StrL	5.71*	0.75ns	0.32ns	22.32***
Vrty	1.74ns	0.66ns	1.19ns	3.83**
Rhz*StrL	4.70*	0.23ns	0.06ns	0.09ns
Rhz*Vrty	0.88ns	0.50ns	0.84ns	0.20ns
StrL*Vrty	0.86ns	0.54ns	0.95ns	4.86**
Rhz*StrL*Vrty	2.40ns	1.46ns	0.69ns	0.06ns

+R: With *Rhizobium*, −R: Without *Rhizobium*. S_1: No water stress, S_2: Water stress imposed at Vegetative Stage, S_3: Water stress imposed at Flowering Stage. V_1: *KAT B9*, V_2: *KAT B1*, V_3: *F9 Kidney Selection*, V_4: *F8 Drought Line*, V_5: *JESCA*. Values presented are means ± SE. *, **, *** = significant at $p \leq 0.05$, at $p \leq 0.01$, and at $p \leq 0.001$ respectively, ns = Not significant. Means followed by similar letter(s) in a given column are not significantly difference from each other at $p = 0.05$.

Table 15: Number of leaves in *P.vulgaris* as influenced by rhizobial inoculation and water stress periods in field experiments for two consecutive seasons

	1st Season		2nd Season	
	Vegetative Stage	**Flowering Stage**	**Vegetative Stage**	**Flowering Stage**
Treatments				
R+	6.23±0.31a	10.95±0.53a	6.80±0.18a	10.47±0.21a
R-	6.17±0.20a	10.10±0.41a	6.47±0.17a	10.37±0.20a
Stress Levels				
S_1	5.97±0.26a	10.55±0.56a	6.57±0.19a	10.60±0.20a
S_2/S_3	6.43±0.24a	10.50±0.39a	6.70±0.16a	10.23±0.21a
Varieties				
V_1	6.33±0.19a	8.58±0.43c	5.67±0.14d	9.33±0.19d
V_2	6.00±0.58a	10.58±0.66b	6.17±0.17cd	10.17±0.24bc
V_3	6.50±0.44a	12.00±0.72a	7.75±0.22a	11.83±0.27a
V_4	6.50±0.34a	11.78±0.91a	7.17±0.21b	10.75±0.22b
V_5	5.67±0.40a	9.68±0.61bc	6.42±0.23c	10.00±0.17c
3-Way Anova (F-Statistics)				
Rhz	0.04ns	2.37ns	3.70ns	0.25ns
StrL	2.11ns	0.01ns	0.59ns	3.36ns
Vrty	1.01ns	5.36**	18.20***	17.64***
Rhz*StrL	1.55ns	0.05ns	2.37ns	1.36ns
Rhz*Vrty	0.90ns	1.06ns	0.28ns	0.67ns
StrL*Vrty	3.72ns	4.06**	0.31ns	0.72ns
Rhz*StrL*Vrty	2.09ns	0.97ns	1.72ns	0.81ns

−R: Without *Rhizobium*, +R: With *Rhizobium.* S_1: No water stress, S_2: Water stress imposed at Vegetative Stage. S_3: Water stress imposed at Flowering Stage. V_1: *KAT B9,* V_2: *KAT B1,* V_3: *F9 Kidney Selection,* V_4: *F8 Drought Line,* V_5: *JESCA.* Values presented are means ± SE. *, **, *** = significant at $p \leq 0.05$, at $p \leq 0.01$, and at $p \leq 0.001$ respectively, ns = Not significant. Means followed by similar letter(s) in a given column are not significantly difference from each other at $p = 0.05$.

Table 16: Stem girth (mm) in *P. vulgaris* as influenced by rhizobial inoculation and water stress periods in field experiments for two consecutive seasons

	1st Season		2nd Season	
	Vegetative Stage	**Flowering Stage**	**Vegetative Stage**	**Flowering Stage**
Treatments				
R+	3.93±0.17a	6.91±0.11a	3.80±0.10a	6.64±0.10a
R-	3.75±0.08a	6.78±0.13a	3.69±0.11a	6.55±0.07a
Stress Levels				
S_1	3.97±0.06a	6.95±0.13a	3.81±0.11a	6.66±0.09a
S_2/S_3	3.70±0.18b	6.75±0.11a	3.68±0.10a	6.53±0.08a
Varieties				
V_1	3.02±0.35c	6.95±0.16a	3.79±0.18a	6.21±0.16d
V_2	3.81±0.09b	6.93±0.23a	3.75±0.14a	6.35±0.04cd
V_3	4.31±0.09a	6.75±0.16a	3.71±0.22a	7.12±0.08a
V_4	4.15±0.08ab	6.84±0.14a	3.75±0.14a	6.76±0.09b
V_5	3.91±0.07ab	6.78±0.25a	3.72±0.16a	6.51±008bc
3-Way Anova (F-Statistics)				
Rhz	1.91ns	0.54ns	0.48ns	0.91ns
StrL	4.24**	1.37ns	0.59ns	2.08ns
Vrty	11.68***	0.21ns	0.03ns	12.33***
Rhz*StrL	0.58ns	0.78ns	0.02ns	3.83ns
Rhz*Vrty	1.99ns	0.72ns	0.38ns	0.11ns
StrL*Vrty	3.44*	0.57ns	0.46ns	0.40ns
Rhz*StrL*Vrty	1.82ns	1.47ns	0.80ns	0.59ns

−R: Without *Rhizobium*, +R: With *Rhizobium.* S_1: No water stress, S_2: Water stress imposed at Vegetative Stage. S_3: Water stress imposed at Flowering Stage. V_1: *KAT B9,* V_2: *KAT B1,* V_3: *F9 Kidney Selection,* V_4: *F8 Drought Line,* V_5: *JESCA.* Values presented are means ± SE. *, **, *** = significant at $p \leq 0.05$, at $p \leq 0.01$, and at $p \leq 0.001$ respectively, ns = Not significant. Means followed by similar letter(s) in a given column are not significantly difference from each other at $p = 0.05$.

Table 17: Leaf Area (cm^2) in *P. vulgaris* as influenced by rhizobial inoculation and water stress periods in field experiments for two consecutive seasons

	1st Season		2nd Season	
	Vegetative Stage	**Flowering Stage**	**Vegetative Stage**	**Flowering Stage**
Treatments				
R+	110.86±1.02a	149.29±9.57a	128.00±5.43a	163.71±5.61a
R-	113.54±1.02a	161.82±8.75a	105.86±5.41b	161.32±4.79a
Stress Levels				
S_1	111.89±1.11a	144.84±9.12a	116.03±5.31a	164.22±4.92a
S_2/S_3	112.51±0.99a	166.27±8.93a	117.84±6.24a	160.81±5.49a
Varieties				
V_1	114.47±1.68a	181.75±12.81a	126.44±5.65a	130.10±4.25e
V_2	109.95±1.40a	131.06±14.30a	114.36±8.89a	142.72±3.47d
V_3	112.32±1.66a	142.02±15.85a	107.64±7.94a	200.07±4.67a
V_4	112.54±1.51a	157.77±11.97a	109.60±7.76a	178.15±3.76b
V_5	111.72±1.95a	165.16±14.85a	126.61±13.36a	161.54±3.45c
3-Way Anova (F-Statistics)				
Rhz	3.79ns	1.01ns	8.08**	0.43ns
StrL	0.20ns	2.96ns	0.05ns	0.88ns
Vrty	1.11ns	2.02ns	1.09ns	47.02***
Rhz*StrL	3.51ns	0.01ns	0.62ns	1.40ns
Rhz*Vrty	2.03ns	0.67ns	0.46ns	0.80ns
StrL*Vrty	1.57ns	1.82ns	1.50ns	0.49ns
Rhz*StrL*Vrty	0.29ns	0.47ns	0.83ns	0.05ns

−R: Without *Rhizobium*, +R: With *Rhizobium.* S_1: No water stress, S_2: Water stress imposed at Vegetative Stage, S_3: Water stress imposed at Flowering Stage. V_1: *KAT B9,* V_2: *KAT B1,* V_3: *F9 Kidney Selection,* V_4: *F8 Drought Line,* V_5: *JESCA.* Values presented are means ± SE. *, **, *** = significant at $p \leq 0.05$, at $p \leq 0.01$, and at $p \leq 0.001$ respectively, ns = Not significant. Means followed by similar letter(s) in a given column are not significantly difference from each other at $p = 0.05$.

Table 18: Shoot dry weight (g plant^{-1}) and Root Dry weight (g plant^{-1}) in *P.vulgaris* as influenced by rhizobial inoculation and water stress periods in field experiments for two consecutive seasons

	Shoot dry weight (g plant^{-1})				**Root Dry weight (g plant^{-1})**			
	1st Season		2nd Season		1st Season		2nd Season	
	Vegetative Stage	**Flowering Stage**	**Vegetative Stage**	**Flowering Stage**	**Vegetative Stage**	**Flowering Stage**	**Vegetative Stage**	**Flowering Stage**
Treatments								
R+	1.36±0.04a	3.81±0.15a	3.37±0.21a	5.45±0.33a	0.33±0.01a	0.43±0.02a	1.59±0.04a	2.59±0.17a
R-	0.93±0.05b	3.70±0.19a	3.25±0.14a	5.12±0.32a	0.34±0.01a	0.41±0.01a	1.10±0.05b	2.06±0.19b
Stress Levels								
S_1	1.20±0.05a	3.91±0.17a	4.05±0.15a	5.67±0.33a	0.36±0.01a	0.43±0.02a	1.43±0.06a	2.96±0.14a
S_2/S_3	1.09±0.06b	3.60±0.17a	2.57±0.08b	4.91±0.30b	0.33±0.01b	0.41±0.01a	1.26±0.07b	1.69±0.15b
Varieties								
V_1	1.00±0.07cd	3.07±0.15b	2.83±0.25d	3.86±0.26b	0.29±0.01c	0.33±0.02b	1.17±0.08cd	1.89±0.28b
V_2	0.95±0.06d	2.90±0.18b	2.95±0.20cd	3.86±0.37b	0.28±0.01c	0.37±0.02b	1.13±0.08d	1.80±0.21b
V_3	1.42±0.09a	4.25±0.21a	3.96±0.32a	6.74±0.47a	0.40±0.02a	0.47±0.02a	1.65±0.10a	2.93±0.29a
V_4	1.10±0.07c	4.36±0.27a	3.35±0.28bc	6.15±0.34a	0.35±0.01b	0.47±0.03a	1.32±0.09bc	2.47±0.26a
V_5	1.25±0.08b	4.18±0.16a	3.47±0.28b	5.82±0.46a	0.35±0.02b	0.46±0.02a	1.45±0.11b	2.53±0.30a
3-Way Anova (F-Statistics)								
Rhz	97.64***	0.39ns	0.77ns	0.89ns	1.15ns	0.85ns	89.53***	10.05**
StrL	6.52*	2.78ns	123.64***	4.75*	26.05***	0.31ns	10.42**	56.80***
Vrty	15.47***	12.07***	9.20***	11.73***	19.05***	6.90***	13.86***	6.32***
Rhz*StrL	2.65ns	1.17ns	3.58ns	2.55ns	2.60ns	5.35ns	1.18ns	1.30ns
Rhz*Vrty	0.33ns	0.67ns	0.86ns	0.57ns	0.37ns	0.56ns	0.31ns	0.22ns
StrL*Vrty	0.48ns	0.67ns	1.21ns	0.48ns	0.95ns	0.18ns	0.19ns	0.41ns
Rhz*StrL*Vrty	0.15ns	0.77ns	0.65ns	0.26ns	0.13ns	0.09ns	0.28ns	0.97ns

+R: With *Rhizobium*, −R: Without *Rhizobium*. S_1: No water stress, S_2: Water stress imposed at Vegetative Stage, S_3: Water stress imposed at Flowering Stage. V_1: *KAT B9*, V_2: *KAT B1*, V_3: *F9 Kidney Selection*, V_4: *F8 Drought Line*, V_5: *JESCA*. Values presented are means ± SE. *, **, *** = significant at $p \leq 0.05$, at $p \leq 0.01$, and at $p \leq 0.001$ respectively, ns = Not significant. Means followed by similar letter(s) in a given column are not significantly difference from each other at $p = 0.05$.

Table 19: Seed yields (kg ha^{-1}) in *P.vulgaris* as influenced by rhizobial inoculation and water stress periods in field experiments for two consecutive seasons

	1st Season		2nd Season	
	Vegetative Stage	**Flowering Stage**	**Vegetative Stage**	**Flowering Stage**
Treatments				
R+	674.97±33.91a	557.12±24.87a	979.33±64.63a	828.53±67.86a
R-	319.11±30.23b	227.04±13.77b	655.73±36.31b	575.60±44.43b
Stress Levels				
S_1	509.29±48.92a	409.23±39.08a	941.60±69.44a	841.87±68.49a
S_2/S_3	484.78±42.94a	374.93±33.75a	693.47±37.65b	562.27±40.53b
Varieties				
V_1	316.40±67.89b	291.78±45.05b	635.00±74.23c	427.33±92.58d
V_2	400.98±59.79b	340.98±53.10b	628.00±96.43c	608.33±71.23c
V_3	586.94±73.79a	446.33±63.47a	859.00±71.40b	706.33±71.67bc
V_4	624.77±58.84a	459.18±58.75a	988.67±98.41a	778.00±81.67b
V_5	556.10±67.51a	422.15±58.88a	977.00±84.28ab	990.33±97.16a
3-Way Anova (F-Statistics)				
Rhz	96.22***	189.27***	64.50***	43.67***
StrL	0.46ns	2.04ns	37.93***	53.36***
Vrty	10.60***	7.30***	15.48***	23.64***
Rhz*StrL	0.53ns	5.04*	50.01***	65.04***
Rhz*Vrty	0.32ns	0.46ns	0.71ns	0.45ns
StrL*Vrty	0.97ns	0.71ns	0.68ns	1.01ns
Rhz*StrL*Vrty	0.60ns	0.11ns	0.22ns	0.42ns

−R: Without *Rhizobium*; +R: With *Rhizobium.* S_1: No water stress, S_2: Water stress imposed at Vegetative Stage, S_3: Water stress imposed at Flowering Stage. V_1: *KAT B9,* V_2: *KAT B1,* V_3: *F9 Kidney Selection,* V_4: *F8 Drought Line,* V_5: *JESCA.* Values presented are means ± SE. *, **, *** = significant at $p \leq 0.05$, at $p \leq 0.01$, and at $p \leq 0.001$ respectively, ns = Not significant. Means followed by similar letter(s) in a given column are not significantly difference from each other at $p = 0.05$.

Table 20: Plant height and Leaf area in *P.vulgaris* as influenced by rhizobial inoculation and water stress periods in the screen house experiment

	Plant height (cm)		Leaf area (cm^2)	
	Vegetative Stage	**Flowering Stage**	**Vegetative Stage**	**Flowering Stage**
Treatments				
R+	25.09±0.90a	33.09±0.82a	153.91±4.54a	171.98±3.75a
R-	24.33±0.92a	32.82±0.96a	118.44±1.45b	181.09±4.23a
Stress Levels				
S_1	25.87±0.73a	33.46±0.84a	133.83±3.21a	177.43±4.05a
S_2/S_3	23.55±1.03a	32.44±0.94a	138.52±5.32a	175.64±4.08a
Varieties				
V_1	24.49±1.24a	30.64±0.95a	130.14±6.22a	169.93±5.23a
V_2	24.89±1.55a	32.29±1.35a	134.52±5.38a	180.49±5.46a
V_3	22.30±1.85a	34.40±1.79a	139.83±6.90a	177.51±6.96a
V_4	25.07±0.85a	33.56±1.51a	143.38±9.74a	175.60±8.38a
V_5	26.79±1.43a	33.88±1.26a	133.01±5.94a	179.13±5.91a
3-Way Anova (F-Statistics)				
Rhz	0.35ns	0.04ns	61.82***	2.33ns
StrL	3.26ns	0.60ns	1.08ns	0.09ns
Vrty	1.24ns	1.04ns	1.13ns	0.38ns
Rhz*StrL	4.42ns	0.82ns	3.76ns	0.10ns
Rhz*Vrty	0.31ns	0.05ns	0.71ns	0.62ns
StrL*Vrty	0.46ns	0.55ns	1.74ns	0.87ns
Rhz*StrL*Vrty	0.60ns	0.97ns	2.04ns	0.64ns

−R: Without *Rhizobium*, +R: With *Rhizobium.* S_1: No water stress, S_2: Water stress imposed at Vegetative Stage, S_3: Water stress imposed at Flowering Stage. V_1: *KAT B9*, V_2: *KAT B1*, V_3: *F9 Kidney Selection*, V_4: *F9 Drought Line*, V_5: *JESCA*. Values presented are means ± SE. *, **, *** = significant at $p \leq 0.05$, at $p \leq 0.01$, and at $p \leq 0.001$ respectively, ns = Not significant. Means followed by similar letter(s) in a given column are not significantly difference from each other at $p = 0.05$.

Table 21: Number of leaves and Stem girth in *P.vulgaris* as influenced by rhizobial inoculation and water stress periods in the screen house experiment

	Number of leaves/plant		Stem girth (mm)	
	Vegetative Stage	**Flowering Stage**	**Vegetative Stage**	**Flowering Stage**
Treatments				
R+	7.17±0.16a	10.38±0.28a	4.03±0.43a	5.41±0.37a
R-	4.35±0.22b	7.22±0.27b	2.87±0.26b	4.31±0.29b
Stress Levels				
S_1	6.16±0.32a	10.06±0.31a	4.56±0.42a	5.16±0.23a
S_2/S_3	5.36±0.25b	7.53±0.32b	2.34±0.18b	4.56±0.42a
Varieties				
V_1	5.59±0.57a	8.76±0.68a	3.07±0.55a	4.79±0.55a
V_2	5.87±0.45a	9.02±0.51a	3.63±0.82a	5.70±0.68a
V_3	5.95±0.48a	8.40±0.57a	2.96±0.37a	4.21±0.46a
V_4	6.25±0.39a	8.92±0.65a	3.22±0.38a	4.58±0.39a
V_5	5.16±0.42a	8.90±0.61a	4.36±0.64a	5.01±0.54a
3-Way Anova (F-Statistics)				
Rhz	134.06***	122.62***	7.07*	5.62*
StrL	10.77**	78.94***	25.99**	1.68ns
Vrty	2.29ns	0.58ns	1.38ns	1.15ns
Rhz*StrL	2.57ns	0.01ns	1.47ns	1.63ns
Rhz*Vrty	0.73ns	1.01ns	1.04ns	1.25ns
StrL*Vrty	0.94ns	0.96ns	0.73ns	0.87ns
Rhz*StrL*Vrty	1.13ns	0.66ns	0.66ns	0.57ns

−R: Without *Rhizobium*, +R: With *Rhizobium.* S_1: No water stress, S_2: Water stress imposed at Vegetative Stage, S_3: Water stress imposed at Flowering Stage. V_1: *KAT B9,* V_2: *KAT B1,* V_3: *F9 Kidney Selection,* V_4: *F8 Drought Line,* V_5: *JESCA.* Values presented are means ± SE. *, **, *** = significant at $p \leq 0.05$, at $p \leq 0.01$, and at $p \leq 0.001$ respectively, ns = Not significant. Means followed by similar letter(s) in a given column are not significantly difference from each other at $p = 0.05$.

Table 22: Shoot Dry weight and Root dry weight in *P. vulgaris* as influenced by rhizobial inoculation and water stress periods in the screen house

	Shoot Dry weight (g plant^{-1})		**Root dry weight (g plant^{-1})**	
	Vegetative Stage	**Flowering Stage**	**Vegetative Stage**	**Flowering Stage**
Treatments				
R+	1.34±0.14a	2.21±0.29a	0.19±0.01a	1.11±0.08a
R-	0.96±0.09b	2.14±0.15a	0.13±0.01b	0.80±0.03b
Stress Levels				
S_1	1.52±0.14a	2.83±0.26a	0.18±0.01a	0.98±0.07a
S_2/S_3	0.78±0.06b	1.52±0.14b	0.13±0.01b	0.93±0.06a
Varieties				
V_1	1.02±0.18a	1.96±0.30a	0.11±0.01c	0.79±0.08a
V_2	1.21±0.27a	2.31±0.41a	0.10±0.02c	0.83±0.11a
V_3	0.99±0.12a	2.03±0.35a	0.23±0.02a	1.06±0.11a
V_4	1.07±0.13a	1.79±2.23a	0.16±0.02b	1.07±0.10a
V_5	1.45±0.21a	2.78±0.49a	0.19±0.02ab	1.03±0.10a
3-Way Anova (F-Statistics)				
Rhz	7.07*	0.04ns	25.44***	10.97**
StrL	25.99***	18.88***	22.93***	0.27ns
Vrty	1.38ns	1.32ns	15.64***	1.75ns
Rhz*StrL	1.47ns	4.27*	0.26ns	0.34ns
Rhz*Vrty	1.04ns	0.29ns	0.39ns	0.06ns
StrL*Vrty	0.73ns	0.37ns	1.10ns	0.19ns
Rhz*StrL*Vrty	0.66ns	0.79ns	2.50ns	0.06ns

−R: Without *Rhizobium*, +R: With *Rhizobium.* S_1: No water stress, S_2: Water stress imposed at Vegetative Stage, S_3: Water stress imposed at Flowering Stage. V_1: *KAT B9,* V_2: *KAT B1,* V_3: *F9 Kidney Selection,* V_4: *F8 Drought Line,* V_5: *JESCA.* Values presented are means ± SE. *, **, *** = significant at $p \leq 0.05$, at $p \leq 0.01$, and at $p \leq 0.001$ respectively, ns = Not significant. Means followed by similar letter(s) in a given column are not significantly difference from each other at $p = 0.05$.

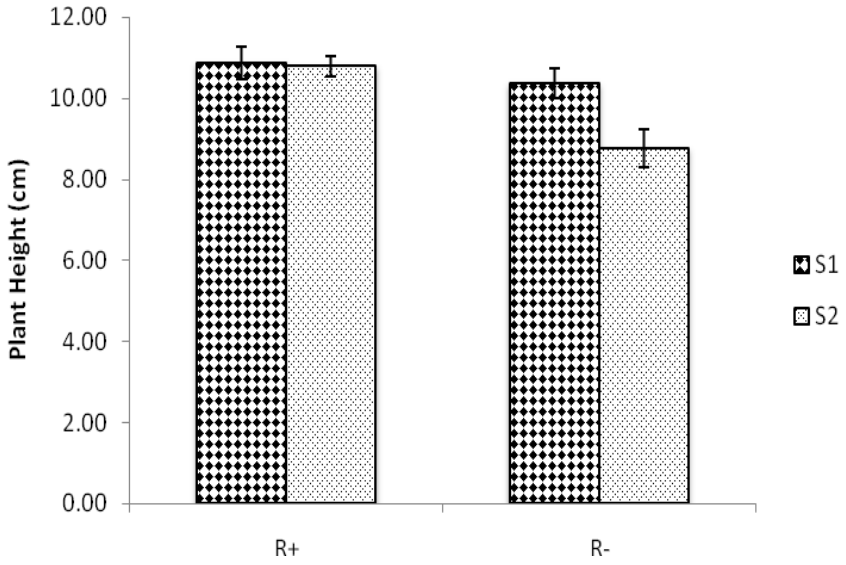


Figure 29: Interactive effects of *Rhizobium* and stress levels on plant height (cm) in season one at vegetative stage. +R: With *Rhizobium*, -R: Without *Rhizobium*. S1: Control, S2: Water stress imposed at vegetative stage

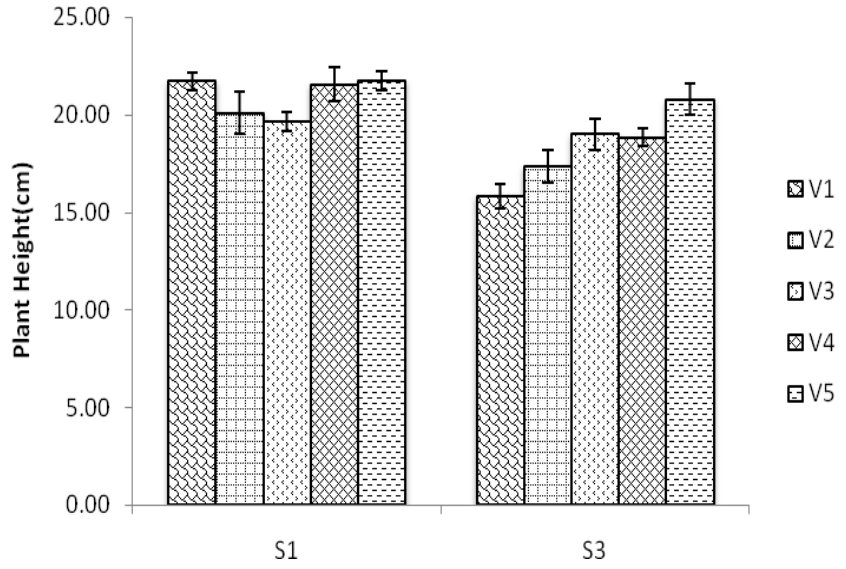


Figure 30: Interactive effects of stress level and five (5) *P. vulgaris* on Plant height (cm) in season two at flowering stage. S1: Control, S3: Water stress imposed at flowering stage. V1: *KAT B9*, V2: *KAT B1*, V3: *F9 Kidney Selection*, V4: *F8 Drought Line*, V5: *JESCA*

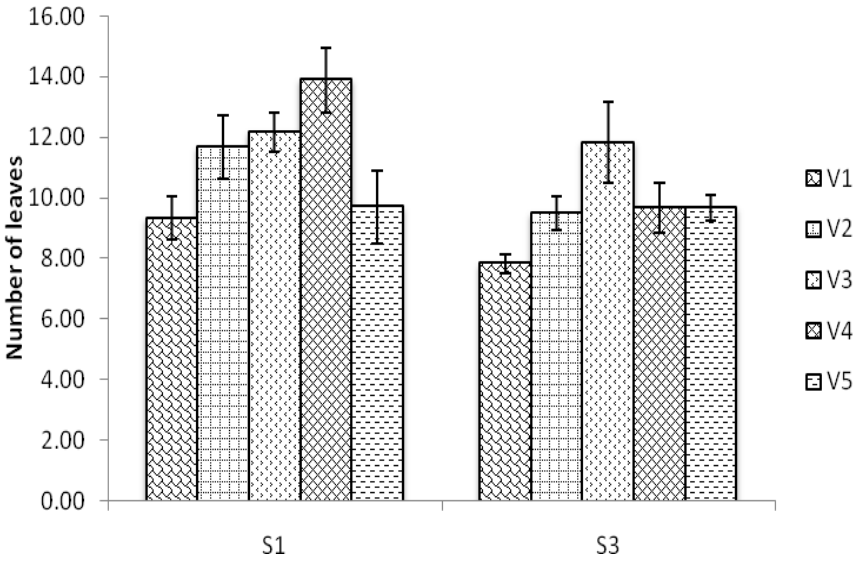


Figure 31: Interactive effects of stress level and five (5) *P. vulgaris* (L.) on number of leaves in season one at flowering stage. S1: Control, S3: Water stress imposed at flowering stage. V1: *KAT B9*, V2: *KAT B1*, V3: *F9 Kidney Selection*, V4: *F8 Drought Line*, V5: *JESCA*

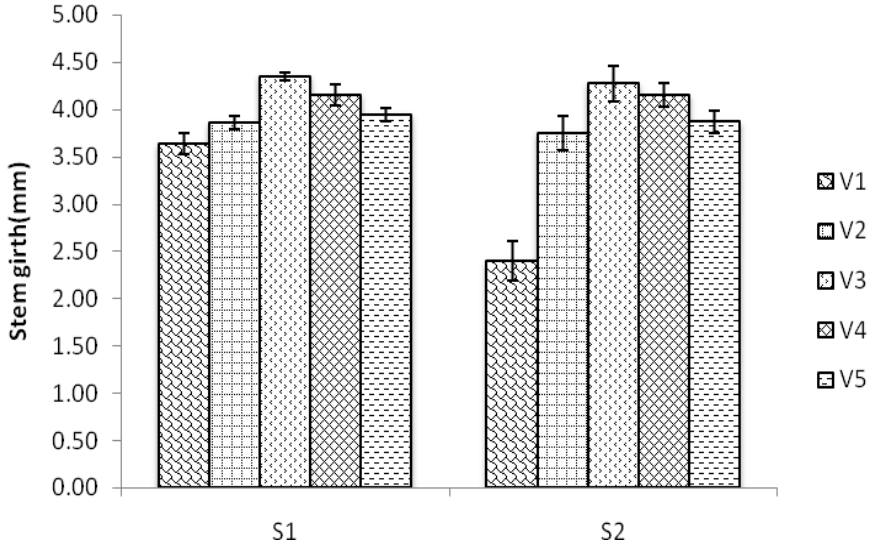


Figure 32: Interactive effects of stress level and five (5) *P. vulgaris* (L.) on stem girth (mm) in season one at vegetative stage. S1: Control, S2: Water stress imposed at vegetative

stage. V1: *KAT B9*, V2: *KAT B1*, V3: *F9 Kidney Selection*, V4: *F8 Drought Line*, V5: *JESCA*

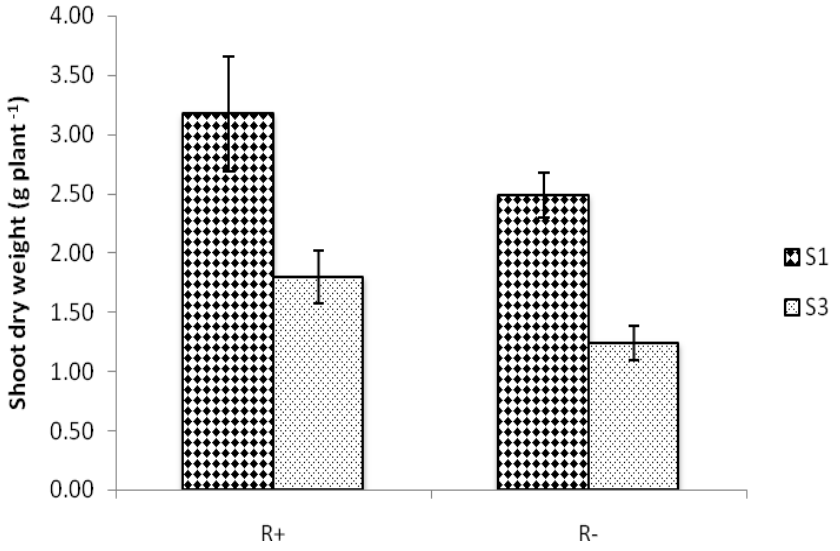


Figure 33: Interactive effects *Rhizobium* and stress levels on Shoot Dry weight (g plant^{-1}) in screen house experiment at flowering stage. +R: With *Rhizobium*, -R: Without *Rhizobium*. S1: Control, S3: Water stress imposed at flowering stage

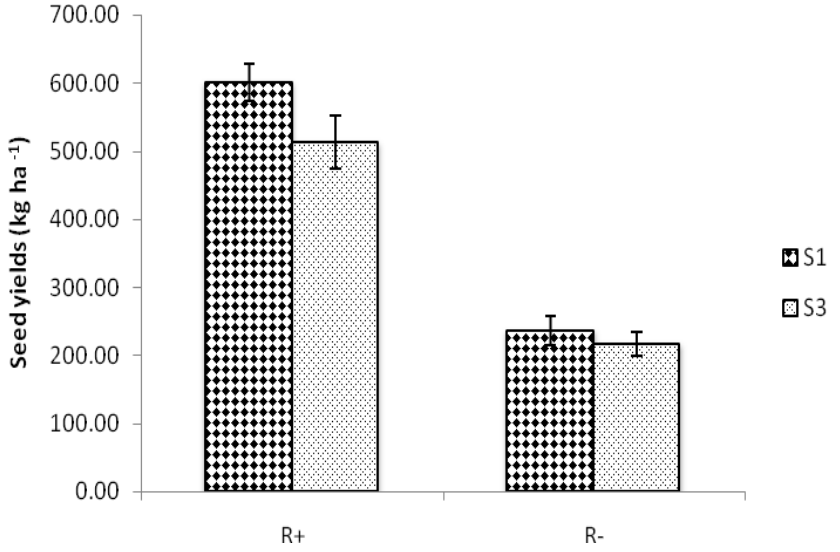


Figure 34: Interactive effects of *Rhizobium* and stress level on seed yields (kg ha^{-1}) in season one at flowering stage. +R: With *Rhizobium*, -R: Without *Rhizobium*. S1: Control, S3: Water stress imposed at flowering stage

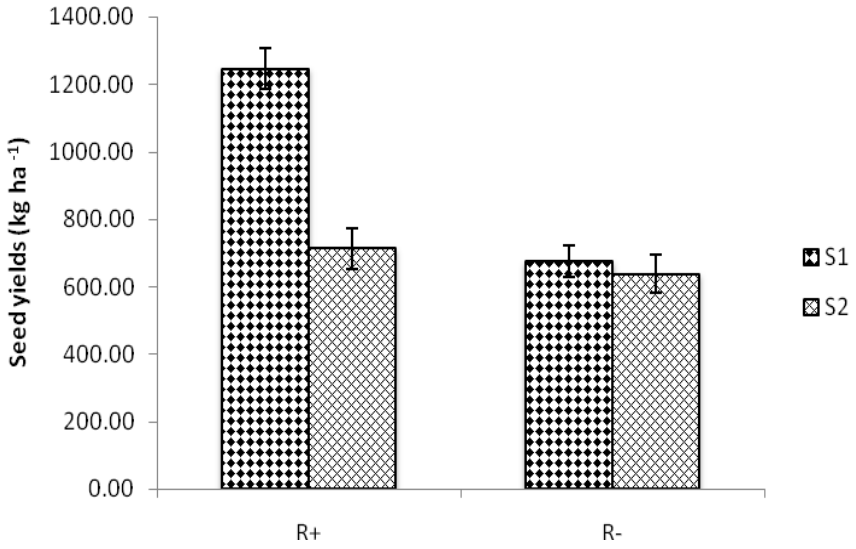


Figure 35: Interactive effects of *Rhizobium* and stress level on seed yields (kg ha^{-1}) in season two at vegetative stage. +R: With *Rhizobium*, -R: Without *Rhizobium*. S1: Control, S2: Water stress imposed at vegetative stage

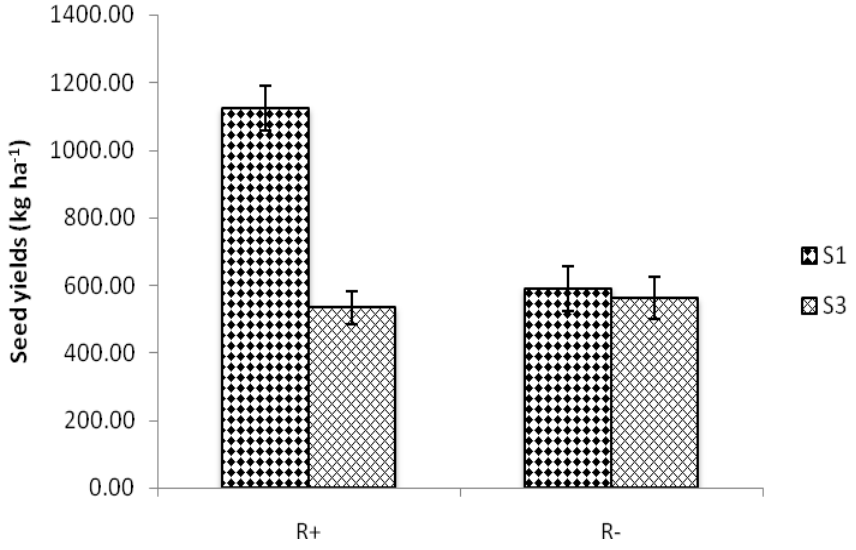


Figure 36: Interactive effects of *Rhizobium* and stress level on seed yields (kg ha^{-1}) in season two at flowering stage. +R: With *Rhizobium*, -R: Without *Rhizobium*. S1: Control, S3: Water stress imposed at flowering stage

8.4 Discussion

In the present study, we assessed the effects of *Rhizobium* inoculation and water stress periods on growth parameters in common bean (*P.vulgaris)*. This study clearly showed that *Rhizobium* inoculation was supportive in improving growth parameters of the common bean. *Rhizobium* inoculation had great positive effects on plant height at vegetative and flowering stages, number of leaves per plant, stem girth (mm) in both growth stages at screen house experiment and Leaf area (Tables 1, 4-6) as compared with the control. Significant observation were also observed in shoot dry weight (g^{-1} plant) and root dry weight (g $plant^{-1}$) as well as seed yields (kg ha^{-1}) (Tables 1-2 & 4) as compared with the control. These improvements in inoculated treatments could be attributed to the legume inoculants BIOFIX, which increased nitrogen supply to the plants and consequently improved the growth parameters of the plant. Our results are similar to those reported by Uchida, (2000) in which plant growth potential was enhanced as a result of Biological Nitrogen Fixation; and Tairo and Ndakidemi, (2013) who reported the improvement of growth parameters in *B.japonicum* inoculated soybeans. The plant height was significantly affected by *Rhizobium* inoculation. The least plant height was recorded in non- inoculated control. Findings by (Amany, 2007; Caliskan *et al*., 2008; Aminifard*et al*., 2010, Tairo and Ndakidemi, 2013; Mfilinge *et al*., 2014; Nyoki and Ndakidemi, 2014) showed that plant height was increased byrhizobial inoculation in different legumes.Moreover inoculated plants showed more dry matter and seed yields than non-inoculated plants. Inoculation with *Rhizobium* bacteria increased the shoot and root dry weight (g $plants^{-1}$) and seed yields (kg ha^{-1}) as compared with the non-inoculatedcontrol. Nitrogen is known to be an essential nutrient for plant growth and development. In this study, rhizobial inoculation increasedthe production of total dry matter in plants (Salvagiotti *et al*., 2008) which enhanced the potential of the plant growth and ultimately resulted in higher seed yields.

Water stress significantly reduced plant height (cm), number of leaves, stem girth (mm) and Leaf area (cm^2), shoot and root dry weight (g $plant^{-1}$) as well as seed yields (kg ha^{-1}) as compared

with control treatments which received adequate water supply. These findings are in line with studies by Hiler *et al*. (1972); Afolabi, (1998) and Aderolu, (2000) which showed decreased in plant height (cm) and number of leaves as a result of water stress. Thedecrease in the assessed growth parameters may be due to the impairment of cell division, cell enlargement caused by loss of turgor and inhibition of various growth metabolisms (Yordanov *et al*., 2003; Farooq *et al*., 2012). Common bean has been reported to respond differently to soil moisture stress during various stages depending on the severity of water stress (Emman *et al*., 2010). For example, in a study by Hayatu and Mukhtar, (2010) in cowpea genotypes, it was reported that drought affected dry matter production and many other aspects of plant growth such as plant height, stem diameter, leaf area and number of leaves, results similar to our study. In closely related studies involving maize, Khan *et al*. (2001) conducted a study comprising of six irrigation treatments and concluded that plant height, stem diameter, leaf area decreased noticeably with increasing water stress. The reduction in plant height could be attributed to decline in the cell enlargement and more leaf senescence in the plant under water stress (Manivannan *et al*., 2007a). Furthermore, Akinci and Losel (2009) also reported that water stress caused major reductions in plant height, leaf number and leaf area index of some *Cucurbitaceae* members. Apart from diseases, water stress has been reported to be the second major constraints in the legume seed yields (Rao, 2001). The reduced seed yields in bean yields as a result of water stress can be attributed to reduction in individual yield components such as dry matter yields, number of pods per plant, number of seeds per pod, seed weight as well as harvest index (Ramirez-Vallejo & Kelly, 1998; Shenkut & Brick, 2003). Report by Nielsen and Nelson (1998) on bean showed that seed yields were reduced due to reduced number of pods per plant and seeds per pod during water stress at flowering and/or reproductive stage. Similarly, in a study by Remenyik and Nemeske, (2010) in French bean (*Phaseolus vulgaris* (L.)) great variation was reported in yields as a result of irregular occurrence of drought periods accompanied by high temperature. Our findings are also similar with studies by (Molina *et al*., 2001; Nielsen and Nelson, 1998; Emam, 1985; Emam and Seghatoleslami, 2005). They all reported a reduction in grain yields and dry weight following water stress and this is attributed by lower percentage of pod production when the water stresses occurring especially during flowering.

P.vulgaris (L.) varieties *F9 Kidney Selection*, *F8 Drought line* and *JESCA* showed significant increase in seed yields (kg ha^{-1}), shoot and root dry weight (g $plant^{-1}$) compared with varieties

KAT B9 and *KAT B1*. The reduced yields in varieties *KAT B9* and *KAT B1* might be attributed by their low genetic potential to deal with water stress imposed at either vegetative or flowering growth stages. Study by Singh (1995) showed that water stress during flowering and grain filling reduced seed yield and seed weight and accelerated maturity of dry bean. It has been reported that the quality and the yield of beans were negatively affected by short periods of water shortage (Ramirez-Vallejo and Kelly 1998).

There was a significant interaction between *Rhizobium*,water stress treatments and varieties in plant height, number of leaves per plant, stem girth, shoot dry weight and seed yields of *P. vulgaris.*The interactions between inoculations showed that *Rhizobium* inoculation in water stress treatment imposed at vegetative and flowering stage had greater effect on the above parameters as compared with un-inoculated treatments. These results suggest that inoculating beans with rhizobial inoculants enhanced growth even in water stressed environment. However, further studies on the mechanism involved warrants further studies. Furthermore, the interactive effects of varieties, *F9 Kidney Selection*, *F8 Drought Line* and *JESCA* under water stressed environmentshows the potential of these varieties to be used in drought tolerant studies. In a closely salt stress related study by Ndakidemi and Makoi, (2009) bean variety *JESCA* showed moderate tolerance to salinity, suggesting the potentiality of this variety in adverse environmental condition such as water stress.

8.5 Conclusion

In conclusion, rhizobial inoculation significantly improved plant height (cm), number of leaves per plant, stem girth (mm), shoot and root dry weight (g plant^{-1}) as well as seed yields (kg ha^{-1}) as compared with un-inoculated treatments. Furthermore, water stress treatments imposed at vegetative and flowering stage significantly reduced plant height (cm), number of leaves, stem girth (mm), shoot and root dry weight (g plant^{-1}) as well as seed yields (kg ha^{-1}) as compared with plants supplied with water optimally. Varieties *F9 Kidney Selection*, *F8 Drought Line* and *JESCA* recorded best bean yields as compared with *KAT B9* and *KAT B1*, hence indicating their genetic potential in performing in adverse water supply.The interactions between inoculations showed that *Rhizobium* inoculation in water stress treatment imposed at vegetative and flowering stage had greater positive effects on growth and yield as compared with un-inoculated treatments. These results suggest that inoculating beans with rhizobial inoculants enhanced

growth even in water stressed environment.The interactive effects of varieties *F9 Kidney Selection*, *F8 Drought Line* and *JESCA* by performing well under water stressed environment demonstrates their potential of being used in drought tolerance studies.

CHAPTER NINE

9.0 GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

9.1 DISCUSSION

The study showed significant effects of rhizobial inoculationon proline content (µmol g^{-1}.FW) in the leaves of *P.vulgaris* (L.). The un-inoculated treatments were, however, characterized by lower proline accumulation in the leaves of *P.vulgaris* cultivars. Study by Andersen *et al*. (1995) indicated thatthere is a positive relationship between N availability and proline accumulation in plants. Further evidence in this study showed significantly higher proline accumulation as results of water stress. It has been reported that water stressed plants produce proline as an adaptive and survival mechanism under water stress conditions (Farooq *et al.,* 2009).Varieties *F8 Drought Line* and *JESCA* significantly recorded higher proline content as compared with other tested varieties and hence are potential candidates in stress tolerant studies (Chapter 3).

The study further indicated the potential of rhizobial inoculation and water stress in the accumulation of secondary compounds (i.e. Flavonoids and Anthocyanins) in the shoots of *P. vulgaris* cultivars. The results showed that non- inoculated treatments significantly increased the level of Flavonoids and Anthocyanis in the common bean cultivars. These suggest the up regulation of some enzymes in the shoots of the identified *P. vulgaris* cultivars such as Phenylalanine ammonia-lyase (PAL) under non-inoculated treatments. Kondorosi *et al*. (1995) reported the enhanced level of Phenylalanine ammonia-lyase (PAL) under low N condition, which also ressemble our findings. Additionally, the study also indicate that water stress treatments significantly increased the level of flavonoids and antocyanins (g DM^{-1}) concentration in the common bean cultivars (Tairo *et al*., 2017). Generally, various studies indicated the accumulation of secondary compounds as a result of different stresses for instance drought and/or water stress (Tairo *et al*., 2017; Farooq *et al*., 2009). It has been reported that this compounds protects the macro-molecules against several oxygen species produced following stress condition (Agati *et al*., 2012). The high contents of these compounds showed their potentiality under water stress. Varieties *F8 Drought Line*, *JESCA* and *F9 Kidney Selection* significantly contained more flavonoids and anthocyanins as compared with the other studied varieties, therefore can be involved in other drought studies (Chapter 4).

In the current study, rhizobial inoculation increased the level of chlorophyll contents in the common bean cultivars. This signifies the presence of N through rhizobial inoculation as nitrogen is the major component of the chlorophyll molecules and plays an essential function in photosynthesis process. However water stress proves negative effects by diminishing chlorophyll *a*, *b* and total chlorophyll contents among the *P.vulgaris* cultivars. The decrease in chlorophyll could be attributed by chloroplasts damage caused by reactive oxygen species (ROS) (Verbruggen and Hermans, 2008) occurring during the stress periods of either vegetative or flowering growth stages.This study revealed that chlorophyll content decreased with increased water stress indicating that photosynthetic pigments are sensitive to water stress conditions. Varieties *F9 Kidney Selection* and *KAT B1* significantly increased chlorophyll *a*, *b* and total chlorophyll contents in this study which suggests it's capability in chlorophyll metabolism as compared with the other studied common bean cultivars (Chapter 5).

In chapter 6, the study indicated that the rhizobial inoculation and un-stressed water treatments enhanced the relative leaf water contents and cell membrane stability in *P vulgaris*. These results suggest that through rhizobial inoculants and the presence of moisture had great input in water relations in this study. The high relative leaf water contents in this study as a result of rhizobial inoculation verify the findings of Namvar *et al*. (2013) on chick pea who showed a significant increase in relative leaf water contents as a result of rhizobial inoculation. The low relative leaf water contents and reduced cell membrane stability in stressed water treatments might be attributed to the decrease in leaf water potential and decreased availability/absorption and translocation of water from soil to roots and ultimately to leaves in the studied common bean cultivars. Among the studied cultivars, varieties *F9 Kidney Selection*, *F8 Drought line* and *JESCA* had great leaf relative water contents as compared with *KAT B9* and *KAT B1*and hence demonstrating their potential against drought.The interaction between rhizobial inoculation, water stress and the tested varieties on relative water content and electrolyte leakage implies the genetic potentiality of some of the identified varieties in relation of moisture and rhizobial inoculation.

In chapter 7 the study showed the significant effects on nutrients uptake (N, P, K, Ca and Mg) as a results of N through rhizobial inoculation and moisture in *P. vulgaris* (L.). The inoculated treatments increased the uptake of the nutrients assessed as compared with non inoculated

treatments. Rhizobia are known to secrete some chemical substances and stimulate plant growth of which eventually increased the uptake of nutrients in plant tissues (Perveen, *et al*., 2002; Khan and Zaidi 2007). In this study, water stress reduced the uptake on N, P, K, Ca and Mg as compared with un-stressed treatments. This reduction might be attributed by root shrinkage which was created during water stress. It has been reported thatwater stress affects nutrient transport to the root surface due to reduced contact between root and soil, hence limited transport to the other plant organs (Yordanov *et al*., 2003). The common bean cultivars *F8 Drought line, JESCA* and *F9 Kidney Selection* were efficient on mineral nutrients uptake as compared with *KAT B9* and *KAT B1*. The interaction effects in the current study indicate the capability of rhizobial inoculants and moisture status in the soil in enhancing nutrient uptake in the specific tested common bean cultivars.

In this study, *Rhizobium* inoculation was supportive in improving growth parameters and seed yields of the common bean cultivars. However water stress significantly reduced the parameters assessed. Nitrogen is the major elements and its availability plays a significance role as it influences photosynthesis activity of the plants (Uchida, 2000), which ultimately enhances growth and yields. Therefore, rhizobial inoculation had a positive function in improving growth parameters assessed indicating its potential for maximizing yields. The reduction in growth parameters due to water stress might be caused byimpairment of cell division and cell enlargement which could be attributed by loss of turgor associated by water stress in the growth stages. The varieties which showed positive results in growth and seed yields signify their genetic potential under water stress condition. The interactive effects of varieties, *F9 Kidney Selection*, *F8 Drought Line* and *JESCA* under water stressed environment shows the potential of these varieties to be used in drought tolerant studies (Chapter 8).

9.2 CONCLUSION

In this study rhizobial inoculation enhanced the accumulation of proline, chlorophyll *a*, *b* and total chlorophyll in the leaves of the *P. vulgaris* cultivars. Significant effects were also observed in relative leaf water contents and electrolyte leakage as well as nutrients uptake and seed yields respectively. However, negative effects were observed in flavonoids and anthocyanins as a result of rhizobial inoculation. Furthermore, water stress during the production phase affected the physiology and morphological characteristics of the tested plants and hence influencing growth,

productivity and final yields. Generally,water stress imposed at either of the growth stages (vegetative and flowering) showed negative effects by reducing leaf chlorophyll contents, relative leaf water contents, electrolyte leakage, mineral nutrients uptake, growth and seed yields. However, proline accumulation as well as flavonoids and anthocyanins significantly increased as a result of water stress treatments. Based on proline, flavonoids and anthocyanins accumulation; the tested cultivars *F8 Drought line, JESCA* and *F9 Kidney Selection*can be promoted for agricultural production especially in drought prone areas, while still involved for further examination. These study suggest that water stress had positive effects on secondary compounds assessed (flavonoids and anthocyanins) and amino acids (proline), however negative effects were observed in growth parameters and seed yields, nutrients uptake, chlorophyll contents and relative leaf water contents and electrolyte leakage as a results of rhizobial inoculation and water stress in five *P. vulgaris* cultivars studied.

9.3 RECOMMENDATION

For improvement of soil fertility and sustainable productivity of *P vulgaris*, the use of rhizobial inoculation is recommended because growth and yield improvements were achieved in this study. Furthermore, rhizobial inoculation is also recommended as they facilitated plant uptake of Ca, P, Mg N, and K. Therefore, appropriate rhizobial strain specific to a particular legume should be applied in order to improve yields and attain sustainable economic performance in legume production. The uses of rhizobial inoculants in the country can be promoted to farmers along with government subsidy or through national agriculture inputs in Tanzania.

Water stress resulted into the accumulation of proline and secondary plant metabolites such as flavonoids and anthocyanin. These compounds played significant role in protecting the plants under water stress conditions. However, further investigations are recommended to explore different functions of these metabolites in defending the plants and improving their growth and development. Varieties *F8 Drought line, JESCA* and *F9 Kidney Selection* demonstrated the capability to accumulate proline and secondary plant metabolites such as flavonoids and anthocyanin as compared with other tested cultivars. Research evidence elsewhere has demonstrated positive association of these compounds and tolerance to stress. From these preliminary results, the varieties are recommended for further studies under different levels of water stress conditions.

REFERENCES

Abate, T. (ed.). (2012). Four Seasons of Learning and Engaging Smallholder Farmers: Progress of Phase 1. PO Box 39063, Nairobi, Kenya. International Crops Research Institute for the Semi-Arid Tropics. 258 pp.

Abd-Alla, M H. (1994). Phosphatases and the Utilization of Organic P by *Rhizobium leguminosarum* Biovar Viceae. *Letters Applied Microbiology*. **18**(5): 294-296.

Abd-Alla, M.H. (1992). Nodulation and nitrogen fixation in faba bean (*Vicia faba* L.) plants under salt stress. *Symbiosis*. **12**: 311-319.

Abu-Muriefah, S.S. (2013). Effect of chitosan on common bean (*Phaseolus vulgaris* L.) plants grown under water stress conditions. *Global Journal of Agricultural Sciences*. **1**(1): 001-008.

Acosta Gallegos, J.A. and Kohashi Shibata, J. (1989). Effects of water stress on growth and yield of indeterminate dry-bean (*Phaseolus vulgaris*) cultivars. *Field Crops Research*. **20**: 81-93.

Aderolu, M.A. (2000). The effect of water stress at different growth stages on yield and seed quality of cowpea varieties. B. Agric. Project Submitted to the Department of Crop Production University Ilorin. Nigeria.

Adnane, B., Mainassara, M.A., Mohamed, F., Mohamed, L., Jean-Jacques, D., Rim, T.M. and Georg, C. (2015). Physiological and Molecular Aspects of Tolerance to Environmental Constraints in Grain and Forage Legumes. *International Journal of Molecular Sciences*. **16**: 18976-19008.

Afolabi, E.S. (1998). The effects of water stress at different stages on growth and yield of cowpea (*Vigna unguiculata* (L) Walp) cultivars. Unpublished Dissertation for the Award of Msc. Degree at Department of Crop Production University of Ilorin, Nigeria.

Agati, G., Azzarello, E., Pollastri, S. and Tattini, M. (2012). Flavonoids as antioxidants in plants: location and functional significance. *Plant Science*. **196**: 67-76.

Agbicodo, E.M., Fatokun, C.A., Muranaka, R.G.F., Visser, R.G.F. and Linden van der, C.G. (2009). Breeding drought tolerant cowpea; constraints, accomplishments, and future prospects. *Euphytica*. **167**: 353-370.

Ahmadi, N. (1985). Plant physiology (photosynthesis and nutrition). First Edition. Center for Academic Publication. **Pp.** 14-16.

Akinci, S. and Losel, D.M. (2009). The soluble sugars determination in *Cucurbitaceae* species under water stress and recovery periods. *Advances in Environmental Biology*. **3**(2):175-183.

Ali, O., Ashraf, M., Shahbaz, M. and Humera, H. (2008). Ameliorating effect of foliar applied proline on nutrient uptake in water stressed maize (*Zea mays* (L.)) plants. *Pakistan Journal of Botany*. **40**(1): 211-219.

Alizade A. (2002). Soil, Water and Plants Relationship. 3rd Edn. Emam Reza University Press, Mashhad, Iran, ISBN: 964-6582-21-4.

Amany, A.B. (2007). Effect of plant density and urea foliar application on yield and yield components of chickpea (*Cicer arietinum* L.). *Research Journal of Agriculture and Biological Sciences*. **3**(4): 220-223.

Aminifard, M.H., Aroiee, H., Fatemi, H., Ameri, A. and Karimpour, S. (2010). Responses of Eggplant (*Solanum Melongenal* L.) to different rates of nitrogen under field conditions. *Journal of Central European Agriculture*. **11**(4): 453-458.

Amtmann, A. and Blatt, M.R. (2009). Regulation of macronutrient uptake. *New Phytologist*. **181**:35-52.

Andersen, P.C., Brent, V.B. and Ruseel, F.M. (1995). Water stress and nutrient solution mediated changes in water relations and amino acids, organic acids, and sugars in xylem fluid of *Prunus salicina* and *Lagerstroemia indica. Journal of the American Society for Horticultural Science*. **120**: 36-42.

Andrade, J.L., Larquee-Saavedra, A. and Trejo, C.L. (1995). Proline accumulation in leaves of four cultivars of *Phaseolus vulgaris* L. with different drought resistance. *Fyton International Journal of Experimental Botany*. **57**: 149-157.

Anjum, M.S., Ahmed, Z.I. and Rauf, C.A. (2006). Effect of *Rhizobium* Inoculation and Nitrogen Fertilizer on Yield and Yield Components of Mungbean. *International Journal of Agriculture and Biology*. **8**(2): 1560-8530.

Anjum, S.A., Farroq, M., Wang, L.C., Xue, L.L., Wang, S.G., Wang, L., Zhang, S. and Chen, M. (2011). Gas exchange and chlorophyll synthesis of maize cultivars are enhanced by exogenously-applied glycinebetaine under drought conditions. *Plant Soil Environment*. **57**(7): 326-331.

Aoki, T., Akashi, T. and Ayabe, S. (2000). Flavonoids of leguminous plants: structure, biological activity, and biosynthesis. *Journal of Plant Research*. **113**: 475- 488.

Arnon, D.I. (1949). Copper Enzymes in Chloroplasts, Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology*. **24**(1): 1-15.

Arrese-Igor, C., Gonzalez, E.M., Marino, D., Ladrera, R., Larrainzar, E. and Gil-Quintana, E. (2009). Physiological Responses of legume nodules to drought. *Plant stress*. **5**(1): 4-31.

Ashraf, M. and Foolad, M.R. (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental Experimental Botany*. **59**(2): 206–216.

Ashraf, M. and Iram, A. (2005). Drought stress induced changes in some organic substances in nodules and other plant parts of two potential legumes differing in salt tolerance. *Flora*. **200**: 535-546.

Athar, A. (1998). Drought tolerance by lentil rhizobia (*Rhizobium leguminosarum*) from arid and semi arid areas of Pakistan. *Letters in Applied Microbiology*. **26**: 38-42.

Awad, M.A. and Jager, A. (2002). Relationship between fruit nutrients and concentrations of flavonoids and chlorogenic acid in 'Elstar' apple skin. *Scientia Horticulturae*. **92**: 265-276.

Ayoola, O.T. (2010). Yield Performance of Crops and Soil Chemical Changes under Fertilizer Treatments in a Mixed Cropping System. *African Journal of Biotechnology*. **9**(26): 4018-4021.

Balakumar, T., Hani Babu Vincent, V. and Paliwal, K. (1993). On the interaction of UV-B radiation (280-3 15nm) with water stress in crop plants. *Physiologia Plantarum*. **87**: 217-222.

Baligar, V.C., Fageria, N.K. and He, Z.L. (2001). Nutrient use efficiency in plants. *Communications in Soil Science Plant Analysis*. **32**: 921-950.

Bambara, S. and Ndakidemi, P.A. (2009). Effects of *Rhizobium* inoculation, lime and molybdenum on photosynthesis and chlorophyll content of Phaseolus vulgaris L. *African Journal of Microbiology Research*. **3**(11): 791-798.

Baqual, M. F. and Das, P. K. (2006). Influence of Biofertilizers on Macronutrient Uptake by the Mulberry Plant and Its Impact on Silkworm Bioassay. *Caspian Journal of Environmental Sciences*. **4**(2): 98-109.

Bargaz, A., Zaman-Allah, M., Farissi, M., Lazali, M., Drevon, J., Maougal, R.T. and Carlsson, G. (2015). Physiological and Molecular Aspects of Tolerance to Environmental Constraints in Grain and Forage Legumes. *International Journal of Molecular Sciences*. **16**: 18976-19008.

Barnabas, B., Jager, K. and Feher, A. (2008). The effect of drought and heat stress on reproductive processes in cereals. *Plant cell Environment*. **31**: 11-38.

Baroowa, B. and Gogoi, N. (2012). Effect of induced drought on different growth and biochemical attributes of black gram (*Vigna mungo* L.) and green gram (*Vigna radiata* L.). *Journal of Environmental Research and Development*. **6**(3A): 584-593.

Barret, R.P. (1990). Legume species as leaf vegetables. In: J. Janick and J.E. Simon (Eds), Advances In New Crops. Timber Press, Portland, OR. Pp. 391-396.

Bates, L.S., Waldren, R.P. and Teare, I.D. (1973). Rapid determination of free proline for water stress studies. *Plant Soil*. **39**: 205-208.

Beebe, S., Ramirez, J., Jarvis, A., Rao, I.M., Mosquera, G., Bueno, J.M. and Blair, M.W. (2011). Genetic Improvement of Common Beans and the Challenges of Climate Change. Crop Adaptation to Climate Change, First Edition. Pp. 356-369.

Beebe, S.E., Rao, I.M. and Blair, M.W. (2010). Phenotyping common beans for adaptation to drought. In: JM, Ribaut and P Monneveux (eds) Drought Phenotyping in Crops: From theory to Practice. Generation Challenge Program Special Issue on Phenotyping. Pp. 311–334.

Beebe, S.E., Rao, I.M., Blair, M.W. and Acosta-Gallegos, J.A. (2013). Phenotyping common beans for adaptation to drought. *Frontiers in Physiology*. **4**: 35.

Bergman, W. (1992). Nutritional disorders of plants development, visual and analytical diagnosis, Gustav Fischer Vela Jena, Stuttgart, New York.

Bharambe, P. R. and Joshi, P. S. (1993). Effect of soil water potential on growth, yield and some biochemical changes in Sorghum. *Journal of the Indian society of Soil Science*. **41** (2): 342-343.

Blackman, S.A., Obendorf, R.L. and Lepold A.C. (1995) Desiccation tolerance in developing soybean seeds: The role of stress proteins. *Plant Physiology*. **93**: 630-638.

Blokhina, O., Virolainen, E. and Fagerstedt, K.V. (2003). Antioxidants, oxidative damage and oxygen deprivation stress. *Annals of Botany*. **91**: 179-194.

Blum, A. and Ebercon, A. (1981). Cell membrane stability as a measure of drought and heat tolerance in wheat. *Crop Science*. **21**: 43-47.

Bojovic, B. and Markovic, A. (2009). Correlation between nitrogen and chlorophyll content in wheat (*Triticum aestivum* L.). *Kragujevac Journal of Science*. **31**: 69-74.

Bongue-Bartelsman, M. and Phillips, D.A. (1995). Nitrogen stress regulates gene expression of enzymes in the flavonoid biosynthetic pathway of tomato plant. *Physiology and Biochemistry Paris*. **33**: 539-546.

Brear, E.M., Day, D.A. and Smith, P.M (2013). Iron: An essential micronutrient for the legume–Rhizobium symbiosis. *Frontier in Plant Science and Plant Nutrition*. **4**: 1–15.

Bremner, J. M. (1965). *Total Nitrogen*, In: C. A. Black, Ed., Methods of Soil Analysis, Part 2, American Society of Agronomy. Madison. Pp. 1149-1178.

Brockwell, J., Bottomley, P.J. and Thies, J.E. (1995). Manipulation of rhizobia microflora for improving legume productivity and soil fertility: a critical assessment. *Plant Soil*. **174**: 143-180.

Broekaert, J.A.C. (2002). Analytical Atomic Spectrometry with Flames and Plasmas. Weinheim, Germany: Wiley-VCH Verlag GmbH.

Brown, C.E., Pezeshki, S.R. and DeLaune, R.D. (2006). The effect of salinity and soil drying on nutrient uptake and growth of Spartina alterniflora in a simulated tidal system. *Environmental Experimental Botany*. **58**: 140-148.

Burns, I.G. (1992). Influence of plant nutrient concentration on growth rate: use of a nutrient interruption technique to determine critical concentrations of N, P and K in young plants. *Plant Soil*. **142**: 221–233.

Busse, M.D. and Bottomley, P.J. (1989). Growth and nodulation responses of Rhizobium meliloti to water stress induced by permeating and non-permeating solutes. *Applied Environmental Microbiology*. **55**: 2431-2436.

Caliskan, S., Ozkaya, I., Caliskan, M.E. and Arslan, M. (2008). The effect of nitrogen and iron fertilization on growth, yield and fertilizer use efficiency of soybean in Mediterranean type soil. *Field Crops Research*. **108**: 126-132.

Chalker-Scott, L. (1999). Environmental Significance of Anthocyanins in Plant Stress Responses. *Photochemistry and Photobiology*. **70**(1): 1-9.

Chaves, M.M., Pereira, J.S., Maroco, J., Rodriques, M.L., Ricardo, C.P.P., Osorio, M.L., Carvatho, I., Faria, T. and Pinheiro, C. (2002). How plants cope with water stress in the field photosynthesis and growth? *Annals of Botany*. **89**: 907–916.

Chiang, H.H. and Dandekar, A.M. (1995). Regulation of proline accumulation in Arabidopsis thaliana (L.) Heynh during development and in response to desiccation. *Plant Cell Environment*. **18**: 1280–1290.

Chu, W., Zhengquan, Hailong, S. and Shenglei, G. (2005). Effects of Different Concentrations of Nitrogen and Phosphorus on Chlorophyll Biosynthesis, Chlorophyll *a* Fluorescence, and Photosynthetic Rate in Larix olgensis Seedlings. **41**(4): 31 – 36.

Cooper, J.E. (2004). Multiple responses of rhizobia to flavonoids during legume root infection. *Advances in Botanical Research*. **41**: 1–62.

Cornic, G. (2000). Drought stress inhibits photosynthesis by decreasing stomatal aperture: Not by affecting ATP synthesis. *Trends in Plant Science*. **5**: 187-188.

Dakora, F.D. (1995). Plant flavonoids-biological molecules for useful exploitation. *Australian Journal of Plant Physiology*. **22**: 87–99.

Dakora, F.D. (2003). Defining new roles for plant and rhizobial molecules in sole and mixed plant cultures involving symbiotic legumes. *New Phytologist*. **158** (1): 39-49.

Dakora, F.D. and Keya, S.O. (1997). Contribution of legume nitrogen fixation to sustainable agriculture in sub-Saharan Africa. *Soil Biology and Biochemistry*. **29**: 809–817.

Dakora, F.D. and Phillips, D.A. (1996). Diverse functions of isoflavonoids in legumes transcend anti-microbial definitions of phytoalexins. *Physiology and Molecular Plant Pathology*. **49**: 1-20.

Dakora, F.D. Matiru, V.N. and Kanu, A.S. (2015). Rhizosphere ecology of lumichrome and riboflavin, two bacterial signal molecules eliciting developmental changes in plants. *Frontiers in Plant Science*. **6**: 700.

Dandekar, A.M. and Uratsu, S.L. (1988). A simple base pair change in proline biosynthesis genes causes osmotic stress tolerance. *Journal of Bacteriology*. **170**: 5943–5945.

Daniel, M., Pierre, F., Ruben, L., Ana, Z., Alain, P., Cesar, A.I. and Esther, M.G. (2007). Nitrogen fixation control under drought stress. Localized or Systemic? *Plant Physiology*. **143**(4): 1968-1974.

Daniells, J.W. and Watson, B.J. (1984). Effects of water stress on bananas. *Banana Newsl*. **7**:3.

Das, K. and Roychoudhury, A. (2014). Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. *Frontier in Environmental Science*. **2**: 53.

Davey, A.G. and Simpson, R.J. (1990). Nitrogen fixation by subterranean clover at varying stages of nodule dehydration. *Journal of Experimental Botany*. **41**: 1175-1187.

De Carvalho, M.H.C. (2008). Drought stress and reactive oxygen species: Production, scavenging and signaling. *Plant Signaling Behaviour*. **3**: 156-165.

De Magalhaes, A.A., Millar, A.A. and Choudhury, E.N. (1978). Effect of water deficit on reproductive period about the production of bean. *Pesquisa Agropecuaria Brasileira*. **13**: 55-60.

Delauney, A.J. and Verma, D.P.S. (1993). Proline biosynthesis and osmoregulation in plants. *The Plant Journal*. **4**(2): 215-223.

Delauney, A.J., Hu, C.A.A., Kavi Kishor, P.B. and Verma D.P.S. (1993). Cloning of ornithineamirwtransferase cDNA from *Vigna aconitifolia* by trans-complementation in Escherichia coli and regulation of proline biosynthesis. *Journal of Biological Chemistry*. **268**: 18673–18678.

Devries, J.D. Bennett, J.M. Albrecht, S.L. and Boote, K.J. (1989). Water relations, nitrogenase activity and root development of three grain legumes in response to soil water deficits. *Field Crop Research*. **21**: 215–226.

Dhanda, S.S., Sethi, G.S. and Behl, R.K. (2004). Indices of drought tolerance in wheat genotypes at early stages of plant growth. *Journal of Agronomy and Crop Science*. **190**: 6-12.

Di Ferdinando, M., Brunetti, C., Agati, G. and Tattini, M. (2014). Multiple functions of polyphenols in plants inhabiting unfavourable Mediterranean areas. *Environmental and Experimental Botany*. **103**: 107-116.

Di Ferdinando, M., Brunetti, C., Fini, A. and Tattini, M. (2012). Flavonoids as antioxidants in plants under abiotic stresses. In: Ahmad P, Prasad MNV, editors. Abiotic Stress Responses in Plants: Metabolism Productivity and Sustainability. New York: Springer Verlag. Pp. 159-179.

Dixon, R.A. and Paiva, N.L. (1995). Stress-induced phenylpropanoid metabolism. *The Plant Cell*. **7**: 1085–1097.

Dixon, R.A. and Steele, C. (1999). Flavonoids and isoflavonoids-gold mine for metabolic engineering. *Tropical Plant Science*. **4**: 394-400.

Djibril, S., Mohamed, O.K., Diaga, D., Diégane, D., Abaye, B.F., Maurice, S. and Alain, B. (2005). Growth and development of date palm (*Phoenix dactylifera* L.) seedlings under drought and salinity stresses. *African Journal of Biotechnology*. **4**: 968-972.

Elboutahiri, N., Thami-Alami, I. and Udupa, S.M. (2010). Phenotypic and genetic diversity in Sinorhizobium meliloti and S. medicae from drought and salt affected regions of Morocco. *BMC Microbiology*. **10**: 15.

Emam, Y. (1985). Effects of N levels and moisture regimes on agronomic characteristics of four cultivars of dry beans (*Phaseolus vulgaris* L.). Unpublished Thesis for the Award of Msc. Degree at Shiraz University Shiraz, Iran, pp, 41.

Emam, Y. and Seghatoleslami, M.J. (2005). *Crop Yield*: Physiology and Processes. First edition. Shiraz University Inc., Shiraz. Pp. 593. (in Farsi).

Emam, Y., Shekoofa, A., Salehi, F. and Jalali, A.H. (2010). Water Stress Effects on Two Common Bean Cultivars with Contrasting Growth Habits. *American-Eurasian Journal of Agriculture and Environmental Sciences*. **9**(5): 495-499.

Enyiukwu, D.N., Awurum, A.N., Ononuju, C.C. and Nwaneri, J.A. (2014). Significance of characterization of secondary metabolites from extracts of higher plants in plant disease management. *International Journal of Advance Agricultural Research*. **2**: 8-28.

Espevig, T., Xu, C., Aamlid, T.S., DeCosta, M. and Huang, B. (2012). Proteomic responses during cold acclimation in association with freezing tolerance of velvet bent grass. *Journal of the American Society for Horticultural Sciences*. **137**: 391–399.

Fabiano, E., Gualtieri, G., Pritsch, C., Polla, G. and Arias. A. (1994). Extent of high-affinity iron transport systems in field isolates of rhizobia. *Plant Soil*. **164**: 177-185.

Fairbairn, J.N. (1993). Evaluation of Soils, Climate and Land Use Information at Three Scales: The case study of Low Income Bean Farming in Latin America, University of Reading UK.

Falcone Ferreyra, M.L., Rius, S.P. and Casati, P. (2012). Flavonoids: biosynthesis, biological functions, and biotechnological applications. *Frontiers in Plant Science*. **3**: 222.

Farhad, M-S., Babak, A.M., Reza, Z.M., Hassan, R-S.M. and Afshin, T. (2011). Response of proline, soluble sugars, photosynthetic pigments and antioxidant enzymes in potato (*Solanum tuberosum* L.) to different irrigation regimes in green house condition. *Australian Journal of Crop Science*. **5**(1): 55-60.

Farooq, M., Basra, S.M.A., Wahid, A., Cheema, Z.A., Cheema, M.A. and Khaliq, A. (2008). Physiological role of exogenously applied glycinebetaine in improving drought tolerance of fine grain aromatic rice (*Oryza sativa* L.). *Journal of Agronomy and Crop Science*. **194**: 325-333.

Farooq, M., Hussain, M., Wahid, A. and Siddique, K.H.M. (2012). Drought stress in plants: An overview. In Plant Responses to Drought Stress from Morphological to Molecular Features; Aroca, R., Ed.; Springer-Verlag: Berlin, Germany. Pp. 1–36.

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D. and Basra, S.M.A. (2009). Plant drought stress: effects, mechanisms and management. *Agronomy for Sustainable Development*. **29**: 185-212.

Farooq, Sh. and Azam, F. (2006). The use of cell membrane stability (CMS) technique to screen for salt tolerant wheat varieties. *Journal of Plant Physiology*. **163**: 629-637.

Faye, I., Diouf, O., Guisse, A., Sene, M. and Diallo, N. (2006). Characterizing root responses to low phosphorus in pearl millet [*Pennisetum glaucum* (L.) R. Br.]. *Agronomy Journal*. **98**: 1187-1194.

Fini, A., Brunetti, C., Di Ferdinando, M., Ferrini, F. and Tattini, M. (2011). Stress-induced flavonoid biosynthesis and the antioxidant machinery of plants. *Plant Signaling and Behavior*. **6**(5): 709-711.

Ford, C.W. (1984). Accumulation of low molecular weight solutes in water stressed tropical legumes. *Phytochemistry*. **23**: 1007–1015.

Foyer, C.H., Descourvieres, P. and Kunert, K.J. (1994). Photo oxidative stress in plants. *Plant Physiology*. **92**: 696-717.

Fuzhong, W., Weikai, B., Fanglan, L. and Ning, W. (2008). Effect of drought stress and N supply on the growth, biomass partitioning and water- use efficiency of Sophora davidii seedling. *Environmental Experimental Botany*. **63**: 248-255.

Gadallah, M.A.A. (1999). Effects of proline and glycinebetaiene on *Vicia faba* responses to salt stress. *Biological Plantarum*. **42**(2): 249-257.

Gama, P.B.S., Inanaga, S., Tanaka, K. and Nakazawa, R. (2007). Physiological response of common bean (*Phaseolus vulgaris* L.) seedlings to salinity stress. *African Journal of Biotechnology*. **6**: 079-088.

Garg, B.K. (2003). Nutrient uptake and management under drought: nutrient-moisture interaction. *Current Agriculture*. **27**: 1–8.

Ghanbari, A., Siahsar, B., Tavassoli, A., Esmaeilian, Y. and Babaeian, M. (2011). Effects of Uniconazole and Cycocel on Growth, Yield and Nutrient uptake of Pearl Millet under Drought Stress Condition. *American-Eurasian Journal of Agriculture and Environmental Sciences*. **10**(5): 857-862.

Ghanbari, A.A., Shakiba, M.R., Toorchi, M. and Choukan, R. (2013). Morpho-physiological Responses of Common Bean Leaf to Water Deficit Stress. *European Journal of Experimental Biology*. **3**(1): 487-492.

Ghassemi-Golezani, K., Ghanehpoor, S. and DabbaghMohammadi-Nasab, A. (2009). Effect of water limitation on growth and grain filling of faba bean cultivars. *Journal of Food Agriculture & Environment*. **7**(3 &4): 442-447.

Gholinezhad, E., Aynaband, A., Ghorthapeh, A.H., Noormohamadi, G. and Bernousi, I. (2009). Study the effect of drought stress on yield, yield components and harvest index of sunflower hybrid iroflor at different levels of nitrogen and plant population. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*. **37**(2): 85-94.

Gordon, A.J., Minchin, F.R., Skot, L. and James, C.L. (1997). Stress-induced declines in soybean N2 fixation are related to nodule sucrose synthase activity. *Plant Physiology*. **114**: 937-946.

Graham, P.H. (1992). Stress tolerance in Rhizobium and Bradyrhizobium and nodulation under adverse soil conditions. *Canadian Journal of Microbiology*. **38**: 475-484.

Graham, P.H. and Vance, C.P. (2000). Nitrogen fixation in perspective: an overview of research and extension needs. *Field Crop Research*. **65**: 93–106.

Grossman, A. and Takahashi, H. (2001) Macronutrient utilization by photosynthetic eukaryotes and the fabric of interactions. *Annual Review of Plant Physiology*. **52**: 163-210.

Guerin, V., Trichant, J. C. and Rigaud, J. (1990). Nitrogen fixation (C_2H_2 reduction) by broad bean (*Vicia faba* L.) nodules and bacteroids under water-restricted conditions. *Plant Physiology*. Baltimore. **92**: 595-601.

Gunes, A., Cicek, N., Inal, A., Alpaslan, M., Eraslan, F., Guneri, E. and Guzelordu, T. (2006). Genotypic response of chickpea (*Cicer arietinum* L.) cultivars to drought stress implemented at pre and post-anthesis stages and its relations with nutrient uptake and efficiency. *Plant Soil Environment*. **52**: 368–376.

Gupta, V.K., Simlai, A., Tiwari, M., Bhattacharya, K. and Roy, A. (2014). Phytochemical contents, antimicrobial and antioxidative activities of *Solanum sisymbriifolium*. *Journal of Applied Pharmaceutical Science*. **4**(03): 075-080.

Gyaneshwar, P., Kumar, G.N., Parekh, L.J. and Poole, P.S. (2002). Role of soil microorganisms in improving P nutrition of plants. *Plant and Soil*. **245**: 83-93.

Halder, A.K. and Chakrabartty, P.K. (1993). Solubilization of Inorganic Phosphate by *Rhizobium*. *Folia Microbiology*. **38**(4): 325-330.

Hansch, R. and Mendel, R.R. (2009). Physiological functions of mineral micronutrients (Cu, Zn, Mn, Fe, Ni, Mo, B, and Cl). *Current Opinion in Plant Biology*.**12**: 259–266.

Hare, P.D. and Cress, W.A. (1997). Metabolic implications of stress-induced proline accumulation in plants. *Plant Growth Regulation*. **21**: 79–102.

Hare, P.D. and Cress, W.A. (1997). Metabolic responses of mesophytes to plant water deficits. *Annual Review of Plant Physiology*. **33**: 163-203.

Hare, P.D. Cress, W.A. and Van Staden, J. (1999). Proline synthesis and degradation: a model system for elucidating stress-related signal transduction. *Journal of Experimental Botany*. **50**: 413-434.

Harris, D., Tripathi, R.S. and Joshi, A. (2002). On-farm seed priming to improve crop establishment and yield in dry direct-seeded rice, in: Pandey S, Mortimer M, Wade L, Tuong TP, Lopes K, Hardy B. (Eds.). Direct seeding: Research Strategies and Opportunities, International Research Institute, Manila, Philippines. Pp. 231-240.

Hassan, S. and Mathesius, U. (2012). The role of flavonoids in root rhizosphere signalling: opportunities and challenges for improving plant microbe interactions. *Journal of Experimental Botany*. **63**(9): 3429 -3444.

Hayat, S., Hayat, Q., Alyemeni, M.N., Wani, A.S., Pichtel, J. and Ahmad, A. (2012). Role of proline under changing environments. *Plant Signalling & Behaviour*. **7**(11): 1456-1466.

Hayatu, M. and Mukhtar, F.B. (2010). Physiological responses of some drought resistant cowpea genotypes (*Vigna unguiculata* (L.) Walp) to water stress. *Bayero Journal of Pure and Applied Sciences*. **3**(2): 69-75.

Hesse, P.R. (1971). A Textbook of Soil Chemical Analysis, Murray, London. Pp. 120-309.

Hiler, E.A., Van Bauel, C.H.M., Hossain, M.M. and Jordan, W.R. (1972). Sensitivity of Southern peas to plant water deficits at three growth stages. *Agronomy Journal*. **64**: 60-64.

Hiscox, J.D. and Israelstam, G.F. (1979). A Method for the Extraction of Chlorophyll from Leaf Tissues without Maceration. *Canadian Journal of Botany*. **57**(12): 1332-1334.

Hokmalipour, S. and Darbandi, M.H. (2011). Effects of Nitrogen Fertilizer on Chlorophyll Content and Other Leaf Indicate in Three Cultivars of Maize (Zea mays L.). *World Applied Sciences Journal*. **15**(12): 1780-1785.

Horbowicz, M., Kosson, R., Grzesiuk, A. and Dębski, H. (2008). Anthocyanins in fruit and vegetables - their occurrence, analysis and role in human nutrition. V*egetable Crops Research Bulletin*. **68**: 5-22.

Hose, E., Clarkson, D.T., Steudle, E., Schreiber, L. and Hartung, W. (2001). The exodermis: a variable apoplastic barrier. *Journal of Experimental Botany*. **52**: 2245-2264.

Hussain, M., Malik, M.A., Farooq, M., Ashraf, M.Y. and Cheema, M.A. (2008). Improving drought tolerance by exogenous application of Glycine betaine and salicylic acid in sunflower. *Journal of Agronomy and Crop Sciences*. **194**: 193-199.

Hussain, M.B., Zahar, Z.A., Asghar, H.N. and Asghar, M. (2014). Can catalase and exopolysaccharides producing rhizobia ameliorate drought stress in wheat? *International Journal of Agriculture Biology*. **16**: 3-13.

Ibrahim, M.H., Jaafar, H.Z.E., Rahmat, A. and Rahman, Z.A. (2011). The Relationship between Phenolics and Flavonoids Production with Total Non Structural Carbohydrate and Photosynthetic Rate in Labisia pumila Benth, under High CO_2 and Nitrogen Fertilization. *Molecules*. **16**: 162-174.

Jaleel, C.A., Gopi, R., Sankar, B., Manivannan, P., Kishorekumar, A., Sridharan, R. and Panneerselvam, R. (2007). Studies on germination, seedling vigour, lipid peroxidation and proline metabolism in *Catharanthus roseus* seedlings under salt stress. *South African Journal of Botany*. **73**: 190–195.

Kaewpradit, W., Toomsan, B., Cadisch, G., Vityakon, P., Limpinuntana, V., Saenjan, P., Jogloy, S. and Patanothai, A. (2009). Mixing groundnut residues and rice straw to improve rice yield and use efficiency. *Field Crops Research*. **110**: 130–138.

Kaiser, W.M. (1987). Effects of water deficit on photosynthetic capacity. *Physiologia Plantarum*.**71**: 142-249.

Kaiser, W.M., Prachuab, G., Kaiser, G., Wildmann, S.G. and Heber, U. (1981). Photosynthesis under osmotic stress. Inhibition of photosynthesis of intact chloroplasts, protoplasts and leaf 62 slices at high osmotic potentials. *Plantarum*. **153**: 416-422.

Kapuya, J.A., Barendse, G.W.M. and Linskens, H.F. (1995). Water stress tolerance and proline accumulation in *Phaseolus vulgaris*. *Acta Botanica Neerlandica*. **34**: 295-300.

Kavikishor, P.B., Sangam, S., Amrutha, R.N., Sri Laxmi, P., Naidu, K.R., Rao, K.R.S.S., Rao, S., Reddy, K.J., Theriappan, P. and Sreenivasulu, N. (2005). Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: It's Implication in plant growth & abiotic stress tolerance. **88(**3): 424-438.

Keyvan, S. (2010). The effects of drought stress on yield, relative water content, proline, soluble carbohydrates and chlorophyll of bread wheat cultivars. *Journal of Applied Physiology*. **8**(3): 1051-1060.

Khadem, S.A., Galavi, M., Ramordi, M., Mousavi, S.R., Rousta, M.J. and Rezvani-Moghadam, P. (2010). Effect of animal manure and superabsorbent polymer on corn leaf relative water content, cell membrane stability and leaf chlorophyll content under dry condition. *Australian Journal of Crop Science*. **4**(8): 642-647.

Khan, M.B., Hussain, N. and Iqbal, M. (2001). Effect of water stress on growth and yield components of maize variety YHS 202. *Journal of Research Science*. **12**(1): 15-18.

Khan, M.S. and Zaidi, A. (2007). Synergistic Effects of the Inoculation with Plant Growth-Promoting Rhizobacteria and an Arbuscular Mycorrhizal Fungus on the Performance of Wheat. *Turkish Journal of Agriculture and Forestry*. **31**(6): 355-362.

Khanna-Chopra, R. and Selote, D.S. (2007). Acclimation to drought stress generates oxidative stress tolerance in drought-resistant than-susceptible wheat cultivar under field conditions. *Environmental Experimental Botany*. **60**: 276–283.

Kiani, S.P., Maury, P., Sarrafi, A. and Grieu, P. (2008). QTL analysis of chlorophyll fluorescence parameters in sunflower (*Helianthus annuus* L.) under well-watered and water-stressed conditions. *Plant Science*. **175**: 565–573.

Kirda, C., Danso, S.K.A. and Zapata, F. (1989). Temporal water stress effects on nodulation, nitrogen accumulation and growth of soybean. *Plant Soil*. **120**: 49-55.

Kishor, P.B.K., Sangama, S., Amrutha, R.N., Laxmi, P.S., Naidu, K.R. and Rao, K.S. (2005). Regulation of proline biosynthesis degradation, uptake and transport in higher plants: its implications in plant growth and abiotic stress tolerance. *Current Science*. **88**: 424-438.

Kohl, D.H., Kennelly, D.J., Zhy, Y., Schubert, K.R. and Sheader, G. (1991). Proline accumulation, nitrogenase (C_2H_2 reducing) activity and activities of enzymes related to proline metabolism in drought-stressed soybean nodules. *Journal of Experimental Botany*. **42**: 831-837.

Kohl, D.H., Straub, P.F. and Shearer, G. (1994). Does proline play a special role in bacteroids metabolism? *Plant Cell and Environment*. **17**: 1257-62.

Kondorosi, A., Ratet, P. and Coronado, C. (1995). Alfalfa root flavonoid production is nitrogen regulated. *Plant Physiology*. **108**: 533-542.

Konwar, P.B. (2009). Characterization of some selected rice cultivars under moisture stress condition. Unpublished Thesis for the Award of Msc. Degree at, Assam Agricultural University, Jorhat.

Kotchoni, S.O. and Bartels, D. (2003). Water stress induces the up-regulation of a specific set of genes in plants: Aldehyde dehydrogenases as an example. *Bulgarian Journal of Plant Physiology*. Special Issue. 37-51.

Kpyoarissis, A., Petropoulou, Y. and Manetas, Y. (1995). Summer survival of leaves in a soft-leaved shrub (*Phlomis fruticosa* L., Labiatae) under Mediterranean field conditions: avoidance of photo inhibitory damage through decreased chlorophyll contents. *Journal of Experimental Botany*. **46**: 1825–1831.

Kramer, P.J. and Boyer J.S. (1995). Water relation of plants and soils. Academic Press, London

Kramer, P.J. and Boyer, J.S. (1997). *Water relations of Plants and Soils*, Academic Press, San DiagoArrese, I., Gonzalez, E.M., Mariano, D., Landera, R., Larraiza, E. and Gil-Quintana, E. 2009. Physiological response of legume nodules to drought. Plant stress. Global Science Book, 5 (Special Issue): 24-31.

Kuhad, M.S. Sheoran, I.S. Kundu, B.S. and Naudwal, A.S. (1990). Relative performance of pentafoliate and trifoliate mungbean (*Vigna radiate* Wilczeck), under water stress. *Indian Journal of Plant Physiology*. **2**: 168-171.

Kumar, A., Omae, H., Egawa, Y., Kashiwaba, K. and Shono, M. (2006). Adaptation and drought stresses in Snap Bean (*Phaseolus vulgaris* L.) during the reproductive stage of Development. *Japan Agricultural Research Quarterly*. **40**(3): 213-216.

Kumar, S. and Pandey, A.K. (2013). Chemistry and Biological Activities of Flavonoids: An Overview. *Scientific World Journal*. Volume 2013 (2013), Article ID 162750, 16 pages.

Lalitha, S. and Santhaguru, K. (2012). Improving soil physical properties and effect on tree legume seedlings growth under barren soil. *Agricultural Science Research Journal*. **2**: 126 -130.

Larcher, W. (2006). *Physiological Plant Ecology*, (4th Edn), Rima, Sao Carlos. Pp. 550

Larson, R.A. (1988). The antioxidants of higher plants. *Phytochemistry*. **27**: 969-78.

Laurentius, H., Nugroho, and Marianne, C. (2002). Activities of enzymes involved in the phenylpropanoid pathway in constitutively salicylic acid-producing tobacco plants. *Plant Physiology and Biochemistry*. **40**: 755-760.

Lawlor, D.W. and Cornic, G. (2002). Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. *Plant Cell Environment*. **25**: 275-294.

Lazacano-Ferrat, I. and Lovat, C.J. (1999). Relationship between relative water content, nitrogen pools and growth of *Phaseolus vulgaris* L. and *P. acutifoolius* A. Gray during water deficit. *Crop Science*. **39**: 467-475.

Leung, K. and Bottomley, P.J. (1994). Growth and nodulation characteristics of subclover (*Trifolium subterraneum* L.) and *Rhizobium leguminosarum* bv trifolii at different soil water potentials. *Soil Biology Biochemistry*. **26**: 805–812.

Lindoo, S.J. and Caldwell, M.M. (1978). Ultraviolet-B radiation induced inhibition of leaf expansion and promotion of anthocyanin production. Lack of the involvement of the low irradiation phytochrome system. *Plant Physiology*. **61**: 278-282.

Lipiec, J., Doussan, C., Nosalewicz, A. and Kondracka, K. (2013). Effect of drought and heat stresses on plant growth and yield: a review. *International Agrophysics*. **2**: 463-477.

Lippi, D., De Paolis, M.R., Osmi, M., Pietrini, F., Pietrosanti, T., Villani, M.C. and Massacci, A. (1999). Effect of a *Rhizobium* sp. inoculation on N_2-fixing and photosynthetic activities of two different cowpea [*Vigna unguiculata* (L.) Walp.] genotypes. *Photosynthetica*. **37**: 413-422.

Liu, W., Zhu, D.W., Liu, D.H., Geng, M.J., Zhou, W.B., Mi, W.J., Yang, T.W. and Hamilton, D. (2010). Influence of nitrogen on the primary and secondary metabolism and synthesis of flavonoids in *Chrysanthemum morifolium*, ramat. *Journal of Plant Nutrition*. **33**(2): 240-254.

Lobato, A.K.S., Oliveira Neto, C.F., Costa, R.C.L., Santos Filho, B.G., Cost, R.C.L., Cruz, F.J.R., Neves, H.K.B. and Lopes, M.J.S. (2008). Physiological and biochemical behavior in soybean (*Glycine max* cv. Sambabia) plants under water deficit. *Australian Journal of Crop Science*. **2**: 25-23.

Mabrouk, Y. and Belhadj, O. (2012). Enhancing the biological nitrogen fixation of leguminous crops grown under stressed environments. *African Journal of Biotechnology*. **11**(48): 10809-10815.

Mafakheri, A., Siosemardeh, A., Bahramnejad, B., Struik, P.C. and Sohrabi, Y. (2010). Effect of drought stress on Yield, Proline and Chlorophyll contents in three chickpea cultivars. *Australian Journal of Crop Science*. **4**(8): 580-585.

Mager, W.H., de Boer, A.H., Siderius, M.H. and Voss, H-P. (2000). Cellular responses of oxidative and osmotic stress. *Cell Stress Chaperones*. **5**(2): 73-75.

Mahouachi, J., Socorro, A.R. and Talon, M. (2006). Responses of papaya seedlings (Carica papaya L.) to water stress and re-hydration: Growth, photosynthesis and mineral nutrient imbalance. *Plant & Soil*. **281**: 137-146.

Makoi, J.H. and Ndakidemi, P.A. (2007). Biological, Ecological and Agronomic Significance of Plant Phenolic Compounds in Rhizosphere of the Symbiotic Legumes. *African Journal of Biotechnology*. **6**: 1358-1368.

Makoi, J.H.J.R., Bambara, S. and Ndakidemi P.A. (2013). *Rhizobium* Inoculation and the Supply of Molybdenum and Lime Affect the Uptake of Macronutrients in Common Bean (*P. vulgaris* L.) Plants. *Australian Journal of Crop Science*. **7**(6): 784-793.

Makoi, J.H.J.R., Bambara, S. and Ndakidemi, P.A. (2010). Rhizosphere phosphatase enzyme activities and secondary metabolites in plants as affected by the supply of Rhizobium, lime and molybdenum in *Phaseolus vulgaris* L. *Australian Journal of Crop Sciences*. **4**(8): 590-597.

Makoi, J.H.J.R., Bambara, S. and Ndakidemi, P.A. (2013). *Rhizobium* Inoculation and the Supply of Molybdenum and Lime Affect the Uptake of Macronutrients in Common Bean (P. vulgaris L.) plant. *Australian Journal of Crop Science*. **7**(6): 784-793.

Makoi, J.H.J.R., Belane, A., Chimphango, S.M.B. and Dakora. F.D. (2010b). Flavonoids and anthocyanins in seed exudates enhance nodulation and defense in symbiotic cowpea (*Vigna unguiculata* L. Walp.). *Field Crops Research*. **118**: 21-27.

Makunde, G. and Pombi, A. (2004). Agronomy Research Institute, AREX, Crop Facts Sheets. Harare Zimbabwe.

Manavalan, L.P., Guttikonda, S.K., Tran, L.P. and Nguyen, H.T. (2009). Physiological and molecular approaches to improve drought resistance in soybean. *Plant Cell Physiology*. **50**(7): 1260-1276.

Manivannan, P., Jaleel, C.A., Kishorekumar, A., Sankar, B., Somasundaram, R., Sridharan, R and Panneerselvam, R. (2007a). Changes in antioxidant metabolism of Vigna unguiculata L. Walp. Bypropiconazole under water deficit stress. *Colloids Surf BBiointerfaces*. **57**: 69-74.

Manjeru, P., Madanzi, T., Makeredza, B., Nciizah, A. and Sithole, M. (2007). Effects of water stress at different growth stages on components and grain yields of Common bean (Phaseolus vulgaris L.). *African Crop Science Conference Proceedings*. **8**: 299-303.

Mansouri-far, C., Sanavy, S.A.M.M. and Saberali, S.A. (2010). Maize yield response to deficit irrigation during low-sensitive growth stages and nitrogen rate under semi-arid climatic conditions. *Agricultural Water Management*. **97**: 12-22.

Marscher H. (1995). *Mineral nutrition of High Plants* (2nd Edn), London Academic Press, London. 889pp.

Martínez, J.P. Silva, H. Ledent, J.F. and Pinto, M. (2007). Effect of drought stress on the osmotic adjustment, cell wall elasticity and cell volume of six cultivars of common beans (*Phaseolus vulgaris* L.). *Eurasian Journal of Agronomy*. **26**: 30-38.

Masoudi-Sadaghiani, F., Abdollahi, M.B., Zardoshti M. R., Rasouli-Sadaghiani M. H. and Tavakoli, A. (2011). Response of proline, soluble sugars, photosynthetic pigments and antioxidant enzymes in potato (*Solanum tuberosum* L.) to different irrigation regimes in greenhouse condition. *Australian Journal of Crop Science*. **5**(1): 55-60.

Massacci, A., Nabiev, S.M., Pietrosanti, L., Nematov, S.K., Chernikova, T.N., Thor, K. and Leipner, J. (2008). Response of the photosynthetic apparatus of cotton (*Gossypium hirsutum*) to the onset of drought stress under field conditions studied by gas-exchange analysis and chlorophyll fluorescence imaging. *Plant Physiology and Biochemistry*. **46**: 189-195.

Mattson, M., Lundborg, T., Larsson, M. and Larsson, C.M. (1991). Nitrogen utilization in N-limited barley during vegetative and generative cultures grown at different relative addition rates of nitrate-N. *Journal of Experimental Botany*. **43**: 15-23.

Matysik, J.A., Bhalu, B. and Mohanty, P. (2002). Molecular mechanism of quenching of reactive oxygen species by proline under stress in plants. *Current Science*. **82**(5): 525-532.

Mazid, M., Khan, T.A. and Mohammad, F. (2011). Role of Secondary metabolites in defense mechanisms of plants. *Biology and Medicine*. **3**(2): 232-249.

McWilliams, D. (2003). Drought Strategies for Cotton, Cooperative Extension Service Circular 582, College of Agriculture and Home Economics, New Mexico State University, USA.

Mduruma, Z.O., Nchimbi-Msolla, S., Reuben, S.O.W.M. and Misangu, R.N. (1998). Evaluation of maturity characteristics and yield components of high protein (*Phaseolus vulgaris* L.) varieties in Morogoro, Tanzania. *TAJAS*. **1**(2): 131-140.

Mengel, K. (2007). *Potassium*. In: Barker, AV, Pilbeam, DJ (Eds) Handbook of Plant Nutrition (1st Edn), CRC Taylor and Francis. NY. Pp. 91-120.

Mengel, K. and Kirkby, E.A. (2001). *Principles of Plant Nutrition* (4th Edn), International Potash Institute, Switzerland. 687Pp.

Mfilinge, A., Mtei, K. and Ndakidemi, P. (2014). Effect of *Rhizobium* Inoculation and Supplementation with Phosphorus and Potassium on Growth and Total Leaf Chlorophyll (Chl) Content of Bush Bean *Phaseolus vulgaris*, L. *Agricultural Sciences*. **5**: 1413-1426.

Mierziak, J., Kostyn, K. and Kulma, A. (2014). Flavonoids as Important Molecules of Plant Interactions with the Environment. *Molecules*. **19**: 16240-16265.

Miklas, N.P., James, D.K., Steve, E.B. and Mathew, W.B. (2006). Common bean breeding to resistance against biotic and abiotic stresses: from classical to MAS breeding. *Euphytica*. **147**: 105-131.

Mirecki, R.M. and Teramura, A.H. (1984). Effects of UV-B irradiance on soybean. V. The dependence of plant sensitivity on the photosynthetic photon flux density during and after leaf expansion. *Plant Physiology*. **74**: 475-480.

Molina, J.C.V., Moda-Cirino, N., Da, S.F., Júnior, R.T. and De FariaDestro, D. (2001). Response of common bean cultivars and lines to water stress. *Crop Breeding and Applied Biotechnology*. **1**: 363-372.

Montanaro, G., Dichio, B., Xiloyannis, C. and Celano, G. (2005). Light influences transpiration and calcium accumulation in fruit of kiwifruit plants (*Actinidiadeliciosa vardeliciosa*). *Plant Science*. **170**: 520-527.

Mortier, V., Holsters, M. and Goormachtig, S. (2012). Never too many? How legumes control nodule numbers. *Plant Cell Environment*. **35**: 245-225.

Mouhouche, B., Ruget, F. and Delecolle (1998). Effects of water stress applied at different phonological phases on yield components of dwarf bean (*Phaseolus vulgaris* L.). *Agronomie*. **18**: 197-205.

Mukankusi, C.M., Nkalubo, S., Katungi, E., Awio, B., Luyima, G., Radeny, M. and Kinyangi, J. (2015). Participatory Evaluation of Common Bean for Drought and Disease Resilience Traits in Uganda. CCAFS Working Paper no. 143. CGIAR Research Program on Climate Change, Agriculture and Food Security (CCAFS). Copenhagen, Denmark.

Munns, R. (2002). Comparative physiology of salt and water stress. *Plant Cell & Environment*. **25**: 239-250.

Murphy, J. and Riley, J. P. (1962). A Modified Single Solution Method for the Determination of Phosphate in Natural Water. *Analytica Chimica Acta*. **27**: 31-36.

Nahar K. and Gretzmacher, R. (2002). Effect of water stress on nutrient uptake, yield and quality of tomato (Lycopersicon esculentum Mill.) under subtropical conditions. *Die Bodenkultur*. **53**(1): 45-51.

Namvar, A., SeyedSharifi, R., Sedghi, M., AsghariZakaria, R., Khandan, T. and Eskandarpour, B. (2011). Study the effects of organic and inorganic nitrogen fertilizer on yield, yield components and nodulation state of chickpea (*Cicer arietinum* L.). *Communications in Soil Science Plant Analysis*. **42**(9): 1097-1109.

Namvar, A., Sharifi, E.S., Khandan, T. and Moghadam, M.J. (2013). Seed Inoculation and Inorganic Nitrogen Fertilization Effects on Some Physiological and Agronomical Traits of Chickpea (Cicer arietinum L.) in Irrigated Condition. *Journal of Central European Agriculture*. **14**(3): 28-40.

Ndakidemi, P.A. and Dakora, F.D. (2003). Legume seed flavonoids and nitrogenous metabolites as signals and protectants in early seedling development. *Functional Plant Biology*. **30**: 729 - 745.

Ndakidemi, P.A. and Makoi, J.H.R. (2009). Effect of NaCl on the productivity of four selected bean cultivars (*Phaseolus vulgaris* L.). *Scientific Research and Essays*. **4**(10): 1066-1072.

Ndakidemi, P.A. Bambara, S. and Makoi, J.H.J.R. (2011). Micronutrient uptake in common bean (*Phaseolus vulgaris* L.) as affected by *Rhizobium* inoculation and supply of molybdenum and lime. *Plant Omics Journal*. **4**(1): 40-52.

Ndakidemi, P.A., Dakora, F.D., Nkonya, E.M., Ringo, D. and Mansoor, H. (2006). Yield and economic benefits of common bean (*Phaseolus vulgaris* L.) and soybean (*Glycine max* L. Merr.) inoculation in northern Tanzania. *Australian Journal of Experimental Agriculture.* **46**(4): 571-577**.**

Ney, B., Duthion, C. and Frontaine, E. (1993). Timing of reproductives absorption in relation to cell, water content and growth of pea seeds. *Crop Sciences*. **33**: 267-270.

Nielsen, D.C. and Nelson, N.O. (1998). Black bean sensitivity to water stress at various growth stages. *Crop Science*. **38**: 422-427.

Nonami, H. (1998). Plant water relations and control of cell elongation at low water potentials. *Journal of Plant Research*. **111**: 373–382.

Nyachiro, J.M., Briggs, K.G., Hoddinott, J. and Johnson-Flanagan, A.M. (2001). Chlorophyll content, chlorophyll fluorescence and water deficit in spring wheat. *Cereal Research Communication*. **29**: 135 -142.

Nyoki, D. and Ndakidemi, P.A. (2014). Effects of Phosphorus and *Bradyrhizobium japonicum* on Growth and Chlorophyll Content of Cowpea (*Vigna unguiculata* (L) Walp). *American Journal of Experimental Agriculture*. **4**(10): 1120-1136.

Nyoki, D. and Ndakidemi, P.A. (2014). Effects of *Bradyrhizobium japonicum* Inoculation and Supplementation with Phosphorus on Macronutrients Uptake in Cowpea (*Vigna unguiculata* (L.) Walp). *American Journal of Plant Sciences*. **5**: 442-451.

Nyoki, D. and Ndakidemi, P.A. (2014). Effects of *Bradyrhizobium japonicum* and Phosphorus supplementation on the productivity of Legumes. *International Journal of Plant and Soil Science*. **3**(7): 894-910.

O'Hara, G.W. (2001). Nutritional constraints on root nodule bacteria affecting symbiotic nitrogen fixation: A review. *Australian Journal of Experimental Agriculture*. **41**: 417-433.

Odjegb, V.J. and Alokolaro, A.A. (2013). Stimulated Drought and Salinity Modulates the Production of Phytochemicals in *Acalypha wilkesiana*. *Journal of Plant Studies*. **2**(2): 105-112.

Okigbo, R.N., Anuagasi, C.L. and Amadi J.E. (2009). Advances in selected medicinal and aromatic plants indigenous to Africa. *Journal of Medical Plants Research*. **3**(2): 86-95.

Ommen, O.E., Donnelly, A., Vanhoutvin, S., van Oijen, M. and Manderscheid, R. (1999). Chlorophyll content of spring wheat flag leaves grown under elevated CO_2 concentrations and other environmental stresses within the ESPACE-wheat project. *European Journal of Agronomy*. **10**: 197-203.

Parida, A.K., Dagaonkar, V.S., Phalak, M.S., Umalkar, G.V. and Aurangabadkar, L.P. (2007). Alterations in photosynthetic pigments, protein and osmotic components in cotton genotypes subjected to short-term drought stress followed by recovery. *Plant Biotechnology*. **1**: 37-48.

Peksen, E. (2007). Non-destructive leaf area estimation model for faba bean (*Vicia faba* L.). *Scientia Horticulturae*. **113**: 322–328.

Pena-Cabriales, J.J. and Castellanos, J.Z. (1993). Effects of water stress on N_2 fixation and grain yield of *Phaseolus vulgaris* L. *Plant and Soil*. **152**: 151-155.

Perveen, S., Khan, M. S. and Zaidi, A. (2002). Effect of Rhizospheric Microorganisms on Growth and Yield of Greengram (*Phaseolus radiatus*). *Indian Journal of Agricultural Sciences*. **72** (7): 421-423.

Peters, A. (1993). China. Michigan Dry Bean Digest. **17**(4): 18-20.

Phillips, D.A. and Dakora, F.D. (2002). Root Exudates as Mediators of Mineral Acquisition in Low-Nutrient Environments. *Plant and Soil*. **245**(1): 35-47.

Postel, S. (2001). Growing more food with less water. *Scientific American*. **284**: 46-51.

Prasad, P.V.V., Staggenborg, S.A. and Ristic, Z. (2008). Impacts of drought and/or heat stress on physiological, developmental, growth and yield process of crop plants. In: Ahuja LR, Reddy VR, Saseendran SA, Yu Q, editors. Response of Crops to Limited Water: Understanding and Modeling Water Stress Effects on Plant Growth Processes. Madison, WI, USA: *American Society of Agronomy*. Pp. 301–351.

Quan, R., Shang, M., Zhang, H., Zhao, Y. and Zhang, J. (2004). Engineering of enhanced glycine betaine synthesis improves drought tolerance in maize. *Plant Biotechnology Journal*. **2**(6): 477- 486.

Rahaman, S., Shaheen, M.S., Rahaman, T. and Malik, T.A. (2000). Evaluation of excised leaf water loss and relative water content as screening techniques for breeding drought 67 resistant wheat. *Pakistan Journal of Biological Sciences*. **3**: 663-665.

Rahman, M. M., Sofian-Azirun, M. and Boyce, A.N. (2013). Response of nitrogen fertilizer and legumes residues on biomass production and utilization in rice-legumes rotation. *Journal of animal and Plant Science*. **23**(2): 589-595.

Ramachandra, R.S. and Ravishankar, G.A. (2002). Plant cell cultures: chemical factories of secondary metabolites. *Biotechnology Advances*. **20**: 101-153.

Ramakrishna, A. and Ravishankar, G.A. (2011). Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signaling & Behaviour*. **6**(11): 1720-1731.

Ramirez-Vallejo, P. and Kelly, J.M. (1998). Traits related to drought resistance in common bean. *Euphytica*. **99**: 127-136.

Ramos, M.L.G., Gordon, A.J., Minchin, F.R. and Sprent, J.I. and Parsons, R. (1999). Effect of water stress on nodule physiology and biochemistry of a drought tolerant cultivar of common bean (*Phaseolus vulgaris* L.). *Annals of Botany*. **83**: 57-63.

Ramos, M.L.G., Parsons, R. and Sprent, J.I. (2005). Differences in ureide and amino acid content of water stressed soybean inoculated with Bradyrhizobium japonicum and B. elkanii. *Pesquisa Agropecuária Brasileira*. **40**: 453-458.

Ramos, M.L.G., Parsons, R., Sprent, J.I. and James, E.K. (2003). Effect of water stress on nitrogen fixation and nodule structure of common bean. *Pesquisa Agropecuária Brasileira*. **38**: 339-347.

Ranawake, A.L., Dahanayaka, N., Amarasingha, U.G.S., Rodrigo, W.D.R.J. and Rodrigo, U.T.D. (2011). Effect of water stress on growth and yield of Mung bean (*Vigna radiate* L.). *Tropical Agricultural Research and Extension*. **14**(4): 76-79.

Rao, I.M. (2001). *Role of physiology in improving crop adaptation to abiotic stresses in the tropics*: The case of common bean and tropical forages. Pp. 583-613. In M. Pessarakli (ed.) Handbook of plant and crop physiology. Marcel Dekker, New York.

Rao, S.R. and Ravishankar, G.A. (2002). Plant cell cultures: Chemical factories of Secondary metabolites. *Biotechnology Advances*. **20**: 101-153.

Ravikumar, R. (2012). Growth Effects of *Rhizobium* Inoculation in Some Legume Plants. *International Journal of Current Science*. Pp. 1-6.

Ravishankar, G.A. and Rao, S.R. (2000). Biotechnological production of phyto-pharmaceuticals. *Journal of Biochemistry Molecular Biology and Biophysics*. **4**: 73-102.

Razal, R.A., Ellis, S. and Singh, S. (1996). Nitrogen recycling in phenylpropanoid metabolism. *Phytochemistry*. **41**: 31-35.

Redondo-Nieto, M., Rivilla, R., El-Hamdaoui, A., Bonilla, I. and Bolaños, L. (2001). Boron deficiency affects early infection events in the pea *Rhizobium* symbiotic interaction. *Australian Journal of Plant Physiology*. **28**: 819-823.

Remenyik, J. and Nemeske, E. (2010). Study of the defensive mechanism against drought in French bean (*Phaseolus vulgaris* L.) varieties. *Acta Physiologies' Plantarum*. **32**: 1125–1134.

Restrepo-Diaz, H., Benlloch, M. and Fernandez-Escobar, R. (2008). Plant water stress and K+ starvation reduce absorption of foliar applied K^+ by olive leave. *Scientia Horticulturae*. **116**: 409-413.

Romic, D., Tomic, F., Borosic, J., Dolanjski, D., Romic, M., Jankovic, M., Marusic, J. and Geres, D. (1994). Effects of irrigation on components of secondary crops yield in Croatia. 1e7 Conf. Reg. Europ. Irrig. Drain CIID, Varna (Bulgaria), 16 -22 mai 1994. 19-28.

Roy, R.N., Finck, A., Blair, G.J. and Tandon, H.L.S. (2006). Plant Nutrition for Food Security-A Guide for Integrated Nutrient Management. FAO. 2006.

Sah, S.K., Reddy, K.R. and Li, J. (2016). Abscisic Acid and Abiotic Stress Tolerance in Crop Plants. *Frontiers in Plant Sciences*. **7**: 571.

Sairam, R.K., Deshmukh, P.S. and Shukla, D.S. (2008). Tolerance of drought and temperature stress in relation to increased antioxidant enzyme activity in wheat. J*ournal of Agronomy and Crop Science*. **178**: 171-178.

Salvagiotti, F., Cassman, K.G., Specht, J.E., Walters, D.T., Weiss, A. and Dobermann, A. (2008). Nitrogen uptake, fixation and response to N in soybeans: A review. *Field Crops Research*. **108**: 1-13.

Samarah, N., Mullen, R. and Cianzo, S. (2004). Size distribution and mineral nutrients of soybean seeds in response to drought stress. *Journal of Plant Nutrition*. **27**: 815-835.

Sánchez, E., Garcia P.C., López-Lefebre, L.R., Rivero, R.M., Ruiz, M.R., and Romero, L. (2002). Proline metabolism in response to nitrogen deficiency in French Bean plants (*Phaseolus vulgaris* L. cv Strike). *Plant Growth Regulation*. **36**: 261–265.

Sanchez, F.J., Manzanares, M., de Andres, E.F., Tenorio, J.L. and Ayerbe, L. (1998). Turgor maintenance, osmotic adjustment and soluble sugar and praline accumulation in 49 pea cultivars in response to water stress. *Field Crops Research*. **59**: 225–235.

Sanchez-Rodriguez, E., Rubio-Wilhelmi, M., Cervilla, L.M., Blasco, B., Rios, J.J., Rosales, M.A., Romero, L. and Ruiz, J.M. (2010). Genotypic differences in some physiological parameters symptomatic for oxidative stress under moderate drought in tomato plants. *Plant Sciences*. **178**: 30-40.

Saneoka, H. Moghaieb, R.E.A. Premachandra, G.S. and Fujita, K. (2004). Nitrogen nutrition and water stress effects on cell membrane stability and leaf water relations in Agrostis palustrais Huds. *Environmental Experimental Botany*. **52**: 131-138.

Sangakkara, U.R. (1994). Growth yield and nodule activity of Phaseolus vulgaris L. as affected by soil moisture. *Journal of Agronomy and Crop Science*. **172**: 62-68.

Sangakkara, U.R., Hartwig, U.A. and Nosberger, J. (1996). Soil moisture and potassium affect the performance of symbiotic nitrogen fixation in faba beans and common beans. *Plant and Soil*. **184**: 123-130.

Sankar, B., Jaleel, C.A., Manivannan, P., Kishorekumar, A., Somasundaram, R. and Panneerselvam, R. (2007). Drought-induced biochemical modifications and proline metabolism in *Abelmoschus esculentus* (L.) Moench. *Acta Botanica Croatica*. **66**(1): 43-56.

Santos, M.G., Ribeiro, R.V., Oliveira, R.F. Machado, E.C. and Pimentel, C. (2006). The role of inorganic phosphate on photosynthesis recovery of common bean after a mild water deficit. *Plant Science*. **170**: 659-664.

Sara, S., Morad, M. and Reza, C.M. (2013). Effects of seed inoculation by *Rhizobium* strains on chlorophyll content and protein percentage in common bean cultivars (*Phaseolus vulgaris* L.). *International Journal o Biosciences*. **3**(3): 1-8.

Saradhi, A. and Saradhi, P.P. (1991). Proline accumulation under heavy metal stress. *Journal of Plant Physiology*. **138**: 554-558.

Sardans, J. and Penuelas, J. (2004). Increasing drought decreases phosphorus availability in evergreen Mediterranean forest. *Plant and Soil*. **267**: 367-377.

Sardans, J. Penuelas, J. and Ogaya, R. (2008). Drought's impact on Ca, Fe, Mg, Mo and S concentration patterns in the plant and soil of a Mediterranean evergreen Quercus ilex forest. *Biogeochemistry*. **87**: 49-69.

Sassi-Aydi, S. and Abdelly, C. (2012). Inoculation with the native Rhizobium gallicum 8a3 improves osmotic stress tolerance in common bean drought-sensitive cultivar. *Acta Agriculturae Scandinavica Section B -Soil and Plant Science*. **62**: 179-187.

Schonfeld, M.A. Johnson, R.C. Carwer, B.F. and Mornhinweg, D.W. (1988). Water relations in winter wheat as drought resistance indicators. *Crop Sciences*. **28**: 526-531.

Schwartz, H.F., Brick, M.A., Nuland, D.S. and Franc, G.D. Tech. Eds, (1996). Dry bean production and pest management. Regional bulletin. 562 S. Pp. 106. Universities of Colorado, Nebraska, and Wyoming, USA.

Serraj, R. (2003). Effects of drought stress on legume symbiotic nitrogen fixation: Physiological mechanisms. *Journal of Experimental Biology*. **41**: 1136-1141.

Serraj, R. and Adu-Gyamfi, J. (2004). Role of Symbiotic Nitrogen fixation in the Improvement of Legume Productivity under stressed Environments. *West African Journal of Applied Ecology*. **6**(1): 95-109.

Serraj, R. and Sinclair, T.R. (1998). N_2 Fixation response to drought in common bean (Phaseolus vulgaris L.). *Annals of Botany*. **82**: 229-234.

Serraj, R., Bona, S., Purcell, L.C. and Sinclair, T.R. (1997). Nitrogen fixation response to water-deficits in field-grown 'Jackson' soybean. *Field Crops Research*. **52**: 109-116.

Serraj, R., Sinclair, T.R. and Purcell, L.C. (1999). Symbiotic N_2 fixation response to drought. *Journal of Experimental Botany*. **50**(331): 143-155.

Shao, H.B. Song, W.Y. and Chu, L.Y. (2008). Advances of Calcium signals involved in plant anti-drought. *Comptes Rendus Biologies*. **331**: 587-596.

Sharma, P., Jha, A.B., Dubey, R.S. and Pessarakli, M. (2012). Reactive Oxygen Species, Oxidative Damage, and Antioxidative Defense Mechanism in Plants under Stressful Conditions. *Journal of Botany*.Volume (2012), Article ID 217037, 26 pages.

Shenkut, A.A. and Brick, M.A. (2003). Traits Associated with Dry Edible Bean (*Phaseolus Vulgaris* L.) productivity under diverse soil moisture environments. *Euphytica*. **133**: 339–347.

Shilpa, K., Varun, K. and Lakshmi, B.S. (2010). An Alternate Method of Natural Drug Production: Eliciting Secondary Metabolite Production Using Plant Cell Culture. *Journal of Plant Sciences*. **5**(3): 222-247.

Shirtliffe, S.J., Vessy, J.K., Buttery, B.R. and Park, S.J. (1996). Comparison of growth and N accumulation of common bean (*Phaseolus vulgaris* L. cv. OAC Rico) and its nodulation mutants R69 and R99. *Canadian Journal of Plant Science*. **76**: 73–83.

Shobhkhizi, A., Rayni, M.F., Barzin, H.B. and Noori, M. (2014). Influence of drought stress on photosynthetic enzymes, chlorophyll, protein and relative water content in crop plants. *International Journal of Biosciences*. **5**(7): 89-100.

Siczek, A. and Lipiec, J. (2011). Soybean nodulation and nitrogen fixation in response to soil compaction and surface straw mulching. *Soil Tillage Research*. **114**: 50-56.

Siddiqui, M.H., Al-Khaishany, M.Y., Al-Qutami, M.A., Al-Whaibi, M.H., Grover, A., Ali, H.M., Al-Wahibi, M.S. and Bukhari, N.A. (2015). Response of Different Genotypes of Faba Bean Plant to Drought Stress. *International Journal of Molecular Sciences*. **16**: 10214-10227.

Sierra, S., Rodelas, B., Martínez-Toledo, M.V., Pozo, C. and González-López, J. (1999). Production of B-group vitamins by two Rhizobium strains in chemically defined media. *Journal of Applied Microbiology*. **86**: 851–58.

Silva, E.C., Nogueira, R.J.M.C., Silva, M.A. and Albuquerque, M. B. (2011). *Drought Stress and Plant Nutrition*. Plant Stress 5 (Special Issue 1), 32-41 ©2011 Global Science Books.

Sinclair, T.R., Muchow, R.C., Bennett, J.M. and Hammond, L.C. (1987). Relative sensitivity of nitrogen and biomass accumulation to drought in field-grown soybean. *Agronomy Journal*. **79**: 986–991.

Singh, S.P. (1995). Selection for water-stress tolerance in interracial populations of common bean. *Crop Science*. **35**: 118–124.

Slatni, T., Krouma, A., Aydi, S., Chaiffi, C., Gouia, H. and Abdelly, C. (2008). Growth, nitrogen fixation and ammonium assimilation in common bean (*Phaseolus vulgaris* L.) subjected to iron deficiency. *Plant Soil*. **312**: 49–57.

Smith, D.L., Dijak, M. and Hume, D.J. (1988). The effect of water deficit on N2 (C_2H_2) fixation by white bean and soybean. *Canadian Journal of Plant Science*. **68**: 957-967.

Sogut, T. (2006). *Rhizobium* inoculation improves yield and nitrogen accumulation in soybean (*Glycine max*) cultivars better than fertilizer. *New Zealand Journal of Crop and Horticultural Science*. **34**: 115-120.

Sprent, J.I. and Minchin, F.R (1983). *Environmental factors on the physiology of nodulation and nitrogen fixation*. In Temperate Legumes: Physiology, Genetics and Nodulation. Eds. DG Jones and DR Davies. Pp. 269-317. Pitman Advance Publishing Program, Boston.

Steel, R.G.D. and Torrie, J.H. (1980). *Principles and Procedures of Statistics*: A Biometrical Approach, 2nd Edition, McGraw Hill, New York.

Stewart, A.J., Chapman, W. and Jenkins, G.I. (2001). The effect of nitrogen and phosphorus deficiency on flavonol accumulation in plant tissue. *Plant Cell and Environment*. **24**: 1189-1197.

Stewart, C.R. (1981). *Proline accumulation*: Biochemical aspects. In: Paleg LG, Aspinall D (Eds), Physiology and Biochemistry of drought resistance in plants. pp. 243-251.

Sullivan, C.Y. (1972). *Mechanism of heat and drought resistance in grain sorghum and methods of measurement*. In: Rao, G.P. and House, L.R. (Eds.) Sorghum in Seventies, 247. Oxford and IBH Publ. Co., New Delhi.

Surendar, K.K., Devi, D.D., Ravi, I., Krishnakumar, S., Kumar, S.R. and Velayudham, K. (2013). Water Stress in Banana- A Review. *Bulletin Environmental Pharmacology and Life Science*. **2**(6): 01-18.

Swaraj, K. (1987). Environmental stress and symbiotic N_2 fixation in legumes. *Plant Physiology and Biochemistry*. **14**: 117–130.

Szabados, L. and Savoure, A. (2009). Proline: a multifunctional amino acids. *Trends in Plant Science*. **15**(2): 89-97.

Tairo, E.V. and Ndakidemi, P.A. (2013). *Bradyrhizobium japonicum* Inoculation and Phosphorus Supplementation on Growth and Chlorophyll Accumulation in Soybean (*Glycine max* L.). *American Journal of Plant Sciences*. **4**: 2281-2289.

Tairo, E.V. and Ndakidemi, P.A. (2013). Possible benefits of Rhizobial Inoculation and phosphorus supplementation on nutrition, growth and economic sustainability in grain legumes. *American Journal of Research Communication*. **1**(12): 532-556.

Tairo, E.V. and Ndakidemi, P.A. (2013). Yields and economic benefits of soybean (*Glycine max* L.) as affected by *Bradyrhizobium japonicum* inoculation and phosphorus supplementation. *American Journal of Research Communication*. **1**(11): 159-172.

Tairo, E.V. and Ndakidemi, P.A. (2014). Macronutrients uptake in soybean as affected by *Bradyrhizobium japonicum* inoculation and phosphorus (P) supplements. *American Journal of Plant Sciences*. **5**: 488-496.

Tairo, E.V., Mtei, K.M. and Ndakidemi, P.A. (2017). Influence of Water Stress and Rhizobial Inoculation on Accumulation of Proline in Selected Cultivars of *Phaseolus vulgaris* (L.). *International Journal of Current Microbiology and Applied Sciences*. **6**(3): 2205-2214.

Taiz, L. and Zeiger, E. (2006). *Plant Physiology* (4th Edn), Sinauer Associates., Massachusetts. 690 pp.

Tajini, F., Drevon, J.J., Lamouchi, L. and Aouani, M.E. (2008). Trabelsi M. Response of common bean lines to inoculation: comparison between the *Rhizobium* tropici CIAT899 and the native *Rhizobium* etli 12a3 and their persistence in Tunisian soils. *World Journal of Microbiology and Biotechnology*. **24**: 407- 417.

Tajini, F., Trabelsi, M. and Drevon, J.J. (2012). Comparison between the reference *Rhizobium* tropici CIAT899 and the native *Rhizobium* etli12a3 for some nitrogen fixation parameters in common bean (*Phaseolus vulgaris* L.) under water stress. *African Journal of Microbiology Research*. **6**(18): 4058-4067.

Tatar, O. and Gevrek, M.N. (2008). Influence of Water Stress on Proline Accumulation, Lipid Peroxidation and Water Content of Wheat. *Asian Journal of Plant Science*. **7**(4): 409-412.

Teri, J.M., Silbernagerl, J.M. and Nchimbi, S (1990). *The SUA/WSU Bean/CRSP*: A review of progress in disease resistance. In: Bean research. (Edited by Smith, J.B.), CIAT Africa Workshop. Cal Colombia. **12**(5): 166-189.

Thung, M. and Rao, I.M. (1999). Integrated management of abiotic stresses. In: SP Singh (ed) Common Bean Improvement in the Twenty-First Century. Pp. 331–370. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Tourian, N., Sinaki, J.M., Hasani, N. and Madani H. (2013). Change in photosynthesis pigment concentration of wheat grass (*Agropyron repens*) cultivars response to drought stress and foliar application with Chitosan. *International Journal of Agronomy and Plant Production*. **4**(5): 1084-1091.

Tripathy, J.N., Zhang, J., Robin, S. and Nguyen, H.T. (2000). QTLs for cell-membrane stability mapped in rice (*Oryza sativa* L.) under drought stress. *Theoretical and Applied Genetics*.**100**: 1197-1202.

Tryphone, G.M., Chilagane, L.A., Protas, D., Kusolwa, P.M. and Nchimbi-Msolla, S. (2012). Introgression of common bacterial blight (*Xanthomonas axonopodis* pv. phaseoli) resistance to common bean (*Phaseolus vulgaris* L.) adapted to Tanzania facilitated by marker assisted selection. *International Journal of Agricultural Sciences*. **2**(10): 285-290.

Turner, N.C., Wright, G.C. and Siddique, K.H.M. (2001). Adaptation of grain legumes (pulses) to water-limited environments. *Advances in Agronomy*. **71**: 193-271.

Uchida, R. (2000). Essential Nutrients for Plant Growth: Nutrient Functions and Deficiency Symptoms. Plant Nutrient Management in Hawaii's Soils, College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa. pp. 31-55.

Uddin, S., Parvin, S. and Awal, M.A. (2013). Morpho-Physiological aspects of Mungbean (*Vigna radiate* L.) in response to water stress. *International Journal of Agricultural Science and Research*. **3**(2): 137-148.

Ullah, F., Bano, A. and Nosheen, A. (2012). Effects of plant growth regulators on growth and oil quality of canola (*brassica napus* l.) Under drought stress. *Pakistan Journal of Botany*. **44**(6): 1873-1880.

Utrillas, M.J., Alegre, L. and Simon, E. (1995). Seasonal changes in production and nutrient content of *Cynodon dactylon* (L.) Pers. Subjected to water deficits. *Plant and Soil*. **175**: 153-157.

Vadez, V., Krishnamurthy, L., Kashiwagi, J., Kholova, J., Devi, J.M., Sharma, K.K., Bhatnagar-Mathur, P., Hoisington, D.A., Hash, C.T. and Bidinger, F.R. (2007). Exploiting the functionality of root systems for dry, saline, and nutrient deficient environments in a changing climate. *SAT eJournal*. **4**(1): 1–61.

Valentovic, P., Luxova, M., Kolarovic, L. and Gasparikova, O. (2006). Effect of osmotic stress on compatible solutes content, membrane stability and water relations in two maize cultivars. *Plant Soil Environment*. **52**(4): 186-191.

Van Praag, H.J., De Smedt, F. and Thanh, T.V. (2000). Simulation of Calcium leaching and desorption in an acid forest soil. *European Journal of Soil Science*. **51**:245-255.

Van Rhijn, R. and Vanderleyden, J. (1995). The *Rhizobium*-plant symbiosis. *Microbiology Reviews*. **59**: 124-142.

Vanisree, M., Lee, C-Y., Lo, S-F., Nalawade, S.M., Lin, C.Y. and Tsay, H.S. (2004). Studies on the production of some important secondary metabolites from medicinal plants by plant tissue cultures. *Botanical Bulletin of Academia Sinica*. **45**: 1-22.

Veberic, R., Colaric, M. and Stampar, F. (2008). Phenolic acids and flavonoids of fig fruit (*Ficus carica* L.) in the northern Mediterranean region. *Food Chemistry*. **106**: 153-157.

Verbruggen, N. and Hermans, C. (2008). Proline accumulation in plants: a review. *Amino Acids*. **35**: 753 -759.

Vincent, J.M. (1980). Factors controlling the legume-*Rhizobium* symbiosis. In Nitrogen Fixation. Vol. 2. Eds. W E and W H OrmeJonhson. Pp 103-129. University Park Press. Baltmore, MD.

Wani, P.A., Khan, M.S. and Zaidi, A. (2008). Impact of zinc-tolerant plant growth promoting rhizobacteria on lentil grown in zinc amended soil. *Agronomy for Sustainable Development*. **28**: 449-455.

Wani, S.H. and Kumar, V. (2015). Plant stress tolerance: engineering ABA: a Potent phytohormone. *Transcriptomics*. An Open Access 3: 1000113.

Waraich, E.A., Ahmad, R., Ashraf, S. and Ehsanullah, M.Y. (2011) Role of mineral nutrition in alleviation of drought stress in plants. *Australian Journal of Crop Sciences*. **5**(6): 764-777.

Werner, D. and Newton, W.E. (2005). Nitrogen fixation in agriculture, forestry, ecology and environment. Published by Springer. Pp. 347.

White, J.W. and Singh, S.P. (1991). *Breeding for adaptation to drought*. Pp. 501-551. In A. van Schoonhoven and O. Voysest (ed.) Common beans: Research for crop improvement. International. Wallingford, U.K and CIAT, Cali, Colombia.

White, J.W., Castillo, J.A. and Ehleringer, J. (1990). Associations between productivity, root growth and carbon isotope discrimination in *Phaseolus vulgaris* under water deficit. *Australian Journal of Plant Physiology*. **17**: 189-198.

Wood, C.W., Torbert, H.A. and Weaver D.B. (1993). Nitrogen Fertilizer Effects on Soybean Growth, Yield and Seed Composition. *Journal of Production Agriculture*. **6**(3): 354-360.

Wortmann, C. S., Kirkby, R.A., Eledu, C.A. and Allen, D.J. (1998). Atlas of Common Bean (*Phaseolus vulgaris* L) Production in Africa. (Cali, Colombia: CIAT), Pp. 133.

Wu, M.T. and Wallner, S.J. (1993). Heat stress responses in cultured plant cells: Developmentand comparison of viability tests. *Plant Physiology*. **72**: 817-820.

Xoconostle-Cazares, B., Ramirez-Ortega, F.A., Flores-Elenes, L. and Ruiz-Medrano, R. (2011). Drought Tolerance in Crop Plants. *American Journal of Plant Physiology*. Pp. 1 - 16.

Yates, R.J., Howieson, J.G., Reeve, W.G., Brau, L., Speijers, J. and Nandasena, K. (2008). Host-strain mediated selection for an effective nitrogen-fixing symbiosis between Trifolium spp. and *Rhizobium leguminosarum* biovar trifolii. *Soil Biology Biochemistry*. **40**: 822-833.

Yordanov, I., Velikova, V. and Tsonev, T.M. (2003) .Plant responses to drought and stress tolerance. *Bulgarian Journal of Plant Physiology*. Special Issue. 187–206.

Zadehbagheri, M. (2014). The evaluation of various agronomic traits of Red & Chiti bean genotypes under well-watered and drought stress conditions. *International Journal of Farming and Allied Sciences*. **3**(4): 389-398.

Zahran, H.H. (1999). *Rhizobium*-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. *Microbiology and Molecular Biology Review*. **63**: 968-989.

Zahran, H.H., Karsisto, L.A.M. and Lindstorm, K. (1994). Alteration of lipopolysacharides and protein profiles in SDS-PAGE of rhizobia by osmotic stress. *World Journal of Microbiology and Biotechnology*. **10**: 100 - 105.

Zhang, C.S. Lu, Q. and Verma, D.P.S. (1995). Removal of feedback inhibition of 1-pyrroline-5 carboxylate synthetase, a bifunctional enzyme catalysing the first two steps of proline biosynthesis in plants. *Journal of Biological Chemistry*. **270**: 20491–20496.

Zhang, F. and Smith, D.L. (2002). Interorganismal signaling in suboptimum environments: the legume-rhizobia symbiosis. *Advances in Agronomy*. **76**: 125-161.

Zhao, T.J., Sun, S., Liu, Y., Liu, J.M., Liu, Q., Yan, Y.B. and Zhou, H.M. (2006). Regulating the drought-responsive element (DRE)-mediated signaling pathway by synergic functions of trans-active and trans-inactive DRE binding factors in Brassica napus. *Journal of Biological Chemistry*. **281**: 10752-10759.

Zhou, X.J., Liang, Y., Chen, H, Shen, S.H. and Jing, Y.X. (2006). Effects of rhizobia inoculation and nitrogen fertilization on photosynthetic physiology of soybean. *Photosynthetica*. **44**: 530-535.

Appendix 1: List of Publications from the Research done,

S/No.	Article Title
1.	**Eutropia V. Tairo, Kelvin M. Mtei and Patrick A. Ndakidemi (2017).** Influence of Water Stress and Rhizobial Inoculation on Accumulation of Proline in Selected Cultivars of *Phaseolus vulgaris* (L.). *International Journal of Current Microbiology and Applied Sciences*. **6(3): 2205-2214.**
2.	**Eutropia V. Tairo, Kelvin M. Mtei and Patrick A. Ndakidemi (2017).** Influence of Water stress and Rhizobial inoculation on accumulation of Flavonoids and Anthocyanins in selected Common bean (*Phaseolus vulgaris* L.) cultivars. *International Journal of Biosciences*. **10(3): 333-342.**
3.	**Eutropia V. Tairo, Kelvin M. Mtei and Patrick A. Ndakidemi (2017).** Influence of Water Stress and Rhizobial Inoculation on the Accumulation of Chlorophyll in *Phaseolus vulgaris* (L.) Cultivars. *International Journal of Plant & Soil Science*. **15(4): 1-13.**
4.	**Eutropia V. Tairo, Kelvin M. Mtei and Patrick A. Ndakidemi (2017).** Influence of Water Stress and Rhizobial Inoculation on Relative Leaf Water content and Electrolyte Leakage in Selected Common Bean cultivars (*Phaseolus vulgaris* L.). *SAUSSUREA Journal*, **7(2): 115-128**
5.	**Eutropia V. Tairo, Kelvin M. Mtei and Patrick A. Ndakidemi (2017).** Influence of Water Stress and Rhizobial Inoculation on Growth and Yield of Selected Common Bean cultivars (*Phaseolus vulgaris* L.). *Journal of Biodiversity and Environmental Sciences.* **11(2): 164-178**