

**RESPONSE OF COMMON BEAN (*PHASEOLUS VULGARIS* L.) TO
RHIZOBIA INOCULATION, NITROGEN AND PHOSPHORUS
APPLICATION ON SMALLHOLDER FARMS IN EASTERN ZIMBABWE**



VONGAI CHEKANAI

A thesis submitted in partial fulfillment of the requirements of the

DEGREE OF MASTER OF PHILOSOPHY

University of Zimbabwe

Faculty of Agriculture

Department of Crop Science

June 2019

DECLARATION

I, **Vongai Chekanai**, do hereby declare that this thesis is a result of original research work undertaken by myself except where clearly and specifically acknowledged. It is being submitted for the fulfillment of the degree of Master of Philosophy in Agriculture. It has not been submitted before for any degree or examination in any other University.

Date.....

Signed

At.....

ABSTRACT

Soil fertility depletion ranks as the most important drawback to crop productivity in Sub-Saharan Africa. On-farm experiments were conducted during the 2014/2015 and 2015/2016 cropping seasons to explore the effect of nitrogen (N), phosphorus (P) and rhizobia inoculation on biological nitrogen fixation (BNF), yields, nutritional and mineral components of common bean in smallholder farming areas of Eastern Zimbabwe. Two improved common bean cultivars (Gloria and NUA45), widely grown in Zimbabwe were tested in a split-plot arranged in randomized complete block design. The three sites were chosen to represent soil variability, two fields selected were fairly fertile and one was degraded. The main plot factor was the combination of N (0 and 60 kg ha⁻¹) and P (0 and 20 kg ha⁻¹), and the sub-plot factors were cultivar (Gloria and NUA 45) and inoculation (+/- inoculum). Both N and P were applied at 20 kg ha⁻¹ at planting, with an additional 40 kg N ha⁻¹ top dressing applied at flowering for the treatments receiving N. At peak flowering, nitrogen fixation was estimated using the ¹⁵N natural abundance method for the P, NP, I and NPI treatments. At physiological maturity, grain samples were analyzed for protein, trypsin and phytate. The other micronutrients tested for the grain were iron, zinc, manganese, copper and boron. Number of nodules per plant, and active nodules were all both significantly increased by P application. Nitrogen application was particularly effective in increasing dry biomass, pod loading and number of seeds per pod. Using *Bidens pilosa* as the reference plant, the proportion of nitrogen fixed was not significant in the all treatments. Analysis of variance showed that variety, N and P fertilizers have no influence on nutritional components of grain grown in degraded sites. Non-degraded fields showed significant varietal differences –NUA 45 had higher Cu and Mn while Gloria was richer in Zn. Fertilization significantly increased grain Zn content but there was no benefit of co-application of N and P. Both variety and fertilization had no influence on grain protein, trypsin, phytate or iron content. On degraded sites that had approximately 0.32% SOC, none of the factors significantly increased grain yields ($P > 0.05$). The combined analysis of variance of grain yield obtained on the two sites that had SOC $> 0.6\%$ showed significant simple effects of N and P, as well as a significant NP interaction ($P = 0.03$), but with neither inoculation nor cultivar effect. These results suggest that farmers can invest in both N and P for common bean production, but only targeting soils that are not acutely degraded. Improved common bean cultivars currently on the market barely respond to the local rhizobia inoculum. Farmers can invest in P only for increased common bean zinc, in undegraded soils. Further investigations are recommended to ascertain the exact conditions and management under which the most nutrition gains for common bean are made.

ACKNOWLEDGEMENTS

This study was funded by N2Africa Project from Wageningen University and a research grant from DAAD. Many thanks to Department of Environmental and Earth Sciences, University of Leuven, Belgium for N₂-fixation analysis and the IITA Ibadan Laboratory, Nigeria for grain nutrition analysis. I would like to acknowledge my supervisors Professor Regis Chikowo, Dr Fred Kanampiu, Professor Ken Giller, Professor Bernard Vanlauwe and Professor Pieters Pypers, and the late Dr D. Icishahayo. These academically powerful individuals patiently trained and mentored me for this research to come to life in this excellent quality.

The support of all fellow students and technical staff is greatly appreciated. Special thanks Nyasha Kafesu, Isaac Chabata, MacDonald Mubayiwa, and Shaw Mlambo. I sincerely thank the farmers in Goromonzi and Murehwa districts for their willingness to host these on-farm trials.

Thank you Lord!

DEDICATION

This one is for you Dad

ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of variance
SSA	Sub –Saharan Africa
BNF	Biological nitrogen fixation
SOC	Soil Organic Carbon
N	Nitrogen
P	Phosphorus
I	Inoculation
Zn	Zinc
Fe	Iron
Cu	Copper
Mn	Manganese
MG	Mega grams

TABLE OF CONTENTS

DECLARATION	i
ABSTRACT	ii
ACKNOWLEDGEMENTS	iii
DEDICATION	iv
ABBREVIATIONS AND ACRONYMS	v
TABLE OF CONTENTS	vi
LIST OF TABLES	xi
LIST OF FIGURES	xii
CHAPTER 1	1
INTRODUCTION	1
1.1 Background	1
1.2 Justification and Problem Statement	2
1.3 Hypotheses	3
1.4 Objectives of the study.....	4

1.5 Thesis structure	4
CHAPTER 2	5
LITERATURE REVIEW	5
2.1 Introduction	5
2.2 Common bean: a brief overview and status	5
2.3 Nutritional importance of common bean	6
2.4 Biological nitrogen fixation and common bean	7
2.5 Nitrogen and common bean	9
2.6 Phosphorus and common bean	9
2.7 Improving nutritional value of common bean	10
CHAPTER 3	11
GENERAL MATERIALS AND METHODS	11
3.1 Description of sites	11
3.2 Field sites characterization procedure	13
3.3 Soil analyses.....	13
3.3.1 Total organic carbon determination	13
3.3.2 Soil pH and texture determination	13
3.3.3 Colorimetric determination of total nitrogen	14

3.3.4 Determination of available phosphorus	14
3.3.5 Determination of total exchangeable bases.....	14
3.4 Experimental design and plot establishment.....	17
3.5 Determination of dry matter, yields and yield components of common bean plants.	18
3.6 Statistical analysis and graphical presentation	18
CHAPTER 4	19
RESPONSE OF COMMON BEAN (<i>PHASEOLUS VULGARIS</i> L.) TO NITROGEN, PHOSPHORUS AND RHIZOBIA INOCULATION ACROSS VARIABLE SOILS IN ZIMBABWE	19
4.1 Introduction	20
4.2 Material and methods	22
4.2.1 The study area	22
4.2.2 Characterization of soils for experimental sites	22
4.4.2 Experimental treatments and management	22
4.4.3 Determination of nodulation and dry biomass	24
4.4.4 Determination of yield components and grain yields	25
4.4.5 Statistical analysis	25
4.5 Results	25
4.5.1 Soil characterization.....	25
4.5.2 N, P and +I effects on nodulation and pod loading.....	26

4.5.4 N, P and +I effects on biomass and grain yields	29
4. 6 Discussion	32
4.6.1 Common bean response to management- the soil fertility factor	32
4.6.2 Common bean fertilization strategy	33
4 .7 Conclusions	34
CHAPTER 5	35
NITROGEN, PHOSPHORUS AND RHIZOBIA INOCULATION EFFECTS ON BIOLOGICAL NITROGEN FIXATION AND NUTRITIONAL COMPONENTS OF COMMON BEAN (<i>PHASEOLUS VULGARIS</i> L.) ON LIGHT TEXTURED SOILS.....	35
5.1 Introduction	36
5.2 Materials and methods	38
5.2.1 Site description and characterization	38
5.2.2 Experimental design and management	39
5.3.3 Estimation of N ₂ -fixation	40
5.2.5 Statistical analysis	41
5.3 Results	42
5.3.1 N ₂ -fixation by common bean	42
5.3.2 Effect of N and P on common bean protein and micronutrients	44
5.4 Discussion	50

5.4.1 Biological nitrogen fixation	50
5.4.2. Effect of N and P on common bean protein, trypsin, phytate and micronutrients.....	50
5.5. Conclusions	52
CHAPTER 6	53
OVERALL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS	53
6.1 Introduction	53
6.2 Influence of rhizobia inoculation, nitrogen and phosphorus application on common bean productivity	53
6.2 Effect of rhizobia inoculation, N and P fertilizers on common bean N ₂ -fixation	54
6.3 Influence of N and P on common bean nutritional quality	55
6.6 Conclusions	55
6.7 Recommendations	56
6.7.1 Recommendations for smallholder farmers	56
6.7.2 Further research	56
REFERENCES	58

LIST OF TABLES

Table 3.1 Physical and chemical characteristics of fields used for experiments in Eastern Zimbabwe	16
Table 4.1 Common bean nodulation as influenced by nitrogen, phosphorus and rhizobia inoculation in fairly fertile soils with > 0.7 % SOC	29
Table 4.2 Influence of nitrogen, phosphorus and rhizobia inoculation on number of pods per plant and number of seeds per pod in soils 2 with >0.7 % SOC.	30
Table 5.1: Estimates of % N ₂ -fixed derived from N ₂ -fixation by common bean as determined by the 15N abundance method using <i>Bidens pilosa</i> as the reference plant as a function of nutrient management and rhizobia inoculation at peak flowering.....	49
Table 5.2: Descriptive statistics for nutritional components in dry common bean grain as affected by fertilizers at physiological maturity in non-degraded sites with SOC>0.6%.....	50
Table 5.3. Varietal differences of common bean on Cu, Mn and Zn concentration in a non-degraded soil with SOC >0.7%.....	52

LIST OF FIGURES

- Figure 3.1** Cumulative daily rainfall for 2015/2014 and 2015/2016 cropping seasons in Domboshava and Murehwa, Zimbabwe. Arrows indicate when major agronomic practices were implemented. 12
- Figure 4.1** Common bean dry biomass as influenced by nitrogen, phosphorus and rhizobia inoculation during the 2014/2015 and 2015/2016 cropping seasons in Eastern Zimbabwe.....30
- Figure 4.2** Common bean grain yields as influenced by nitrogen, phosphorus and rhizobia inoculation (+I) during the 2014/2015 and 2015/2016 cropping seasons in Eastern Zimbabwe. A₁ and A₂ show yield gaps associated with degraded soils while B₁ and B₂ are common bean 31
- Figure 5.1:** Zinc concentration as affected by nitrogen at 40 kg ha⁻¹ and phosphorus at 20 kg ha⁻¹ fertilization in non-degraded soils in Eastern Zimbabwe.....56

CHAPTER 1

INTRODUCTION

1.1 Background

Soil fertility in much of Sub-Saharan Africa (SSA) is characteristically low, with soil organic carbon (SOC) being particularly poor on sandy soils. Maize production is disproportionately prioritized compared to legumes in Zimbabwean smallholder farming systems (Kasasa et al. 1999) These smaller farm portions assigned to legumes have to accommodate different grain legume crops commonly grown such as groundnuts (*Arachis hypogea*), common bean (*Phaseolus vulgaris*), cowpea (*Vigna unguiculata*), soybean (*Glycine max*) and Bambara nuts (*Vigna*

subterranea) (Madamba et al. 2003). Common bean is one of the most important legume crops for household nutrition as well as income but the current yields on these farms are approximately 0.6 Mg ha⁻¹ compared to attainable yields of 1.6 Mg ha⁻¹ (Chianu et al. 2011). Besides low inherent soil fertility the other reasons for these paltry yields include use of unimproved varieties, lack of inputs and limited extension services (Fageria and Baligar, 2005).

Legume crops have the ability to fix nitrogen using rhizobia bacteria in a process called biological nitrogen fixation (BNF). Compatible rhizobia bacteria in the soils might not exist or exist in insufficient quantities to effect BNF thus there is need to supplement with artificial inoculants. However, common bean is known to possess the least capacity in fixing atmospheric nitrogen. Artificial fertilizers such as nitrogen (N) and phosphorus (P) can aid in improving grain yields of common bean but smallholder farmers have the tendency of not applying supplementary fertilizers to common bean preferring to fertilize cereals (Madamba et al. 2003).

Common bean plays an important role in household nutrition as 80% of total protein consumed by humans comes from plants and common bean provides dietary protein and micronutrients for the populations in SSA (Sathe, 2002). However, bio-availability of these micronutrients is reduced by increased trypsin inhibitors and phytate content of the common bean grain (Frossard et al. 2000). Approximately two billion people in the world suffer from zinc (Zn) and iron deficiency (Fe) and research advances to improve absorption of these minerals mainly focuses on varietal improvement, processing and bio-fortification (Sathe, 2002). Are these anti-nutritional components sensitive to N and P fertilizers? Can the addition of these fertilizers also increase the protein and micro-nutritional quality of the common bean grain?

1.2 Justification and problem statement

Soil fertility depletion continues to be the most important drawback to crop productivity in SSA with poor N and P contents being the most limiting nutrients (Bationo, 2004; Vanlauwe et al. 2015). Efficient biological nitrogen fixation by legumes is a function of the legume genotype, rhizobia strain, environment and management (Tsigie et al. 2011). Success in soybean production on smallholder farms in southern Africa have been hinged mainly on use of both improved germplasm and appropriate rhizobia strains for specific soybean varieties (Kasasa et al. 1999; Mpeperekwi et al. 2000). Although common bean has the capacity to fix nitrogen, it is reported to have the lowest N₂-fixation rate among the most widely grown grain legumes but with a compatible rhizobia and a nodulating variety, the crop can fix N (Martinez-Romero, 2003). However, little research has been done to improve the yield of common bean using artificial fertilizers, yet as a pulse, increased productivity would have immediate impact on household nutrition as well as income. Malnutrition problems in SSA population can be mitigated if research on the influence of N and P on protein and micro-nutritional quality of common bean along with the capacity of these fertilizers to reduce anti-nutritional components such as trypsin inhibitors and phytate. To date, not much research has been done to investigate the response of available cultivars to rhizobia inoculation in different soils. Once common bean varieties that are responsive to rhizobia inoculation for specific soil conditions are known, the awareness would be used to increase productivity of common bean and design more efficient crop sequences on farms. Improvement of common bean grain quality has been hinged on germplasm and bio-fortification, not much research has been done to obtain the most nutritive gains from common bean as affected by management. This study, therefore, seeks to establish the response of common bean to rhizobia inoculation, and

N and P fertilization for increased BNF, grain yields and nutritional quality of two common bean varieties that are used by farmers.

1.3 Hypotheses

The study was centred on hypotheses listed below.

1. Application of N and P and rhizobia inoculation significantly increases common bean grain yield and yield parameters.
2. Rhizobia inoculation, N and P significantly improve nodulation and biological nitrogen fixation (BNF) in common bean.
3. Application of N and P significantly influences nutritional quality of common bean grain.

1.4 Objectives of the study

The general objective of the study was to improve biological nitrogen fixation, nutritional quality and grain yields of common bean through rhizobia inoculation, N and P fertilizers.

The specific objectives of this study were to:

1. Determine the common bean yield and yield parameters as influenced by rhizobia inoculation, N and P fertilizers.
2. Determine the nodulation and biological nitrogen fixation response of common bean to rhizobia inoculation, N and P fertilization.
3. Investigate the influence of N and P fertilization on micronutrient concentration of common bean grain.

1.5 Thesis structure

Chapter 1 provides the background and justification of the study. Chapter 2 draws attention to relevant background material and the prevailing knowledge gaps. The study site, and general materials and methods employed in the study are presented in Chapter 3 while the more detailed and particular methodologies are in the respective results chapters. Response of common bean to rhizobia inoculation, N and P across variable soils Chapter 4. Nitrogen, phosphorus and rhizobia inoculation effects on biological nitrogen fixation and nutritional components of common bean (*Phaseolus vulgaris* L.) on light textured soils is presented in Chapter 5. Chapter 7 focuses on the major findings, conclusions and recommendations generated in the study.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Many studies have demonstrated that rhizobia inoculation increases BNF in commonly grown legume crops such as soybean, groundnuts and cowpea (Sanginga et al. 2003; Van Kessel and Hartley, 2000). Different legumes respond differently to rhizobia inoculum and there are also varietal differences within the same crop (Tsigie et al. 2011). This literature review probes into the background on common bean productivity in Zimbabwe, biological nitrogen fixation, nutritional quality of common bean, addressing malnutrition in SSA while highlighting knowledge gaps that this research may assist in bridging. Research on the response of common bean in Zimbabwean farming systems is undocumented.

2.2 Common bean: a brief overview and status

Common bean is an essential part of SSA cropping systems and a major source of protein for the poor in Eastern and Southern Africa. While mainly grown for subsistence, mostly by women, currently, common bean productivity is failing to keep up with population growth in many countries because of abiotic and biotic as well as socioeconomic constraints (Kambewa, 1997). Drought is the major and most important factor among the abiotic constraints (Manjeru et al. 2007; Fageria and Moreira, 2011). Erratic rainfall, inadequate total rainfall, poor rainfall distribution and delayed onset and/or early cessation of rains are some of the factors that cause drought. Common bean crop is mainly grown by smallholder farmers in Zimbabwe who contribute 80% of total

annual bean production with the remaining 20% being produced by commercial farmers (Madamba et al. 2003; Madamba and Pompei, 2004). The crop has a potential to alleviate protein and micronutrient deficiency in these resource constrained farmers who cannot afford animal protein. Most smallholder farmers in Zimbabwe practice rainfed crop production hence the poor productivity as drought is one of the major production constraints. Smallholder farmers common bean yields are approximately 0.24 Mg ha⁻¹ compared to between 1.5 Mg ha⁻¹ to 4 Mg ha⁻¹ achieved by commercial farmers (Hikwa and Jiri, 2002). Apart from drought and input shortages, smallholder farmers also tend to target common bean on poorer quality land compared to cereal crops even though the crop fetches higher prices at the market (Fageria and Baligar, 2005; Ramakrishna et al. 2000).

Low common bean productivity is also often associated with declining soil fertility yet the majority of Zimbabwean smallholder farmers are unable to afford fertilizers. There is need for low cost, and sustainable solutions to increase the grain yield. Legume crops are known for their ability to fix N through BNF therefore, rhizobia inoculation becomes an important source of N for resource poor farmers. Success stories have been reported in soybean production where inoculation with *Rhizobium japonicum* strains significantly increased grain yields in Zimbabwe (Mpeperekwi et al. 2000). Smallholder farmers in Zimbabwe are yet to fully adopt rhizobia inoculants in legume production.

2.3 Nutritional importance of common bean

Common bean (*Phaseolus vulgaris* L.) is an essential, high-quality legume crop that provides large quantities of mineral micronutrients and protein to the human diet. Compared to other vegetables,

common bean has the highest value for human nutrition (Fageria et al. 2011). Bean seeds contain approximately 20% protein and supplies protein for roughly 500 million people Africa and Latin America (Fageria et al. 2011). The inclusion of bean in diets is linked to numerous health benefits such as the reduction of coronary heart diseases and cholesterol level (Chen, 2004; Coelho et al. 2002), as well as high antioxidant capacity (Heimler et al. 2005). Functional value of common bean in the human diet is defined by the crops' nutritional value which is determined by the micronutrient concentration, presence or absence of anti-nutritional factors such as polyphenols, trypsin inhibitors and phytates (Frossard et al. 2000). The most abundant phytate is phytic acid which has the highest capability of preventing Fe and Zn absorption in the gastrointestinal tract. At the same time, phytates have been reported to reduce cancer risks in human beings hence there should be an optimum amount of phytate in common bean as a functional food (Nestel et al. 2006). Other anti-nutritional compounds are trypsin inhibitors which bind and inactivate digestive enzyme trypsin. This enzyme breaks down proteins during digestion and its variability in content is influenced by genotype of the common bean variety and environmental conditions (Sotelo et al. 1995; Barampama and Simard, 1993). Diets of people in under-developed countries lack most energy, micronutrients and protein needed for the body and this has led to researchers focusing on breeding for crops for higher micronutrient content and bio-fortification of common bean crops (Welch et al. 2000; Nestel et al. 2006). Iron and Zn are the most deficient micronutrients in the diet of the resource constrained populations and common bean could potentially fix this problem.

2.4 Biological nitrogen fixation and common bean

All living organisms require nitrogen but it is required in the largest amount by plants (Marschner, 2012). Nitrogen exists as a di-nitrogen gas (N₂) which cannot be utilized by plants in this form and

legumes have the ability to fix N in symbiosis with bacteria. Biological nitrogen fixation is the process whereby species of bacteria convert nitrogen into ammonia (NH₃), which can be utilized by plants biologically (Unkovich et al. 2008; Giller, 2001). These bacteria, that are present in the soil, infect the roots of the plant and in symbiosis they form nodules in which biological nitrogen fixation takes place. In legume crops, biological nitrogen fixation occurs on the roots in specialized structures called nodules. Root nodule development starts when roots exude flavonoids into the rhizosphere and the specific bacteria Nod D genes are expressed and cause release of lipochito-oligosaccharides called Nod D factors. Upon binding to flavonoids, activated Nod D factors re-enter the bacterial cell and activate nod genes which induce release of Nod factors. Nod factors are responsible for initiating nodule formation as they promote movement of rhizobia towards root hair surfaces for attachment (Smit et al. 1987). The hair curls, cell wall degrades as the bacterium is absorbed by the plant during the invagination of the plasma membrane forming an infection thread. Rhizobia multiply and penetrate other root hair cells, with infected cells proliferating and forming a nodule initially. Ability of the formed nodule to fix nitrogen is determined by the genetic makeup of the host and environmental factors (Farinelli et al. 2006)

2.5 Nitrogen and common bean

Common bean requires N in highest amounts because it is a component of proteins, amino acids, and enzymes among other various biological processes therefore the nutrient has a marked effect on crop growth and yield (Soratto et al. 2006). Nitrogen promotes growth, photosynthesis, nutrient uptake and improves immunity of plants to environmental stresses when applied in sufficient amounts (Rathke et al. 2006; Wang et al. 2014). Giller et al. (2001), reported that nodule

senescence of common bean often coincides with the development of seed thereby leading to reduced yields. On the other hand, common bean responds to N fertilizer even when grown in conditions where it fixes nitrogen well and as a result, this crop is generally considered to be more responsive N fertilization than other legumes (Graham, 1981). However, blanket nitrogen fertilizer recommendations have limited relevance because smallholder farms have heterogeneous soil types and also influenced by past managements (Zingore et al. 2007; Henson and Bliss, (1991) applied 50 to 60 kg N ha⁻¹ at different growth stages of common bean and higher yields were observed where N was applied during the vegetative stage than at planting. However, the same N showed negative results on nodulation. Recommendations from this study were applying N at vegetative growth as the best management system and the need to identify bean varieties capable of fixing N₂ in the presence of N to maximize economic and biological yields.

2.6 Phosphorus and common bean

Phosphorus deficiency is extensive in regions where the common bean (*Phaseolus vulgaris*), and apart from nitrogen, phosphorus becomes the second most important nutrient. The total amount of P percentage in most soils is about 0.04 to 0.10%, although only limited amounts is available for plant uptake (Cordell et al. 2011). One of the reasons why P is seldom sufficient for optimal plant growth is because of its ability to react with various elements in the soil forming complexes that are not available for plant uptake (Liao et al. 2004; Araujo et al. 2000). In the whole world, a quantity of 17.5 million tons of P is processed yearly from rock phosphates reserves and 85% of it is used in fertilizer production (Cordell et al. 2009). Phosphorus deficiency is estimated to limit over 50% of common bean productivity (CIAT, 1992). Specifically, in legumes, it plays a

significant role in root growth and development, production of protein phytin and phospholipids (Rahman et al. 2008). Yield of common bean is greatly increased by application of phosphorus fertilizer (Turuko and Mohammed, 2014; Singh et al. 2008). Combined application of phosphorus and rhizobia inoculation increases grain yield of bean as well as yield parameters (Ndakidemi et al. 2006; Morad et al. 2013).

2.7 Improving nutritional value of common bean

Although common bean is known to be a good source of protein, over the years, researchers have been attempting to improve the nutritional value of common bean to curb micronutrient deficiency such as iron, zinc prevalent in SSA populations (Frossard et al. 2000). Over 3.5 billion people are affected by micronutrient deficiencies and even prevalent in areas where food is not limiting because of decreased food quality (Graham et al. 1999). The most prevalent micronutrient deficiency is iron and zinc especially in women and iron deficiency causes anemia to pregnant women thereby leading to maternal and perinatal mortality. Zinc deficiency has been reported to have harmful long-term effects on growth, immunity and survival of children (Harika et al. 2017). One approach to increase micronutrient concentration in common bean is through bio-fortification which is the breeding common bean varieties for higher micronutrient concentration (Nestel et al. 2006).

CHAPTER 3

GENERAL MATERIALS AND METHODS

3.1 Description of sites

On-farm experiments were carried out on two sites found in Murehwa Ward 15 and one site in Goromonzi Ward 1, smallholder farming areas of Eastern Zimbabwe during the 2014/15 cropping season. Zimbabwe is divided into five agro-ecological regions with semi-arid NR V receiving an annual average rainfall of <500 mm and NR I having the most favourable season that receives >1000 mm rainfall. The experimental fields lie in NR II receiving a fairly reliable rainfall ranging from 750-1000 mm per year in a unimodal season from November to March. In Zimbabwe, 75-80 % of total area planted to crops is accounted for by NR II and the soils are often acidic and poor in soil organic carbon (SOC) content. The three sites chosen represented soil variability commonly found in smallholder farming systems, two fields selected were fairly fertile and one was degraded as per farmer perception and previous crop performance. Rainfall received for the two seasons is shown in Figure 3.1.

1
3

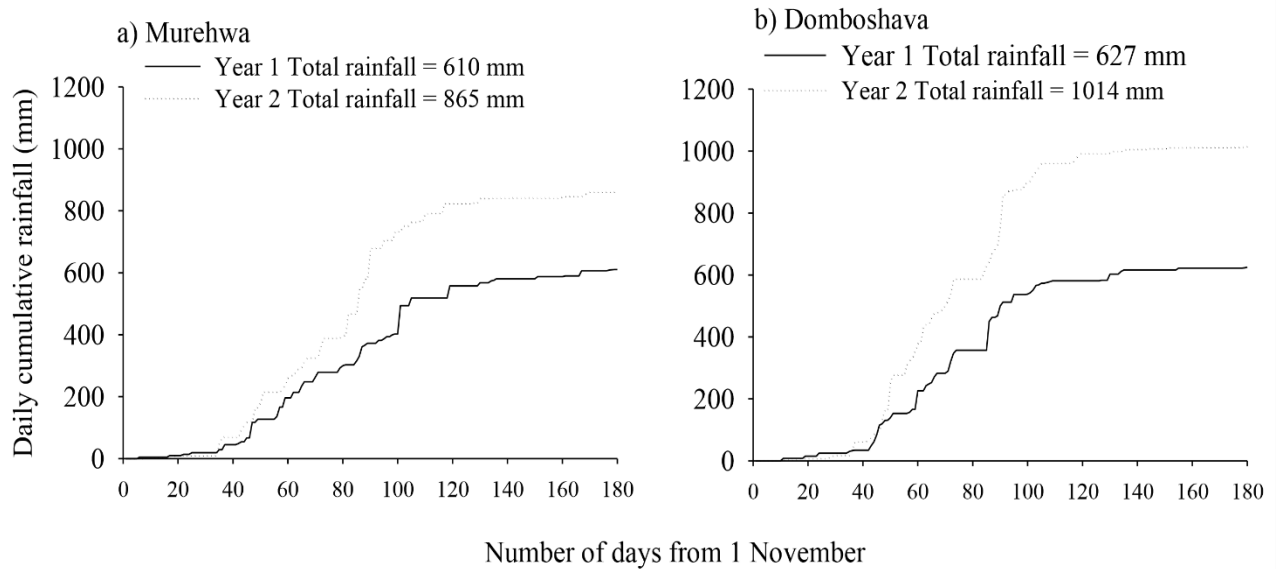


Figure 3.1 Cumulative daily rainfall for 2015/2014 and 2015/2016 cropping seasons in Domboshava and Murehwa, Zimbabwe.

3.2 Field sites characterization procedure

Before experiment establishment, a composite soil sample, from the plough layer (0 - 20 cm depth) was collected. This sample was made from 10 subsamples from randomly selected points in each plot and the samples were used for detailed for chemical analysis. Physical and chemical characteristics are shown in Table 3.1.

3.3 Soil analyses

3.3.1 Total organic carbon determination

Soil organic carbon was determined by a modified Walkley –Black method (Anderson and Ingram, 1993). A 2 mm sieve was used to sieve the soil and 1 g was weighed in a digestion flask containing 2 ml of distilled water. Ten ml of 5% potassium dichromate solution was added to the soil to oxidise the

carbon. Sulphuric acid (20ml) was added to the mixture and gently mixed by swirling. The mixture was digested for 30 minutes, allowed before adding 50 ml of 0.4 % barium chloride and mixing. The mixture was brought to the 100 ml mark and left over night to obtain supernatant solution. A larger portion of the supernatant was transferred into a colorimeter cuvette and absorbance of the standards, sample and blank were measured using a BUCK Scientific 100 VIS spectrophotometer. A graph of absorbance at 600 nm was plotted against a set of standards. Dissolving 11.886 g dry sucrose in 100 ml of distilled water made a stock solution of 50g/ml carbon (making of the standards). 0, 5, 10, 15, 20 and 25 ml of the 50 g/ml C stock solution were transferred different volumetric flasks and made up to the mark in the respective volumetric flasks. Two ml of the standards were pipetted into 100 ml digestion tubes and completely dried by heat. This resulted in dried contents of 0, 5, 10, 15, 20, 25 mg C. They were brought to the mark with distilled water and finally a standard series of 0, 0.05, 0.15, 0.20, and 0.25 mg C/ ml were obtained. The % organic C was calculated as follows:

$$\% \text{ organic C} = (a-b) \times 0.1 / W$$

Where a = Cr^{+3} ions in the blank, b = Cr^{+3} ions in the sample and W = the weight of the soil.

3.3.2 Soil pH and texture determination

Soil pH was determined using the Water method. A soil sample weighing 50 g was added to 125 ml of distilled water, thoroughly swirled to mix the suspension and a pH meter was used to measure the pH. The hydrometer method was used to determine soil texture. Twenty ml of 30 % hydrogen peroxide was added to a soil sample weighing 50 g and saturated with 125 ml of distilled water. The mixture was placed in a boiling water-bath and cooled before placing it on a mechanical shaker for overnight. A plunger was used to stir the mixture until all sediment had disappeared from the bottom of the cylinder, a hydrometer and a thermometer were inserted into the mixture and readings were obtained. The

percentage of clay and silt were calculated and the textural class for the sand was obtained from a textural triangle.

Calculations:

40 seconds (correctional reading) = 2 (40 seconds reading – 40 seconds blank + T)

5 hours (correctional reading) = 2 (5 hours reading – 5 hours blank + T)

Where T = temperature corrections: For every degree above 20 °C (d), T = 0.3 x d for every °C below 20 °C (d); T = - 0.3 x d

d = Temperature difference

% sand = 100-40 seconds (correctional reading)

% silt = 40 seconds (correctional reading) – 5 hours (correctional reading)

% clay = 5 hours (correctional reading)

3.3.3 Colorimetric determination of total nitrogen

The total N content was measured in a digest obtained by treating soil samples with hydrogen peroxide, sulphuric acid, lithium sulphate and selenium as a catalyst. A soil sample weighing 0.5 g was mixed with 5 ml of the digestion mixture and digested at 360 °C for 2 hours. The mixture was cooled, a total of 100 ml of distilled water was added and a clear supernatant was obtained for analysis. Test tubes were labelled and 0.1 ml of the standards and samples were added before adding a reagent containing 25 g potassium tartrate, 25 g trisodium citrate and 34 g sodium salicylate. The solution was made up to 100 ml by distilled water in each test tube. The solutions were mixed well and after 15

minutes, a second reagent made of sodium hypochlorite and sodium hydroxide was added and left for 1 hour for colour development. A series of standards containing 0, 2.5, 5.0, 7.5, 10.0 and 15.0 mg N / litre were made from the digestion mixture and graphs of absorbance at 655 nm were plotted against standard concentration. The % N was calculated as follows:

$$\% N = \{(\text{absorbance of sample} - \text{absorbance of blank}) \times F \times 0.01\} / \text{sample weight}$$

Where F = the mean of (concentration of standards (ppm)/ absorbance of standards

3.3.4 Determination of available phosphorus

The Olsen method was used to obtain available soil phosphorus in the soil. An extracting solution made up of 0.5 M sodium bicarbonate at pH 8.6 was added to soil weighing 2.5 g and shaken for 30 minutes. The suspension was filtered using Whatman No. 42 paper and used for colorimetric measurements of P. Five ml of boric acid and 10 ml of ascorbic acid were added to the filtered solution and left to stand for an hour. Colorimetric measurements from the formed phosphorus molybdate complex was then carried out at 880 nm.

$$P \text{ (mg kg}^{-1}\text{)} = \frac{(a-b) \times V \times F \times 1000}{1000 \times W}$$

Where a = the concentration of P in the sample; b = the concentration of P in the blank; V= volume of extracting solution; F= dilution factor; W= weight of soil.

3.3.5 Determination of total exchangeable bases

A soil sample weighing 5 g was extracted with 100 ml 1M ammonium acetate. The amount of exchangeable K was determined by flame photometry, Ca and Mg were determined by atomic

absorption spectrophotometry. Formation of refractory compounds which may interfere was prevented by adding lanthanum and strontium as releasing agents. The total exchangeable bases was calculated as: $\text{mg kg}^{-1} \text{ K, Ca and Mg} = \{(a-b) \cdot v \cdot f \cdot 1000\} / (1000 \cdot w)$

where a is the concentration of K, Mg and Ca in the sample, b is the concentration of element in blank sample, 2 v is volume of the extract solution, w is weight of the soil sample and f is the dilution factor.

1 **Table 5.1** Physical and chemical characteristics of fields used for experiments in Eastern Zimbabwe

Site	Farmer	Fertility	Clay	Sand	SOC	Total N	Available P (mg kg ⁻¹)	Soil pH (water 1:10)	Exchangeable (cmol ₍₊₎ kg ⁻¹)		
									Ca	Mg	K
Domboshava	Chawonza	High	120	850	7.9	0.7	16.3	5.7	5.52	1.05	0.1
	Kaviya	Low	100	800	3.2	0.5	5.90	4.7	1.26	1.10	0.1
Murehwa	Madziva	High	100	780	7.4	0.7	15.2	5.8	5.31	1.37	0.3
	Faro	Low	80	880	3.7	0.5	5.80	5.3	1.18	1.40	0.3
	Marimo	Low	80	760	3.9	0.5	5.90	4.9	1.80	2.10	0.1

2

3

3.4 Experimental design and plot establishment

Land preparation was done using ox-drawn plough (10-30 cm depth) to establish 4 m × 4 m plots. Two improved common bean cultivars, Gloria and NUA45 widely grown in Zimbabwe. Gloria matures in 93 days and yields up to 2400 kg/ha while NUA 45 matures in 90 and yields 2400 kg/ha. They were planted with inter- row spacing of 45 cm and intra- row spacing of 10 cm to achieve a plant population of 222 000 plants per hectare. Two seeds were planted per station and thinning to one seed per station was carried out two weeks after crop emergence. The plots were kept weed-free by hand-hoeing throughout the growing season. In each field, the experiments were laid out in a split plot arranged in randomized complete block design replicated three times. Experimental treatments were designed to explore the interaction of nitrogen (N), phosphorus (I) and rhizobia inoculation on the two common bean varieties. Nitrogen was applied using a local fertilizer ammonium nitrate with 24.5 % N and phosphorus was applied from single super phosphate which contains 19 % P. Rhizobia inoculation was applied at a rate of 100 kg of seed per 100 g satchet.

The main plot treatment combinations [-F (no fert), N, P and NP] and subplot combinations were variety and rhizobia inoculation as follows:

- i. Control (no fertilizer or rhizobia added)
- ii. . NPI (ammonium nitrate + single super phosphate+ inoculation)
- iii. NP (ammonium nitrate + single super phosphate) iv. NI (ammonium nitrate + inoculation)

- iv. PI (single super phosphate + inoculation)
- v. N (nitrogen only) vii. P (single super phosphate only)
- vi. I (inoculation only)

Common bean inoculant (*Rhizobium tropici* strain CIAT899) was obtained locally from the Soil Productivity Research Laboratory in Marondera. Both N and P were applied at 20 kg ha⁻¹ at planting. Nitrogen top dressing was added at a rate of 40 kg N ha⁻¹ at flowering stage for the treatments receiving N.

3.5 Determination of dry matter yields and yield components of common bean plants.

At peak flowering stage, destructive sampling of plants was done to obtain above ground biomass using a 1 m × 1 m quadrat within each plot, excluding the border rows and the net plot. Fresh weight was determined by immediately weighing the biomass using a digital scale (ACCT 40 x 10g Mini Portable). The samples were then oven-dried at 65 °C for 3 days to determine dry matter. Number of nodules and active nodules per plant was assessed by uprooting 10 random plants outside the net plot, counting the nodules and computing the mean. Active nodules were determined by cutting each nodule in the middle on the 10 plants, activity was shown by a pink to reddish colour inside the nodule. At physiological maturity, the pod loading and number of seeds per pod was obtained by randomly sampling 10 plants within the net plot and the average worked out. Common bean grain yields were assessed in 1.8 m x 1.8 m net plots. Pods were harvested, sun-dried for several days and threshed. Grain was then weighed and grain moisture content was

determined using a John Deere SW moisture meter. Grain yield was then adjusted to 12 % moisture to obtain the final yield.

3.6 Statistical analysis and graphical presentation

Number of nodules and active nodules per plant, pod loading and number of seeds per pod, dry matter and grain yield were analyzed. Genstat Version 14 was used to subject data to analysis variance (ANOVA) after testing for assumptions. Normality was tested using Shapiro-wilk tests and homogeneity of variances was tested using Levene's test. Where appropriate, Fisher's Protected LSD was used to separate means. Graphs were plotted using Sigma plot 10.

CHAPTER 4

RESPONSE OF COMMON BEAN (*PHASEOLUS VULGARIS L.*) TO NITROGEN, PHOSPHORUS AND RHIZOBIA INOCULATION ACROSS VARIABLE SOILS IN ZIMBABWE

Abstract

Common bean is one of the most important crops with potential to curb malnutrition in Sub-Saharan African populations. Yields of common bean (*Phaseolus vulgaris L.*) are, however poor, limited by low soil phosphorus (P), nitrogen (N) and poor biological N₂-fixation. On-farm experiments were carried out to study the effect of N, P and rhizobia inoculation on common bean yield and yield components during the 2014/2015 and 2015/2016 cropping seasons in Zimbabwe. Experiments were conducted on five farmers' fields in Eastern Zimbabwe, three were considered to be degraded and two non-degraded sites. Two common bean varieties (Gloria and NUA45) were tested in a split-plot arranged in randomized complete block design. The main plot factor was the combination of N (0 and 40 kg ha⁻¹) and P (0 and 20 kg ha⁻¹), and the sub-plot factors were variety (Gloria and NUA 45) and inoculation with *Rhizobium tropici* strain CIAT899 (+/- inoculum). At planting, both N and P were applied at 20 kg ha⁻¹, with an additional 20 kg ha⁻¹N top dressing applied at flowering. Analysis of variance indicated common bean did not respond to rhizobia inoculation (P>0.05) whilst P significantly increased the number of nodules and active nodules per plant (P<0.001), and grain yield. Application of 40 kg ha⁻¹ N significantly increased the number of pods per plant and number of seeds per pod, and grain yields. A significant NP interaction was only observed on grain yield for non-degraded soils. Co-application of N and P in non-degraded sites increased grain yields from 0.27 Mg ha⁻¹ to 1.48 Mg ha⁻¹ during the first year and from 0.37 Mg ha⁻¹ to 2.09 Mg ha⁻¹ during the second season. In general, effects of N or P were not significantly different, suggesting that farmers could invest in either of these nutrients for increased common bean grain yields. Strategically, P investments would be more logical as residual P effects on rotational cereals.

This chapter is published as: Chekanai V, Chikowo R and Vanlauwe B 2018. Response of common bean (*Phaseolus vulgaris L.*) to nitrogen, phosphorus and rhizobia inoculation across variable soils in Zimbabwe. Agriculture, Ecosystems and Environment DOI: <https://doi.org/10.1016/j.agee.2018.08.010>

4.1 Introduction

Common bean (*Phaseolus vulgaris* L.) is an important grain legume throughout the world providing a source of protein, dietary fibre, starch and minerals such as potassium, thiamine, vitamin B6 and folic acid in diets affordable by the poor (Garden-Robinson and McNeal, 2013). Edaphic and environmental factors that constrain bean production in most areas where the crop is grown include nitrogen and phosphorus deficiency, soil acidity (including aluminium and manganese toxicity) and drought (Bationo, 2004). Due to these factors, current bean yields in Southern Africa average only 0.6 Mg ha⁻¹ compared to attainable yields of > 1.5 Mg ha⁻¹ (Chianu et al. 2011).

A hallmark trait of agriculturally-useful legumes is their symbiosis with rhizobia bacteria which fix atmospheric nitrogen (N₂) within root nodules and make it available to the host plant (Giller, 2001). Although common bean has good potential for N₂ fixation, it is reported to have the lowest N₂-fixation rate among the most widely grown grain legumes (Giller, 1990; Martinez-Romero, 2003). Inoculation of common bean with rhizobia strains has been shown to be beneficial in increasing nodulation thereby enhancing biological nitrogen fixation, but in many cases effective nodulation is also affected by competition from high populations of competitive but ineffective native rhizobia (Giller, 2001). Sufficient nodulation also depends on the amount of P as the mineral is responsible for root growth and is important for biological N₂-fixation process.

Phosphorus is an essential nutrient for the growth and development of common bean as it is involved in various metabolic processes such as photosynthesis, respiration, and signal transduction, among others. Past research that investigated different rates of P for yields and yield

components of common bean have consistently shown a positive response to P application (Turuko and Mohammed, 2014; Fageria and Baligar, 2016). Nodule number, weight and volume increases with the addition of P, indicating more effective nitrogen fixation (Singh et al. 2008; Rifat et al. 2008). Nitrogen fixation in common bean has also been established to be more affected by P deficiency than in other legume crops such as soybean (Fageria and Baligar, 2016). Adequate P rates for maximum yield and yield attributes as reported by Gidago et al. (2011) are at least 40 kg ha⁻¹.

While most grain legumes only require ‘starter’ N to initiate early growth before the N₂-fixing symbiosis is established, there is need for ‘top dressing’ of common bean with additional N fertilizer. However, large amounts of plant-available N tend to inhibit rates of N₂-fixation (Giller, 2001). Past agronomy research on grain legumes in Zimbabwe has mostly focused on soybean, cowpea and groundnut (Chikowo et al. 1999). Therefore, the main objective of this work was to investigate the effect of N, P and rhizobia inoculants on productivity of common beans across different soils on which common bean is typically grown on smallholder farms.

4.2 Material and methods

4.2.1 The study area

The study was carried in two smallholder farming communities of Murehwa (17°45'S, 31°34'E) and Domboshava (17°36'S, 31°10'E) in Eastern Zimbabwe, during the 2014/15 and the 2015/2016 cropping seasons. Details of the study site are given in section 3.1.

4.2.2 Characterization of soils for experimental sites

Before the cropping season, composite soil samples consisting of five sub-samples collected along the field's diagonal line were collected from the plough layer (0 - 20 cm depth) and bulked for each of the five sites. The soil samples were air-dried, and those for total N, available P, extractable bases analysis were passed through 2 mm sieve, while those for SOC analysis were passed through a 0.5 mm sieve. Total SOC was determined by the modified Walkley-Black) (Okalebo et al. 2002), while total N and available P were determined by the micro-Kjeldahl and Olsen methods, respectively (Anderson and Ingram, 1993). Extractable bases (K, Mg and Ca) were extracted using ammonium acetate. Potassium (K) was determined by flame photometry, and Ca and Mg concentrations were determined by atomic absorption spectrophotometry. Soil pH was determined using the 1:10 water method and soil texture was determined using the hydrometer method (Gee and Bauder, 1986). The soils were mainly acidic with low P and N contents (Table 3.1)

4.4.2 Experimental treatments and management

Experimental treatments were designed to explore the interaction of nitrogen (N), phosphorus (P) and rhizobia inoculation (+I) on two common bean varieties (Gloria and NUA 45) using site as a

random factor. In each field, the experiments were laid out in a split plot arranged in randomized complete block design replicated in three blocks. Main plots were nested within the blocks and sub-plots were nested within the main plots. The main plot treatments were fertilizer management [no fertilizer, N, P or NP], and subplots were randomly assigned to +/- inoculation and variety, resulting in the following treatments:

- i) Control (no fertilizer or rhizobia added),
- ii) NP+I [ammonium nitrate (34.5% N) + single super phosphate (19% P₂O₅) + inoculation],
- iii) NP (ammonium nitrate + single super phosphate),
- iv) N+I (ammonium nitrate + inoculation),
- v) P+I (single super phosphate + inoculation),
- vi) N (ammonium nitrate only),
- vii) P (single super phosphate only), and
- viii) +I (inoculation only).

Two improved varieties, Gloria and NUA 45 that are both maintained by Zimbabwe Crop Breeding Institute and locally available on the market in Zimbabwe, were used. The varieties are both determinate and take approximately 90-100 days to reach physiological maturity. A rhizobia inoculum (*Rhizobium tropici* strain CIAT899) obtained from a local commercial manufacturer (Soil Productivity Research Laboratory, Marondera) was used to inoculate the bean seed prior to sowing, using a rate of 100g inoculum per 25 kg of seed as recommended by the manufacturer. Treatments P, NP, PI and NPI received 20 kg ha⁻¹P at planting. Nitrogen was added at 20 kg ha⁻¹ in

the N, NP, NI and NPI treatments at planting while an additional 20 kg ha⁻¹N was applied at flowering stage. Both N and P were applied at 20 kg ha⁻¹ at planting. Land preparation was done using ox-drawn plough and plots were established at 4 m × 4 m size. Planting for the first season was done on the 28th December 2014 during the 2014/15 cropping season, and on the 1st of January 2016 for the 2015/2016 cropping season. Planting was done after 8 weeks from the onset of the rains to prevent physiological maturity from coinciding with abundant rainfall in February.

Common bean was planted using an inter- row spacing of 45 cm and intra- row spacing of 10 cm for a plant population of 222 000 plants ha⁻¹. Prior laboratory seed germination tests had established a nearly 100% seed viability for both varieties, which was also achieved in the field.

The plots were kept weed-free by hand-hoeing throughout the growing season.

4.4.3 Determination of nodulation and dry biomass

At 6-7 weeks after germination, destructive sampling of plants was done within a 1 m × 1 m quadrat in each plot, excluding the border rows and the net plot. Ten common bean plants were randomly sampled from the uprooted plants, carefully washed in water to remove excess soil. The number of nodules per plant was determined by counting all nodules on each of the 10 plants and computing the average. Number of active nodules was determined by cutting nodules on each of the 10 plants and observing the colour inside the nodule. Active nodules were identified by a pink to reddish internal colour. Fresh weight was then determined by immediately weighing all the uprooted plants using a digital scale. The samples were then oven-dried at 65 °C for 3 days and weighed to determine dry biomass.

4.4.4 Determination of yield components and grain yields

At physiological maturity, all the plants in 1.8 m x 1.8 m net plots (4 rows x 1.8 m long) were cut at soil level and heaped at the centre of the plot. Random sampling of 10 plants was done and all the pods on each plant were counted and recorded to determine the number of pod per plant. A sub-sample of the pods was used to determine the number of seeds per pod. Later, all pods from the net plot were harvested into perforated harvest bags, sun-dried for 14 days and threshed. The grain was then weighed and grain moisture content determined using a John Deere SW moisture meter. Yields reported here are adjusted to 10% moisture content.

4.4.5 Statistical analysis

The number of nodules and active nodules per plant, number of pods per plant and number of seeds per pod were transformed using quantile normalization and subjected to Analysis of Variance (ANOVA) using R Version i3863.32. ANOVA for grain yield was obtained using a split plot model at one site, with different errors for main plots and subplots. The magnitude of the difference in all variables measured from degraded and non-degraded sites was huge; therefore data from these soil fertility domains was analyzed separately with site considered as a random factor.

4.5 Results

4.5.1 Soil characterization

All sites had low clay content, ranging from 8 – 12 %, while two sites that had poor SOC content of less than 0.4 % were concomitantly acidic ($\text{pH} < 5.5$) and acutely deficient in available P (Table

3.1). These infertile fields are subsequently referred to as “degraded” (<0.4% SOC) and the remainder of the sites as “non-degraded” with SOC >0.7%. We evaluated the response of common bean to management separately in the degraded and non-degraded fields due to these distinct differences in soil fertility.

4.5.2 N, P and +I effect on nodulation and pod loading

There was no significant variety effect; therefore, data presentation is at the crop level throughout the paper. In degraded soils with SOC < 0.4%, none of the tested factors significantly influenced nodulation and pod loading. During the first season, analysis of variance showed significant differences in the number of nodules per plant when only 20 kg ha⁻¹P was added (p = 0.006). The number of nodules per plant increased from 3 in the control to 8 in the P treatment (Table 4.1).

Similar results were observed during the second season where P application significantly increased (p < 0.001) number of nodules per plant from 4 in the control to 9 in the P treatment (Table 4.1). Phosphorus also significantly (p < 0.001) increased the number of active nodules per plant for both seasons, from 2 in the control to a maximum of 6 in the P treatment during Year 1. Co-application of phosphorus and rhizobia (P+I) did not result in significant increases in the number of active during Year1 and Year 2.

Analysis of variance showed that pod numbers were significantly increased by 40 kg ha⁻¹ N in the first season (p=0.02) and the second season (p=0.003). Increases in pod number from 4 in the control to 8 in the N only treatment were observed in the first season, and 8 to 11 during the second season (Table 4.2). Number of seeds per pod were significantly (p=0.03) increased by the addition

of N at 40 kg ha⁻¹ for both seasons. In all cases, N application more than doubled the number of pods per plant and number of seeds per pod (Table 4.2).

- 1 Table 4.1 Common bean nodulation as influenced by nitrogen, phosphorus and rhizobia inoculation in fertile soils with > 0.7 %
 2 SOC.

Treatments	Number nodule plant ⁻¹		Number of active nodules plant ⁻¹	
	Year 1	Year 2	Year 1	Year 2
Control	1±0.8 ^a (3)	2±0.8 ^a (4)	1±0.6 ^a (2)	3±0.7 ^a (3)
+Inoculation	1±1.0 ^a (5)	2±1.0 ^a (5)	1±0.8 ^a (3)	3±0.9 ^a (4)
+Nitrogen	3±1.4 ^a (4)	2±1.0 ^a (4)	3±1.4 ^a (2)	3±0.9 ^a (3)
+Phosphorus	13±1.4 ^b (8)	11±1.0 ^b (9)	11±1.4 ^b (6)	9±0.9 ^b (8)
+Phosphorus+Inoculation	13±2.5 ^b (8)	11±1.4 ^b (9)	11±1.6 ^b (6)	9±1.2 ^b (8)
+Nitrogen+Inoculation	3±2.5 ^a (4)	2±1.4 ^a (4)	3±1.6 ^a (3)	3±1.2 ^a (4)
+Nitrogen+Phosphorus	11±2.0 ^b (6)	10±1.3 ^b (8)	9±1.9 ^b (5)	9±1.2 ^b (7)
+Nitrogen+Phosphorus+Inoculation	11±3.0 ^b (6)	11±2.0 ^b (8)	9±2.2 ^b (5)	9±1.8 ^b (7)
CV %	103	103	135	84

- 3 Mean separation was done using transformed values that are outside parenthesis. Values with different letter(s) are significantly different
 4 at 5% probability

1 Table 4.2 Influence of nitrogen, phosphorus and rhizobia inoculation on number of pods per plant and number of seeds per pod in soils 2 with >0.7 % SOC.

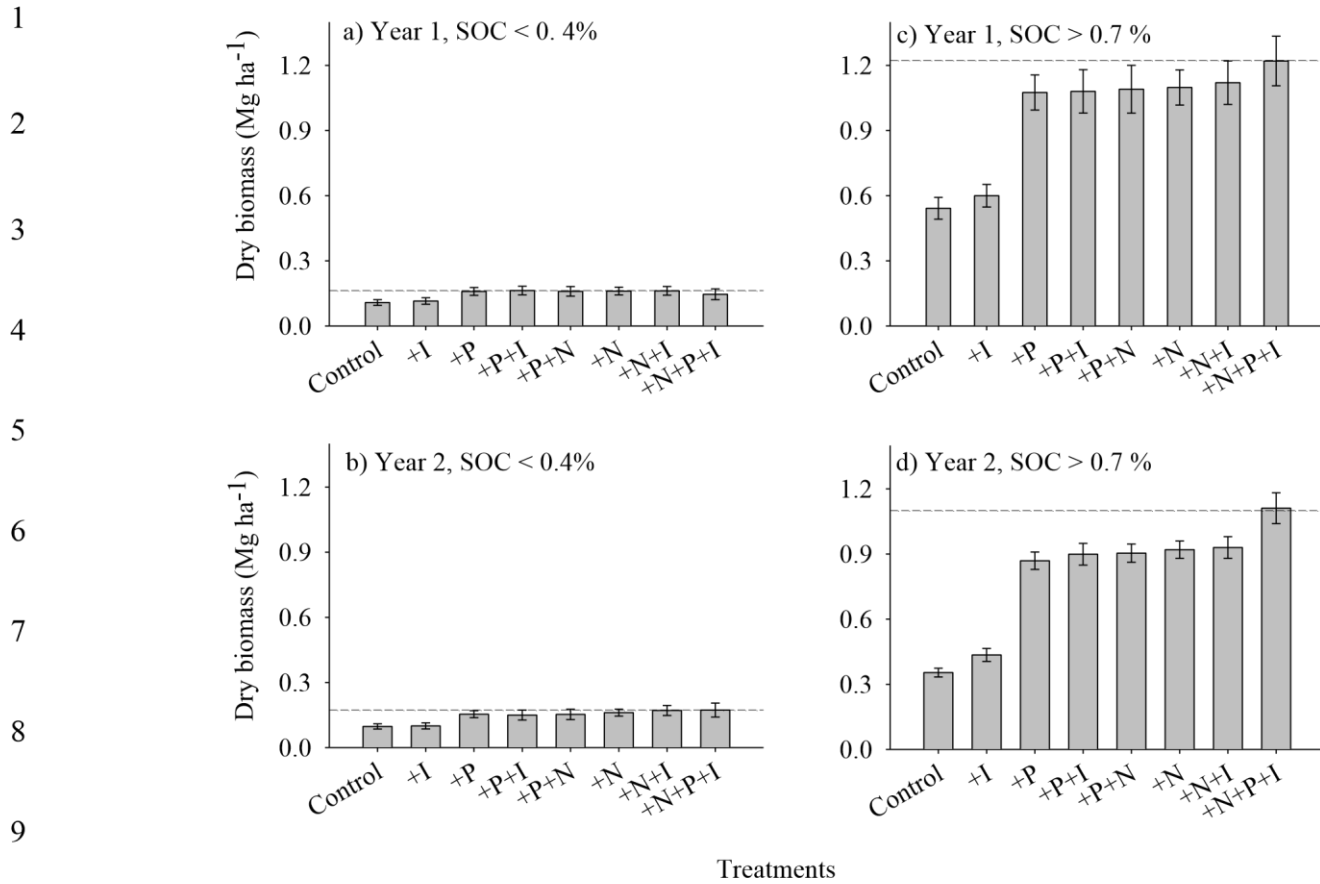
Treatments	Number pods plant ⁻¹		Number of pod ⁻¹ seeds	
	Year 1	Year 2	Year 1	Year 2
Control	3±0.2 ^a (4)	9±0.4 ^a (8)	2±0.1 ^a (2)	2±0.1 ^a (2)
+Inoculation	3±0.3 ^a (4)	9±0.5 ^a (8)	2±0.1 ^a (2)	2±0.1 ^a (2)
+Phosphorus	4±0.4 ^a (4)	8±0.6 ^a (9)	4±0.1 ^b (4)	4±0.1 ^b (4)
+Nitrogen	9±0.4 ^b (8)	12±0.6 ^b (11)	4±0.1 ^b (4)	4±0.1 ^b (4)
+Phosphorus+Inoculation	3±0.5 ^a (4)	8±0.7 ^a (8)	4±0.2 ^b (4)	4±0.2 ^b (4)
+Nitrogen+Inoculation	9±0.5 ^b (8)	12±0.7 ^b (11)	4±0.2 ^b (4)	4±0.2 ^b (4)
+Nitrogen+Phosphorus	9±0.6 ^b (8)	12±0.8 ^b (11)	4±0.2 ^b (4)	4±0.2 ^b (4)
+Nitrogen+Phosphorus+Inoculation	10±0.8 ^b (9)	13±1.0 ^b (12)	4±0.3 ^b (4)	4±0.3 ^b (4)
CV %	53	40	50	37

Mean separation was done using transformed values that are outside parenthesis. Values with different letter(s) are 3 significantly different at 5% probability.

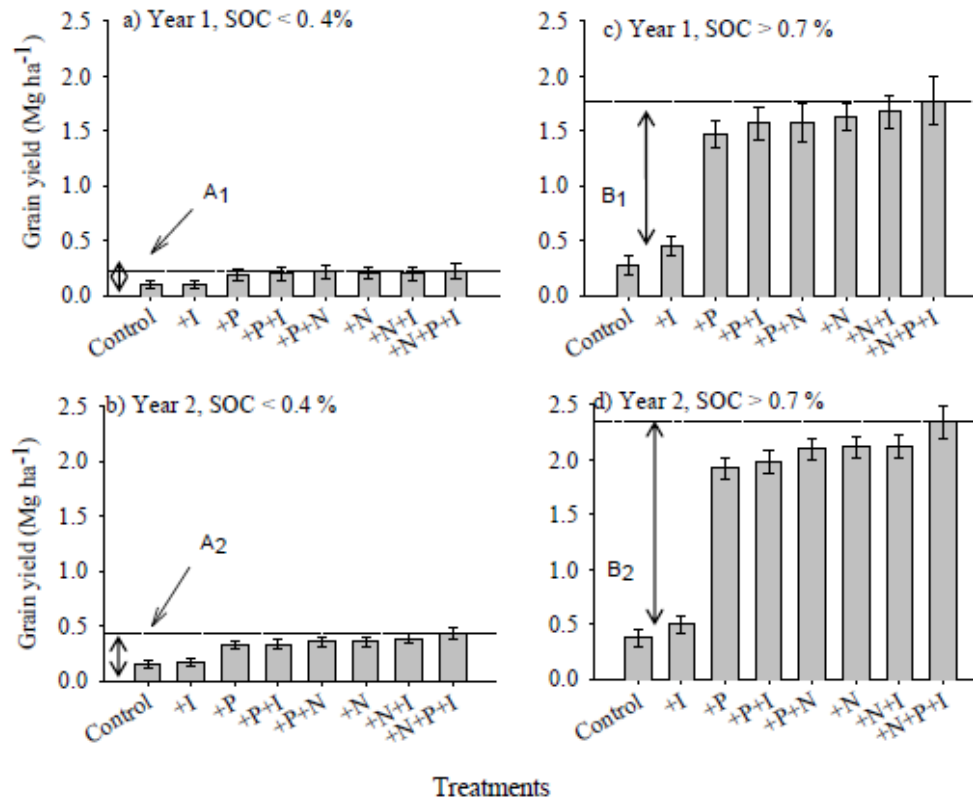
4.5.4 N, P and +I effect on biomass and grain yields

Common bean dry biomass was significantly increased by application of N, P and NP in both degraded and non-degraded soils, but biomass was a maximum of only 0.17 Mg ha⁻¹ under degraded soils compared to 1.2 Mg ha⁻¹ for non-degraded soils (Fig. 4.1). There was no response to rhizobia inoculation on degraded soils for both years, while only marginal biomass gains were observed on non-degraded soils. In all cases, co-application of N and P did not result in biomass yield differences from the N or P only treatments.

Application of N or P equally significantly increased common bean grain yields, but there were generally no benefits of co-application of N and P for both degraded and non-degraded soils (Fig. 4.2). The exception was during Year 2 when NP application resulted in larger yields under nondegraded soils. Under degraded soils, grain yields without any fertilizer were a paltry 0.27 and 0.37 Mg ha⁻¹, for Years 1 and 2, respectively, and only a maximum of 0.43 Mg ha⁻¹ with NPI application during Year 2. For the non-degraded soils, grain yields increased from 0.27 to 1.77 Mg ha⁻¹ when NPI was applied during Year 1, and from 0.37 to 2.3 Mg ha⁻¹ with NPI application. Inoculation with rhizobia only did not result in significant yield increases although grain increased in PI, NI and NPI treatments. These results indicate that base yields for non-degraded soils are comparable or larger than yields obtained with NP fertilization on degraded soils are therefore the practical exploitable yields gaps on degraded soils were 0.13 and 0.28 Mg ha⁻¹ (A₁ and A₂), while the potential benefits of fertilizing non-degraded soils were 1.5 and 1.93 Mg ha⁻¹ (B₁ and B₂) (Fig. 4.2). Inoculation with rhizobia only or in combination with N and P did not influence common bean productivity for both varieties.



10 **Figure 4.1** Common bean dry biomass as influenced by nitrogen, phosphorus and rhizobia inoculation during the 2014/2015 and 11 2015/2016 cropping seasons in Eastern Zimbabwe.



10
11
12
13
14
15
16
17
18
19
20

21 **Figure 4.2** Common bean grain yields as influenced by nitrogen, phosphorus and rhizobia inoculation (+I) during the 2014/2015 and
22 2015/2016 cropping seasons in Eastern Zimbabwe. A₁ and A₂ show yield gaps associated with degraded soils while B₁ and B₂ are
23 common bean yield gaps associated with non-degraded soils.

4. 6 Discussion

4.6.1 Common bean response to management- the soil fertility factor

In many smallholder farming communities in Africa, farmers often preferentially allocate cereal crops to more fertile fields on their farms, with legumes relegated to soils with multiple constraints (Zingore et al., 2007). If soils are not severely depleted in nutrients, this strategy may ensure successful production of both cereals and legumes in cases where legumes' ecological capabilities are sufficient to overcome the soil infertility hurdle. Common bean has little tolerance to low soil fertility (Singh et al., 2003). In our study, the performance of the two varieties we tested was consistently poor at three sites that had SOC < 0.4% and over two seasons. Application of both N and P did not result in any significant yield gains on these soils, this contrasted sharply with soils that had SOC > 0.7% (Fig. 4.1). These results strongly suggest multiple soil fertility limitations for common bean production that cannot be solved by application of only N and P fertilizers that farmers often use in the study sites. Non-responsive soils such as these have been described earlier (Vanlauwe et al., 2015). While common bean originated from regions with moderately fertile soils, globally, the cultivation of common bean by smallholder farmers on degraded soils has contributed to poor productivity (Beebe et al., 2012).

Inoculating common bean with rhizobia gave no significant increase in nodulation, biomass or grain production (Fig. 4.1). Rebeschini et al. (2014) also found that inoculation of beans with *Rhizobium tropici* gave no positive response. Hungria et al. (2000) also reported that inoculation with rhizobia in field experiments rarely increases yield of beans. The poor response of common bean to rhizobia inoculation observed in this study could be attributed to failure by the strains used to adapt to the harsh abiotic of the soils. In other studies, abundant native and ineffective rhizobia strains in the soils competed with the

introduced inoculum to form nodules, while only certain rhizobia strains had the ability to fix N in specific cultivars (Valverde et al. 2003).

Phosphorus fertilization significantly increased nodulation and number of active nodules, but only for non-degraded soils (Tables 4.1). Strong increases of nodulation with P fertilizer have frequently been found when there is little soil P available (Giller et al. 1998; Leidi and Rodríguez-Navarro, (2000); Tang et al. 2001). Phosphorus fertilization improves early root formation facilitating increased nodulation and enhanced common bean productivity. Nitrogen had a significant effect on pod loading, number of seeds per pod, and yields (Table 4.2; Fig. 4.2). Da Silva et al. (1993) reported increased common bean grain yields with application of N in N-deficient soils. Topdressing common bean with N has also been reported to increase common bean yield by Soratto et al. (2014) whose study reported that common bean response to N varies with cultivars and environmental factors.

4.6.2 Common bean fertilization strategy

Application of N or P had comparable effects on common bean productivity, with no clear benefits of co-application of these nutrients in most of the cases (Fig. 4.2). Market-oriented smallholder farmers in Zimbabwe have regularly fertilized common bean with N on sandy soils. With a cropping systems improvement objective, we content that it would be prudent to prioritize P fertilization to common bean and benefit from residual P effects for cereal crops grown in sequence (Rurangwa et al. 2017).

4.7 Conclusions

Application of N or P had equal magnitude of increasing common bean grain yields with no significant benefits of adding both elements. This result is important as farmers in the study area regularly invest more in N fertilizers for common bean than in P. With well documented P residual benefits to crops in

rotations, direct P fertilization to common bean is expected to improve cropping system performance. This study also established that the improved common bean varieties that are currently on the market did not respond to rhizobia inoculum currently marketed in Zimbabwe. We also confirmed the existence of degraded non-responsive soils. While some 'wonder' legumes can be successfully grown on infertile soils, attempts to grow common bean on such soils is uneconomic.

CHAPTER 5

NITROGEN, PHOSPHORUS AND RHIZOBIA INOCULATION EFFECTS ON BIOLOGICAL NITROGEN FIXATION AND NUTRITIONAL COMPOSITION OF COMMON BEAN (*PHASEOLUS VULGARIS* L.) ON LIGHT TEXTURED SOILS

Abstract

Common bean is an important pulse providing protein and micronutrients in the diets of Sub-Saharan African populations. However, productivity is generally poor with yields less than 0.6 Mg ha⁻¹. On-farm experiments were carried out in Zimbabwe, to investigate the influence of nitrogen (N), phosphorus (P) and rhizobia inoculation on bean biological nitrogen fixation and grain nutritional composition. Two varieties (Gloria and NUA45) were used in a split-plot arranged in randomized complete block design on one degraded site and two non-degraded sites. The main plot factor was the combination of N (0 and 40 kg ha⁻¹) and P (0 and 20 kg ha⁻¹), and the sub-plot factors were variety and inoculation with *Rhizobium tropici* strain CIAT899 (+/- inoculum). Both N and P were applied at 20 kg ha⁻¹, with an additional 20 kg N ha⁻¹ top dressing. N₂-fixation was estimated using the ¹⁵N natural abundance method using *Bidens pilosa* as a reference plant. Protein, phytate, trypsin and micronutrients (iron, manganese, zinc, copper) contents were determined in dry grain. The proportion of nitrogen fixed was not significantly different between treatments. Analysis of variance showed that variety, N and P fertilizers had no influence on nutritional components of grain grown in degraded sites. Non-degraded fields showed significant varietal differences –NUA 45 had higher Cu and Mn while Gloria was richer in Zn. Both variety and fertilization had no influence on grain protein, trypsin, phytate or iron content. Fertilization significantly increased grain Zn content but there was no benefit of co-application of N and P. Farmers can invest in P only for increased common bean zinc, in non-degraded soils. More experiments need to be done on other varieties to investigate response to rhizobia inoculation as well as increased protein content.

5.1 Introduction

Common bean (*Phaseolus vulgaris* L.) is one of the most important legume crops for human nutrition and is a major protein and energy supplier in the world (Beebe et al. 2000; Broughton et al. 2003; de Faria Müller et al. 2014). Common bean has been reported to be a poor fixer of N but the potential to fix up to 60 kg ha⁻¹ has been demonstrated in numerous studies (Hardarson et al. 1993; Epping et al. 1994; Asadi et al. 2005; Mnasri et al. 2007). The crop can grow from mineral nitrogen but can also fix N through biological nitrogen fixation in symbiosis with rhizobia bacteria. A number of *Rhizobium* species have the ability fix N with common bean and these include

Rhizobium leguminosarum biovar *phaseoli*, *Rhizobium tropici* and *Rhizobium etli*. In Sub-Saharan Africa (SSA), most soils are N deficient and rhizobia bacteria have the potential to increase yields at lower production costs. Nodulation and response to rhizobia inoculation is mainly dependant on the genetic makeup of the variety, compatibility of the bacteria and the variety. Other factors that reduce nodulation include high N supplementation, soil dryness as well as population of native rhizobia that maybe existing in the soils (Graham, 1981; Giller and Cadisch, 1995). The use of N and P fertilizers in growing common bean is not a common practice by Zimbabwean farmers but this management practice has the potential to increase BNF benefits from common bean as P is known to promote root growth and starter N helps in initial growth (Rahman et al. 2008).

Grain legumes are an important source of calories and dietary protein for the poor within SSA.

About 80% of the total protein consumed by humans in SSA comes from plants (Singh et al. 1991). The primary nutritional problem in SSA is lack of dietary protein also called Protein calorie malnutrition (PCM). Lack of micronutrient intake is also prevalent causing an overall malnutrition among children

and women of reproductive age (Nestel et al. 2006). Common bean is one of the most important grain legumes that provide protein and minerals for direct human consumption. Common bean has the potential to lessen malnutrition problems. The grain is rich in protein (20-28%), energy (32%), fibre (56%) and micronutrients especially iron (70 mg/kg), zinc (33 mg/kg) and vitamin A (Beebe et al. 2000; Welch et al. 2000; Islam et al. 2002). The smallholder populations' diet is mainly composed of legumes as a cheap and affordable source of protein. Common bean seeds are known to contain carbohydrates, proteins, vitamins and minerals and in addition to these nutritional components, the seed may also contain anti-nutritional factors such as polyphenols, tannins and phytates, among others (Frossard et al. 2000; Petry et al. 2010). Several studies have shown that these anti-nutritional factors bind to some essential minerals making them unavailable for absorption in the gastrointestinal tract (Vasic et al. 2012). Polyphenols and plant phytates play an essential role in biological functions of the plant such as provision of phosphorus, protection against pathogens and UV radiations (Lott et al. 2000; Petry et al. 2010). Although the plants need the polyphenols and phytates, these compounds tend to inhibit non-heme iron absorption. In recent years, increased research has been done to combat micronutrient deficiencies in SSA (Smith and Akinbamijo, 2000; Zimmermann and Hurrell, 2002). Iron and zinc deficiencies are common in these cereal-based populations with approximately 2 billion people in the world being iron deficient. Zinc is an important component of enzymes for digestion and metabolism, vital for normal growth and immune system. Most research advances on nutritional value of common bean are mainly through processing and bio-fortification. There is a scarcity of information on the improvement of common bean nutritional value through management practices at farm level.

With all these phenomena explored, the aim of this study was to investigate sensitivity of nutritional components of common varieties to N and P across variable soils and also to evaluate the BNF on

common bean under N, P and rhizobia inoculation. In Zimbabwe, the response of common bean to rhizobia inoculation and fertilizers for increased BNF has not been studied; the objective of this study was to examine effect of inoculation, N and P fertilizers on commonly grown varieties of common bean in Zimbabwean smallholder farmers.

5.2 Materials and methods

5.2.1 Site description and characterization

Three on-farm experiments were conducted in Domboshava (17°36'S, 31°10'E) and Murehwa (17°45'S, 31°34'E), two smallholder farming areas in Eastern Zimbabwe. Zimbabwe is divided into five agro-ecological regions with NR I receiving >1000 mm per year while NR V receives the least rainfall of <500 mm. The experimental fields lie in NR II receiving a fairly reliable rainfall ranging from 750-1000 mm/year in a unimodal season from November to March. Rainfall data and long term averages for the two cropping season are shown in Figure 3.1.

Before experiment establishment, composite soil samples containing five sub-samples by diagonal sampling of the fields were taken from the plough layer (0-20 cm depth) and bulked for each of the three sites. The soil samples were air-dried and passed through a 2 mm sieve for total N, available P and extractable bases analysis. Samples for soil organic carbon (SOC) analysis were passed through a 0.5 mm sieve and total SOC was determined by the modified Walkley-Black)

(Okalebo et al. 2002). Total N was determined by the micro-Kjeldahl method while available P was determined by the Olsen method (Anderson and Ingram, 1993). Extractable bases (Ca, Mg and K) were extracted using ammonium acetate. Potassium (K) was determined by flame photometry, and calcium (Ca) and magnesium (Mg) concentrations were determined by atomic absorption spectrophotometry.

Soil pH was determined using the 1:10 water method and soil texture was determined using the hydrometer method (Gee and Bauder, 1986). The physical and chemical properties of soils for the three field sites are presented in Table 3.1.

5.2.2 Experimental design and management

Treatments were designed to investigate the influence on N, P and rhizobia inoculation on N₂fixation and common bean nutritional components. The experiments were laid out in a split plot arranged in randomized complete block design replicated in three blocks, in each field. The main plot consisted of fertilizer management [control, N, P and NP] and subplot combinations were variety and rhizobia inoculation. The resulting treatments were as follows as follows:

- i) Control (no fertilizer or rhizobia added)
- ii) I (rhizobia inoculation only)
- iii) P (single super phosphate only (19% P₂O₅))
- iv) N (ammonium nitrate only (34.5% N))
- v) NP (ammonium nitrate + single super phosphate)
- vi) NPI (ammonium nitrate + single super phosphate + rhizobia inoculant)

Land preparation was done using ox-drawn plough to establish 4 m × 4 m plots. Two improved common bean cultivars, Gloria and NUA45 widely grown in Zimbabwe were planted with inter- row spacing of 45 cm and intra- row spacing of 10 cm to give a plant population of 222 000 plants ha⁻¹. Two seeds were planted per station and thinning to one seed per station was carried out two weeks after crop emergence. The plots were kept weed-free by hand-hoeing throughout the growing season. Common bean inoculant

(*Rhizobium tropici* strain CIAT899) was obtained locally from Soil Productivity Research Laboratory in Marondera. Both N and P were applied at 20 kg ha⁻¹ at planting. N top dressing was initially targeted at 60 kg ha⁻¹ but due to a prolonged mid-season drought N was added at a rate of 40 kg N ha⁻¹ at flowering stage for the treatments receiving N.

9 **5.3.3 Estimation of N₂-fixation**

10 Determination of BNF was done in control, I, P, N, NP and NPI from one site using the ¹⁵N natural
11 abundance method (Peoples et al. 1989). *Bidens pilosa* from adjacent paths was used as non-fixing
12 reference plants. Whole common bean plants were uprooted in a 1.8 m x 1.8 m sampling plot. The
13 roots were washed in distilled water to remove all the soil. The samples were dried in an oven 60
14 °C for 48 hours and ground to pass a 1 mm sieve. The samples were analyzed for %N and ¹⁵N 15
using a 20-20 stable isotope mass spectrophotometer (**Europa 20-20 CF-IRMS**), coupled to a CN
auto-analyzer and δ¹⁵N was 16 computed as:

$$17 \quad \delta^{15}\text{N} = 1000 \times \frac{(\text{atom\% sample} - 0.3663)}{0.3663}$$

18 The natural abundance ¹⁵N of the air is a constant 0.3663 atom % ¹⁵N (Hogberg, 1997). The amount
19 of N derived from N₂-fixation was calculated as:
%N₂-fixation = 100 × δ¹⁵N (reference crop) - δ¹⁵N (legume N) / δ¹⁵N (reference crop) - B, where
δ¹⁵N (reference crop) was obtained from a non-fixing reference plant *Bidens pilosa*. B is the δ¹⁵N
of the same nodulated N₂-fixing plant when grown with N₂ as the sole source of N, a measure of
isotopic fractionation during N₂- fixation. B values (-2.16) were obtained from the literature

(Unkovich et al. 2008, Peoples et al., 1989).

5.2.4 Grain nutrition analysis

Measurements of grain micronutrients, (Mn, Fe, Cu, Zn, B,), protein, trypsin and phytate were done on samples from the control, NP and P treatments across all the three sites. This was done by selecting unbroken, clean and healthy pods (fully brown). The selected pods were washed with the de-ionised water and air- dried. Seeds were removed from the pods and piled evenly on a clean stainless tray surface. The pile of seeds was flattened, spread into a circle, divided into quarter and mixing the seeds in adjacent sections. The seeds were mixed with the de-ionised water for few seconds and dried overnight at 60°C in a stainless conventional oven. The samples were milled using a 0.5 mm sieve in an analytical miller to homogenize them before analysis of micronutrients. The grain samples were burned and mineralized at 550°C for 4-6 hours and then diluted in a 1:4 hydrochloric acid solution. Micronutrients were read in a spectrometer, AAS6000, by Flame Atomic Absorption Spectrometry.

5.2.5 Statistical analysis

Analysis of variance (ANOVA) for N₂-fixation and grain nutrition data was done using Genstat version 14 after testing for the assumptions of ANOVA. Sigma plot 10 was used to plot the graphs. Fisher's Protected least significant difference (LSD) was used to compare treatment means at $p=0.05$.

5.3 Results

5.3.1 N₂-fixation by common bean

The $\delta^{15}\text{N}$ signatures for *Bidens pilosa* the non-fixing control was 5.9 (ratio of $^{15}\text{N}:^{14}\text{N}$). Some of the treatments had $\delta^{15}\text{N}$ higher than the non-fixing control and for these treatments %N₂-fixed was zero. Analysis of variance was done and neither varietal nor treatment differences were observed. However, the highest proportion of N₂-fixed was due to rhizobia inoculation (22%). Although +inoculation treatment showed the highest rate of N₂-fixation, it did not contribute much to the biomass at peak flowering as compared to the N and NP treatments (Table 5.1).

1

Table 5.1: Estimates of % N₂-fixed derived from N₂-fixation by common bean as determined by the ¹⁵N abundance method using *Bidens pilosa* as the reference plant as a function of nutrient management and rhizobia inoculation at peak flowering.

Treatments	δ ¹⁵ N	N ₂ -fixed (%)	Total biomass (kg ha ⁻¹)	Total biomass N (kg ha ⁻¹)	Total N from BNF (kg ha ⁻¹)
Control	4.7	9	448	11	1.0
Inoculation	3.8	22	482	9	2.0
Phosphorus	4.9	8	603	13	1.1
Nitrogen	3.9	18	1050	24	4.3
NP	4.9	7	980	20	1.4
+Nitrogen + phosphorus +inoculation	4.2	13	1152	29	3.8

B value was -2.16 obtained from Peoples et al. (1989). Control = no inoculum or fertilizer added, + Inoculation = +inoculum, Nitrogen added at 40kg ha⁻¹, Phosphorus added at 20 kg ha⁻¹, + inoculum. Total biomass N = %N in sample x total biomass determined at peak flowering (kg/ha). Total N from BNF= %N₂fixed x Total biomass N

5.3.2 Effect of N and P on common bean protein and micronutrients

Analysis of variance was done separately according to the SOC content. In a degraded site with <0.6% SOC no significant differences between sites, treatments or varieties were observed in all the measured parameters of common bean. Combined ANOVA in non-degraded soils with >0.7 % SOC, Fe was not significantly affected by any of the tested factors (N,P and rhizobia inoculation. (Table 5.2). Similar results were also observed on protein, phytate and trypsin content. Protein ranged from 20.6% to 22.5% whilst trypsin ranged from 1.6 mg g⁻¹ to 2.5 mg g⁻¹. Boron was not detectable in 70% of the tested samples. The protein content of these two varieties ranged between 19.5% and 30.3 %. Similar results were observed across all sites where phytate content N and P had no significant effects on both varieties. Grain phytate content ranged from 1.9 mg g⁻¹ to 2 mg g⁻¹ among the control, NP and P treatments.

1

Table 5.2: Descriptive statistics for nutritional components in dry common bean grain as affected by fertilizers at physiological maturity

2 in non-degraded sites with SOC>0.6%.

Treatment	Cu	Fe	Mn	Zn	Protein	Phytate	Trypsin
		mg kg ⁻¹				mg g ⁻¹	
Control	.0 _a	46.5 ^a	21.7 ^a	45.8 ^a	22.5 ^a	1.9 _a	2.5 _a
NP	6.4 _a	43.9 ^a	16.6 ^a	59.2 ^b	22.4 ^a	2.0 _a	1.6 _a
P	6.7 _a	56.2 ^a	16.2 ^a	56.8 ^b	20.6 ^a	1.6 _a	2.5 _a
LSD	1.6	13.2	7.6	10.0	3.6	0.4	1.4
CV %	27.9	31.8	49.7	22.0	19.7	22.3	74.2

3 Values followed by the same letter are not significantly different according to LSD test (p=0.05). Control = no fertilizer added, P = 4 phosphorus at 20 kg ha⁻¹. NP = nitrogen at 40 kg ha⁻¹ and phosphorus at 20 kg ha⁻¹.

5

Significant varietal differences were observed on grain Cu concentration ($p=0.008$) with NUA 45 variety showing a higher content of 7.6 mg kg^{-1} as compared to Gloria which had 5.8 mg kg^{-1} (Table 4). Variety was also significantly different in grain Mn content ($p<0.001$) and Zn ($p<0.001$) concentration where NUA 45 had a higher Mn concentration of 24.8 mg kg^{-1} than 11.6 mg kg^{-1} observed in Gloria. However, a contrast was observed as NUA 45 had lower Zn concentration (39.9 mg kg^{-1}) compared to 67.9 mg kg^{-1} found in Gloria (Table 5.3).

Table 5.3. Varietal differences of common bean on Cu, Mn and Zn concentration in a non-degraded soil with SOC >0.7%

Variety	Cu (mgkg ⁻¹)	Mn(mgkg ⁻¹)	Zn (mg kg ⁻¹)
Gloria	5.8	11.6	67.9
NUA 45	7.6	24.8	39.9
p	0.008	<0.001	<0.001
LSD	1.29	6.24	8.19
CV %	27.9	49.7	22.0

Significant treatment differences were observed in the grain Zn concentration ($p < 0.001$). Addition of NP significantly increased Zn relative to the control from 45.8 mg kg^{-1} in the control to 59.2 mg kg^{-1} whilst P increased it to 56.8 mg kg^{-1} (Figure 5.1). However, there was no significant difference between NP and P treatments. Variations in Mn content were also observed between the two non-degraded sites. Madziva had 12.7 mg kg^{-1} as compared to Chawonza which had 23.7 mg kg^{-1} .

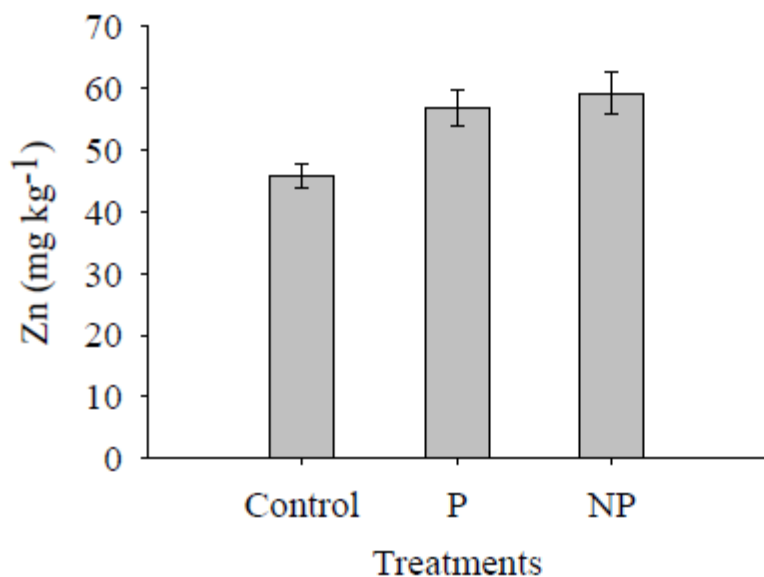


Figure 5.1: Zinc concentration as affected by nitrogen at 40 kg ha^{-1} and phosphorus at 20 kg ha^{-1} fertilization in non-degraded soils in Eastern Zimbabwe

5.4 Discussion

5.4.1 Biological nitrogen fixation

The results of this study showed that common bean varieties Gloria and NUA 45 did not fix N under N, P and rhizobia inoculation. Rhizobia inoculation in common bean only fixed 22 % N₂ which is consistent with research done by Mnasri et al. (2007). Common bean is known to be poor fixer of nitrogen compared to other legume crops. Our N₂-fixation estimates in N treatments were expected to be as low as the control treatment as N is known to prevent BNF but strangely they were similar to the rhizobia inoculation only treatment. This could be due to errors in data collection and variations in soil properties. Phosphorus was also expected to have higher nitrogen fixation than N as P improves root growth hence higher BNF. Tsai et al. (1993) found that high N rates inhibited nodulation and only 30 kg ha⁻¹ N₂ was biologically fixed under 10 kg ha⁻¹ N application. Although inoculation with rhizobia did not give a positive response statistically, visible results of inoculum increasing N fixed from 9% in the control to 22% in the + inoculation treatment were observed (Table 5.1). However, the inoculum on its own produced small amounts dry biomass and consequently less grain yields indicating that it's not sensible for farmers to rely on rhizobia inoculation on these varieties for improved productivity.

5.4.2. Effect of N and P on common bean protein, trypsin, phytate and micronutrients

Shellie-Dessert et al. (1991), reported that the quality of common bean grain is mostly determined by the protein content, digestibility, and presence of anti-nutritional factors and grain mineral content. Half a cup or 90 g of cooked common bean supplies approximately 7 to 8 g protein

(Messina, 1999). Although no significant differences were found in this study, Barampama and Simard, 1993 found a mean protein content of 22.26% in four varieties of common bean which is similar to our findings of 24.5% protein content. Our results are also similar to 20% and 30% reported by Shellie-Dessert et al. (1991) and also agree with previously reported values of common bean protein content (Moraghan and Grafton, (1997); Guzmán-Maldonado et al. 2000; 2003).

Common bean grain stores phosphorus in the form of phytate or phytic acid which has been known as an anti-nutrient, as it binds proteins and minerals, leading to reduced bio-availability (Coelho et al. 2002; Vucenik and Shamsuddin, 2003; Chen, 2004). Phytate content is mainly affected by the genotype as well as processing techniques such de-hulling (Deshpande et al. 1982; Ugen et al. 2012) and the values obtained in this study are lower than values reported by Vasic et al. (2012) and Kumar et al. (2010) whose study showed a mean phytate content of 8.095 mg/g in different common bean cultivars.

Compared to other food proteins, common bean has been reported to have low digestibility due to trypsin inhibitors. Addition of N and P fertilizers did not affect the grain trypsin content. Further fertilization with N makes the trypsin to increase even more as Peric et al. (2009) found that addition of N at different level reduced the trypsin inhibitor content in soybean genotypes.

In this study N and P showed no effect on grain B and Fe content of common bean suggesting that N and P possibly reduce the translocation of B and Fe to the grain of common bean. Micronutrient uptake by plants is carried out by multiple transporters which transport more than one element.

This phenomenon of having similar uptake mechanisms leads to antagonism and this could be the reason why B content was low (Grotz and Guerinot, 2006).

A positive effect of P fertilizer was observed on grain Zn content. Genotypic variations in grain observed between the two varieties are similar to results obtained by other researchers. Bean varieties show variability in Fe content ranging from 30 to 120 ppm and Zn content ranging from 20 to 60 ppm (Graham et al. 1999; Beebe et al. 2000; Cichy et al. 2005; Welch et al. 2000). Our results showed an Fe content which was similar although it was not affected by N and P.

Can farmers invest in phosphorus for increased yields? Yes! Can they rely on phosphorus for improved nutritional quality? Definitely no! The use of phosphorus fertilizers on common bean increases productivity in fields that are not acutely degraded but doesn't necessarily improve its nutritional components. Although grain zinc content was increased by 20 kg ha⁻¹ phosphorus, protein was not increased of which rural nutrition mainly suffers from protein malnutrition. Common bean nutrition can be improved only if anti-nutritional factors are reduced and micronutrients increased.

5.5. Conclusions

Rhizobia inoculation, nitrogen and phosphorus application did not fix N in common bean. Farmers cannot rely on these fertilizers and inoculum for improved biological nitrogen fixation in common bean as it is a poor fixer. Grain Cu, Mn and Zn content varies among different varieties. Nitrogen and phosphorus application does not influence protein, phytate, trypsin, Fe, Mn, Cu and B content. Farmers can apply 20 kg ha⁻¹ P for increased zinc content.

CHAPTER 6

OVERALL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1 Introduction

The main objective of this study was to improve productivity and nutritional quality of common bean through the use of rhizobia inoculation, nitrogen and phosphorus fertilizers in smallholder farms in Zimbabwe. Specifically, the objectives of this study were to: determine the common bean yield and yield parameters when applied with rhizobia inoculation, N and P fertilizers across variable soils (Chapter 4); to determine the amount of N₂-fixed when applied with rhizobia inoculation, N and P fertilizers (Chapter 5). Chapter 5 also addresses the influence of N and P fertilizers on common bean nutritional quality. The study presented results of common bean yields, biological nitrogen fixation and common bean protein, phytate and micronutrient content as affected by N and P fertilizers. This chapter summarises the main findings and conclusion of the study as well as highlighting further research recommendations for farmers and future researchers.

6.2 Influence of rhizobia inoculation, nitrogen and phosphorus application on common bean productivity

The main objective of the study was to investigate the influence of rhizobia inoculation, nitrogen and phosphorus application on common bean yield and yield parameters across variable soils. This was hinged on the hypothesis that rhizobia inoculation, N and P significantly increases common bean grain yield and yield parameters. The results of the study showed that N and P can be used to significantly increase common bean dry biomass and grain yield at the rates 20 kg ha⁻¹P and 40 kg ha⁻¹N, but only for non-degraded soils. Grain yield was significantly increased by co-application

of N and P from 0.27 Mg ha⁻¹ to 1.48 Mg ha⁻¹ during the first year and from 0.37 Mg ha⁻¹ to 2.09 Mg ha⁻¹ during the second season. During the second season, non-significant differences were obtained in degraded sites with grain yields changing from 0.09 to 0.19 Mg ha⁻¹ in year 1 and from 0.16 to 0.28 Mg ha⁻¹. Nitrogen and phosphorus also significantly increased common bean dry biomass in non-degraded soils from a maximum of only 0.17 Mg ha⁻¹ under degraded soils to 1.2 Mg ha⁻¹ for non-degraded soils. Similar findings were reported by Da Silva et al. (1993) and Tang et al. (2001) which clearly show that N and P increase common bean dry biomass and grain yields. Nitrogen had a significant effect on pod loading, number of seeds per pod, and yields. No clear benefits of co-application of N or P were observed as both nutrients had comparable effects on common bean productivity.

6.2 Effect of rhizobia inoculation, N and P fertilizers on common bean N₂-fixation

This second hypothesis for the study was that rhizobia inoculation, N and P significantly improves nodulation and biological nitrogen fixation in common bean. The results of this study showed that on P significantly nodulation of common bean but did not fix N₂. No response to N and rhizobia inoculation was observed on both nodulation and the amount of nitrogen fixed. Other studies have shown that P increases nodulation in common bean (Leidi and Rodriguez-Navarro, (2000); Tang et al. 2001). This comes about as a result of the ability of P in improving early root formation and root growth thereby and enhancing common bean productivity. Common bean has been known to be a poor fixer of nitrogen and the results of our study are consistent with research done by Mnasri et al. (2007).

6.3 Influence of N and P on common bean nutritional quality

Another hypothesis was that N and P fertilizers have the ability to improve common bean nutritional quality. Results were partly supportive of this hypothesis as they showed that P improves grain Zn content from 45.8 to 56.8 mg kg⁻¹. Although zinc concentration increased by adding P at a rate of 20 kg ha⁻¹, it would have been more desirable if protein was also increased as many rural populations suffer from protein malnutrition. However, the amount of protein, phytate, trypsin, Cu, Mn and Fe were not affected by the addition of either N or P. Other differences in Zn, Mn and Cu were due to the variety factor where it showed that NUA 45 is superior in grain Cu and Mn concentration whereas Gloria has higher Zn content. Common bean grain quality is determined by the presence or absence of anti-nutritional factors phytic acid and trypsin. These anti-nutritional factors bind to nutrients (reduced bio-availability) in the gastrointestinal. (Vucenik and Shamsuddin, 2003; Chen, 2004).

6.6 Conclusions

According to the results of this study, common bean does not perform well in soils with <0.6% SOC. Nitrogen and phosphorus fertilizers can be used to increase common bean yields and micronutrients in smallholder farms. The addition of nitrogen and phosphorus fertilizers increase the concentration of Zn and Mn in the common bean grain. Common bean does not respond to rhizobia inoculation that is available on the market. The use of phosphorus fertilizers on common bean increases productivity in fields that are not acutely degraded however, it does not necessarily improve its nutritional components. Farmers must apply nitrogen and phosphorus fertilizers to common bean crop but targeting fields with soil organic carbon > 0.6% for increased yields. Application of N or P had equal

magnitude of increasing common bean grain yields with no significant benefits of adding both elements.

6.7 Recommendations

6.7.1 Recommendations for smallholder farmers

Based on results of this study, farmers must improve the soils organic carbon content of their fields by adding fertilizers and manure before they grow common bean as it has proved to underperform in degraded soils (<0.6% SOC). Smallholder farmers need to apply at least 20 kg ha⁻¹ of phosphorus in common bean in order to realize grain yield benefits as well as improved nutritional quality. Farmers must avoid investing in nitrogen for common bean production as it does not increase grain yields that are significantly from phosphorus. Rhizobia inoculation should be restricted to soya beans as common bean does not respond to the locally available inoculum. Farmers should apply P fertilizers in common bean in order to realize increased grain yields and Zn concentration. There is no benefit of adding both N and P although N also has an equal magnitude of increasing grain yields.

6.7.2 Further research

There is need to conduct research with other common bean varieties and or other strains that are available on the market and assess their performance. Similar research also needs to be done across all regions in Zimbabwe to give accurate recommendations best suited to the area. Other methods of improving common bean nutritional quality such as bio-fortification need to be explored using local varieties as our results showed little to no effect of fertilizers on protein and micronutrient

1 concentration. Biological nitrogen fixation capabilities of common bean needs to be investigated
2 more so that varieties that have the highest capacity to fix N are obtained and made available to 3
the farmers. These investigations will have to include isolation and testing of indigenous
rhizobia bacteria and assessing their N-fixing capacity as well as their specificity to common bean.

REFERENCES

- Anderson, J.M., Ingram, J.S.I. eds., 1993. Tropical Soil Biology and Fertility: A Handbook of method. CAB International.
- Araujo, A. P., Teixeira, M.G., and Almeida, D., 2000. Growth and yield of common bean cultivars at two soil phosphorus levels under biological nitrogen fixation. *Pesquisa Agropecuária Brasileira*, Brasília, 35 (4), 809-817.
- Barampama, Z., and Simard, R.E., 1993. Nutrient composition, protein quality and antinutritional factors of some varieties of dry beans (*Phaseolus vulgaris*) grown in Burundi. *Food Chemistry*, 47(2), 159-167.
- Barbosa Filho, M.P., and da Silva, O.F., 1994. Agroeconomic aspects of liming and fertilization in rice and bean crops irrigated by sprinkling. *Pesquisa Agropecuária Brasileira*, 29 (11), 1657-1667.
- Bationo, A., 2004. Managing Nutrient Cycles to Sustain Soil Fertility in Sub- Saharan Africa. TSBF-CIAT. Academy Science Publishers, Nairobi, Kenya.
- Beebe, S.E., Rao, I. M., Mukankusi, C., Buruchara, R., 2012. Improving resource use efficiency and reducing risk of common bean production in Africa, Latin America, and the Caribbean. Centro Internacional de Agricultura Tropical (CIAT).
- Beebe , S., Gonzalez A. V., Rengifo, J., (2000). Research on traceminerals in the common bean. *Food Nutrition Bulletin*, 21, 387–391.

- Beebe, S., Skroch, P.W., Tohme, J., Duque, M.C., Pedraza, F., and Nienhuis, J., 2000. Structure of genetic diversity among common bean landraces of Middle American origin based on correspondence analysis of RAPD. *Crop Science*, 40(1), 264-273.
- Broughton, W.J., Hernandez, G., Blair, M., Beebe, S., Gepts, P., and Vanderleyden, J., 2003. Beans (*Phaseolus* spp.)—model food legumes. *Plant and Soil*, 252, 55-128.
- Broughton, W.J., Hernandez, G., Blair, M., Beebe, S., Gepts, P., and Vanderleyden, J., 2003. Beans (*Phaseolus* spp.)—model food legumes. *Plant and Soil*, 252, 55-128.
- Chen, Q., 2004. Determination of phytic acid and inositol pentakis phosphates in foods by highperformance ion chromatography. *Journal of Agricultural and Food Chemistry*, 52(15), 4604-4613.
- Chianu, J.N., Nkonya, E.M., Mairura, F.S., Chianu, J.N., and Akinnifesi, F.K., 2011. Biological nitrogen fixation and socioeconomic factors for legume production in Sub-Saharan Africa. *Agronomy and Sustainable Development*, 31,139-154.
- Chikowo, R., Tagwira, F., and Piha, M., 1999. Agronomic effectiveness of poor quality manure supplemented with phosphate fertilizer on maize and groundnut in a maize-groundnut rotation. *African Crop Science Journal*, 7(4), 383-395.
- CIAT (Centro Internacional de Agricultura Tropical), 1992. Constraints to and opportunities for improving bean production. A planning document 1993–98. An achievement document 1987–92, CIAT, Cali Colombia.

- Cichy, K.A., Forster, S., Grafton, K.F., and Hosfield, G.L., 2005. Inheritance of seed zinc accumulation in navy bean. *Crop Science*, 45 (3), 864-870.
- Coelho, C.M.M., Santos, J.C.P., Tsai, S.M., and Vitorello, V.A., 2002. Seed phytate content and phosphorus uptake and distribution in dry bean genotypes. *Brazilian Journal of Plant Physiology*, 14(1), 51-58.
- Cordell, D., Drangert, J.O., and White, S., 2009. The story of phosphorus: Global food security and food for thought. *Global Environmental Change*, 19, 292–305.
- Cordell, D., Rosemarin, A., Schröder, J.J. and Smit, A.L., 2011. Towards global phosphorus security: A systems framework for phosphorus recovery and reuse options. *Chemosphere*, 84(6), pp.747-758.
- Da Silva, P. M., Tsai, S. M., and Bonetti, R., 1993. Response to inoculation and N fertilization for increased yield and biological nitrogen fixation of common bean (*Phaseolus vulgaris* L.) :In *Enhancement of biological nitrogen fixation of common bean in Latin America*. Springer, Netherlands, pp 123-130.
- de Faria Müller, B.S., Sakamoto, T., Silveira, R.D.D., Zambussi-Carvalho, P.F., Pereira, M., Pappas, G.J., do Carmo Costa, M.M., Guimarães, C.M., Pereira, W.J., Brondani, C., and Vianello-Brondani, R.P., 2014. Differentially expressed genes during flowering and grain filling in common bean (*Phaseolus vulgaris*) grown under drought stress conditions. *Plant molecular biology reporter*, 32(2), 438-451.
- Deshpande, S.S., Sathe, S.K., Salunkhe, D.K., and Cornforth, D.P., 1982. Effects of dehulling on phytic acid, polyphenols, and enzyme inhibitors of dry beans (*Phaseolus vulgaris* L.). *Journal of Food Science*, 47(6), 1846-1850.

- Epping, B., Hansen, A., Djilaji, B., Martin, P., 1994. Symbiotic efficiency of four *Phaseolus vulgaris* genotypes after inoculation with different strains of *Rhizobium* under controlled conditions. *Z. Naturforsch* 49, 343-351.
- Fageria, N.K., and Baligar, V.C., 2016. Growth, yield and yield components of dry bean as influenced by phosphorus in tropical acid soil. *Journal of Plant Nutrition*, 3 (4), 562-568.
- Fageria, N. K. and Moreira, A. 2011. The role of mineral nutrition on root growth of crop plants. *Advances in Agronomy*, 110, 251–332.
- Fageria, N.K., and Baligar. V.C., 2005. Enhancing nitrogen use efficiency in crop plants. *Advances in Agronomy* 88,97–185.
- Fageria, N.K., Baligar, V.C., and Jones, C.A. 2011. “mineral nutrition of field crops”. In *Growth and*, 3rd, Boca Raton, Fl.: CRC Press.
- Frossard, E., Bucher, M., Mächler, F., Mozafar, A., and Hurrell, R., 2000. Potential for increasing the content and bioavailability of Fe, Zn and Ca in plants for human nutrition. *Journal of the Science of Food and Agriculture*, 80 (7), 861-879.
- Garden-Robinson, J., and McNeal, K., 2013. Nutrition, health benefits, preparation and use in menus. North Dakota State University Extension Service, North Dakota, USA.
- Gee, G.W., and Bauder, J.W., 1986. Particle size analysis In: Klute, A. (Ed) *Methods of Soil Analysis*, Part 1. Am. Soc. Agron., Madison, WI. pp. 383–411.

Gidago, G., Beyene, S., Worku, W., and Sodo, E., 2011. The response of haricot bean (*Phaseolus vulgaris* L.) to phosphorus application on Ultisols at Areka, Southern Ethiopia. *Journal of Biology, Agriculture and Healthcare*, 1(3), 38-49.

Giller, K.E., 1990. Assessment and improvement of nitrogen fixation in tropical *Phaseolus vulgaris* L. *Soil Use Manag.* 6, 82-84.

Giller, K.E., 2001. Nitrogen fixation Giller, K.E., Amijee, F., Brodrick, S.J., and Edje, O.T., 1998. Environmental constraints to nodulation and nitrogen fixation of *Phaseolus vulgaris* L. in Tanzania II. Response to N and P fertilizers and inoculation with *Rhizobium*. *African Crop Science Journal*, 6, 171-178. in *tropical cropping systems*, 2nd Edition. CAB International, Wallingford, UK.

Giller, K. E., Amijee, F., Brodrick, S. J., Edje, O. T., 1998. Environmental constraints to nodulation and nitrogen fixation of *Phaseolus vulgaris* L. in Tanzania II. Response to N and P fertilizers and inoculation with *Rhizobium*. *Afri. Crop Sci. J.* 6, 171-178.

Giller, K.E., and Cadisch, G., 1995. Future benefits from biological nitrogen fixation: an ecological approach to agriculture. In *Management of Biological Nitrogen Fixation for the Development of More Productive and Sustainable Agricultural Systems*. Springer Netherlands, pp. 255-277 Graham, P.H., 1981. Some problems of nodulation and symbiotic nitrogen fixation in *Phaseolus vulgaris* L.: a review. *Field Crops Research*, 4, 93-112.

Graham, R., Senadhira, D., Beebe, S., Iglesias, C., and Monasterio, I., 1999. Breeding for micronutrient density in edible portions of staple food crops: conventional approaches. *Field Crops Research*, 60 (1), 57-80.

- Grotz, N., and Guerinot, M. L., 2006. Molecular aspects of Cu, Fe and Zn homeostasis in plants. *Biochemica et Biophysica Acta–Molecular Cell Research*, 1763, 595–608.
- Guzmán-Maldonado, S.H., Acosta-Gallegos, J. and Paredes-López, O., 2000. Protein and mineral content of a novel collection of wild and weedy common bean (*Phaseolus vulgaris* L). *Journal of the Science of Food and Agriculture*, 80(13), pp.1874-1881.
- Hardarson, G., Bliss, F.A., Cigales-Rivero, M.R., Henson, R.A., Kipe-Nolt, J.A., Longeri, L., Manrique, A., Pena-Cabriales, J. J., Pereira, P.A.A., Sanabria, C.A., and Tsai, S.M., 1993. Genotypic variation in biological nitrogen fixation by common bean. In *Enhancement of Biological Nitrogen Fixation of Common Bean in Latin America*. Springer Netherlands, pp. 59-70.
- Harika, R., Faber, M., Samuel, F., Kimiywe, J., Mulugeta, A. and Eilander, A., 2017. Micronutrient status and dietary intake of iron, vitamin A, iodine, folate and zinc in women of reproductive age and pregnant women in Ethiopia, Kenya, Nigeria and South Africa: a systematic review of data from 2005 to 2015. *Nutrients*, 9(10), p.1096.
- Henson, R.A. and Bliss, F.A., 1991. Effects of N fertilizer application timing on common bean production. *Fertilizer research*, 29(2), pp.133-138.
- Hikwa, D., and Jiri, O., 2002. Simplified Production Fact Sheet on Selected Field Grown in Zimbabwe. Agronomy Research Institute, Harare, Zimbabwe.
- Högberg, P., 1997. Tansley review no. 95 15 N natural abundance in soil–plant systems. *The New Phytologist*, 137(2), pp.179-203.

- Hungria, M., Vargas, M.A., 2000. Environmental factors affecting N₂ fixation in grain legumes in the tropics, with an emphasis on Brazil. *Field Crop. Res.* 65, 151-164.
- Islam, F.M.A., Basford, K.E., Jara, C., Redden, R.J., and Beebe, S.E., 2002. Seed compositional and disease resistance differences among gene pools in cultivated common bean. *Genetic Resources and Crop Evolution*, 49, 285–293.
- Kambewa, P.S., 1997. The Bean Subsector in Malawi: Historical developments, Current Status, and Policy Issues (No. 1097-2016-88705).
- Kasasa, P., Mpeperekwi, S., Musiyiwa, K., Makonese, F., and Giller, K.E., 1999. Residual nitrogen benefits of promiscuous soyabeans to maize under field conditions. *African Crop Science Journal*, 7: 375–382.
- Kumar, V., Sinha, A.K., Makkar, H.P., and Becker, K., 2010. Dietary roles of phytate and phytase in human nutrition: A review. *Food Chemistry*, 120(4), 945-959.
- Leidi, E.O. and RODRÍGUEZ-NAVARRO, D.N., 2000. Nitrogen and phosphorus availability limit N₂ fixation in bean. *The New Phytologist*, 147(2), pp.337-346.
- Liao, H., Yan, X., Rubio, G., Beebe, S.E., Blair, M.W. and Lynch, J.P., 2004. Genetic mapping of basal root gravitropism and phosphorus acquisition efficiency in common bean. *Functional Plant Biology*, 31(10), pp.959-970.

- Lott, J.N., Ockenden, I., Raboy, V., and Batten, G.D., 2000. Phytic acid and phosphorus in crop seeds and fruits: a global estimate. *Seed Science Research*, 10(01), 11-33.
- Madamba, R., and Pompei I., 2004. Common bean production in Zimbabwe. Crop Breeding Institute, ARES, Harare, Zimbabwe.
- Madamba, R., Mwashireni, A., and Makunde, G., 2003. A Guide to Pulse Production in Zimbabwe. Ministry of Lands, Agriculture and Rural Resettlement. Department of Research and Extension Zimbabwe Government, Harare.
- Manjeru, P., Madanzi, T., Makedredza, B., Nciizah, A. and Sithole, M., 2007. Effect of water stress at different growth stages on components and grain yield of common bean (*Phaseolus vulgaris* L.). In African Crop Science Conference Proceedings (Vol. 8, pp. 299-303).
- Marschner, P. and Rengel, Z., 2012. Nutrient availability in soils. In Marschner's mineral nutrition of higher plants (pp. 315-330). Academic Press.
- Martinez-Romero, E., 2003. Diversity of *Rhizobium-Phaseolus vulgaris* symbiosis: overview and perspectives. *Plant and Soil*, 252 (1), 11-23.
- Messina, M.J., 1999. Legumes and soybeans: overview of their nutritional profiles and health effects. *The American journal of clinical nutrition*, 70(3), 439s-450s.
- Morad, M., Sara S., Alireza, E., Reza, C. M., and Mohammad, D., 2013. Effects of seed inoculation by *Rhizobium* strains on yield and yield components in common bean cultivars (*Phaseolus vulgaris* L.). *International Journal of Biosciences*, 3, 134-141.

- Moraghan, J.T., and Grafton, K., 1997. Accumulation of calcium in bean cultivars differing in seed size. *Journal of the Science of Food and Agriculture*, 74(2), 251-256.
- Mpepereki, S., Javaheri, F., Davis, P. and Giller, K.E., 2000. Soyabeans and sustainable agriculture: promiscuous soyabeans in southern Africa. *Field crops research*, 65(2-3), pp.137-149.
- Ndakidemi, P.A., Dakora, F.D., Nkonya, E.M., Ringo, D. and Mansoor, H., 2006. Yield and economic benefits of common bean (*Phaseolus vulgaris*) and soybean (*Glycine max*) inoculation in northern Tanzania. *Australian Journal of Experimental Agriculture*, 46(4), pp.571-577.
- Nestel, P., Bouis, H.E., Meenakshi, J.V., and Pfeiffer, W., 2006. Biofortification of staple food crops. *The Journal of nutrition*, 136(4), 1064-1067.
- Okalebo, J. R., Gathna, K. W., and Woome, P. L., 2002. *Laboratory methods for soil and plant analysis: A working manual*. 2nd ed. Nairobi: Tropical Soil Fertility and Biology Program.
- Peoples, M.B., Faizah, A.W., Rerkasem, B., and Herridge, D.F., 1989. *Methods for evaluating nitrogen fixation by nodulated legumes in the field*. Monographs.
- Perić, V., Srebrić, M., Jankuloski, L., Jankulovska, M., Žilić, S., Kandić, V., and Mladenović-Drinić, S., 2009. The effects of nitrogen on protein, oil and trypsin inhibitor content of soybean. *Genetika*, 41(2), 137-144.

- Petry, N., Egli, I., Zeder, C., Walczyk, T., and Hurrell, R., 2010. Polyphenols and phytic acid contribute to the low iron bioavailability from common beans in young women. *The Journal of nutrition*, 140(11), 1977-1982
- Rafat, M., and Sharifi, P., 2015. Green Global Foundations©.
- Rahman, M.M., Bhuiyan, M.M.H., Satradhar, G. N. C., and Paul, A. K., 2008. Effect of phosphorus, molybdenum and Rhizobium inoculation on yield and yield attributes of mungbean. *International Journal of Sustainable Crop Production*. 3(6), 26-33.
- Ramakrishna, A., Gowda, C.L.L. and Johansen, C., 2000. Management factors affecting legumes production in the Indo-Gangetic Plain. International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Andhra Pradesh, India, pp. 156-165.
- Rathke, G.W., Behrens, T., and Diepenbrock W., 2006. Integrated nitrogen management strategies to improve seed yield, oil content, and nitrogen efficiency of winter oilseed rape (*Brassica napus* L.): A review. *Agriculture, Ecosystems and Environment* 117, 80–108.
- Rebeschini, A.C., de Araujo, A.S.F., and de Araujo, F.F., 2014. Nitrogen application and inoculation with *Rhizobium tropici* on common bean in the fall/winter. *African Journal of Agricultural Research*, 9(42), 3156-3163.
- Richardson, A.E., and Barea, J.M., 2009. Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. *Plant Soil*, 321, 305-339.

- Rifat, H., Safda, A., Siddique, M.T. and Chatha, T.H., 2008. Biological nitrogen fixation of summer legumes and their residual effects on subsequent rainfed wheat yield. *Pakistan Journal of Botany*, 40(2): 711-722.
- Rurangwa, E., Vanlauwe B., Giller K. E., 2017. Benefits of inoculation, P fertilizer and manure on yields of common bean and soybean also increase yield of subsequent maize. *Agric. Ecosyst. Environ.*
- Sanginga, N., 2003. Role of biological nitrogen fixation in legume based cropping systems; a case study of West Africa farming systems. *Plant and soil*, 252(1), pp.25-39.
- Sathe SK. Dry bean protein functionality. *Critical Reviews in Biotechnology*. 2002 Jan 1;22(2):175-223.
- Sattari, S. Z., van Ittersum, M.K., Bowman, A.F., Smit, A.L. and Janssen, B.H., 2014. Crop yield response to soil fertility and N, P, K inputs in different environments. *Field Crops Research*, 154, 35-46.
- Shellie-Dessert, K.C., Bliss, F.A., Schoonhoven, A.V., and Voysest, O., 1991. Genetic improvement of food quality factors. *Common beans: research for crop improvement*, 12(2), 45-60.
- Singh, R. P., Gupta, S. C., and Yadav, A. S., 2008. Effect of levels and sources of phosphorus and PSB on growth and yield of blackgram. *Legume Research*, 31(2), 139-141.
- Singh, S.P., Gepts, P. and Debouck, D.G., 1991. Races of common bean (*Phaseolus vulgaris*, Fabaceae). *Economic Botany*, 45(3), 379-396.

- Singh, S.P., H. Terán, C.G., Osorno, J.M., Takegami, J.C., and Thung, M.D.T., 2003. Low Soil Fertility Tolerance in Landraces and Improved Common Bean Genotypes. *Crop Science* 43, 110-119. pp.649-677.
- 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 and Soil*, 312, 49–57.
- Soratto, R.P., Perez, A.A., Fernandes, A.M., 2014. Age of no-till system and nitrogen management on common bean nutrition and yield. *Agron. J.* 106, 809-820.
- Smit, G.E.R.R.I.T., Kijne, J.W. and Lugtenberg, B.J., 1987. Involvement of both cellulose fibrils and a Ca²⁺-dependent adhesin in the attachment of *Rhizobium leguminosarum* to pea root hair tips. *Journal of Bacteriology*, 169(9), pp.4294-4301.
- Smith, O.B., and Akinbamijo, O.O., 2000. Micronutrients and reproduction in farm animals. *Animal Reproduction Science*, 60: 549-560.
- Tang, C., Hinsinger, P., Jaillard, B., Rengel, Z. Drevon, J.J., 2001. Effect of phosphorus deficiency on the growth, symbiotic N₂-fixation and proton release by two bean (*Phaseolus vulgaris*) genotypes. *Agronomie*, 21, 683-689.
- Soratto, R.P., Carvalho, M.A.C., and ARF, O., 2006. Nitrogen in Coverage in common bean grown in no-tillage. *Revista Brasileira de Ciência do Solo*, 30, 259-266.

- Ssali, H., and Keya, S.O., 1986. The effects of phosphorus and nitrogen fertilizer level on nodulation, growth and dinitrogen fixation of three bean cultivars. *Tropical Agriculture*.
- Tesfaye, T., and Balcha, A., 2015. Effect of phosphorus application and varieties on grain yield and yield components of common bean (*Phaseolus vulgaris* L.). *American Journal of Plant Nutrition and Fertilization Technology*, 5, 79-84.
- Tsai, S.M., Da Silva, P.M., Cabezas, W.L., and Bonetti, R., 1993. Variability in nitrogen fixation of common bean (*Phaseolus vulgaris* L.) intercropped with maize. *Plant and Soil*, 152(1), 93-101.
- Tsigie, A., Tilak, K.V. and Saxena, A.K., 2011. Field response of legumes to inoculation with plant growth-promoting rhizobacteria. *Biology and Fertility of Soils*, 47(8), p.971.
- Turuko, M., and Mohammed, A., 2014. Effect of different phosphorus fertilizer rates on growth, dry matter yield and yield components of common bean (*Phaseolus vulgaris* L.). *World Journal Agriculture Research*, 2, 88-92.
- Ugen, M., Musoni, A., Cheminingwa, G., Kimani, P., Mucharo, M., and Katafire, M., 2012. Utilization of common bean for improved health and nutrition in eastern and central Africa. ASARECA, <http://www.asareca.org>.
- Van Kessel, C. and Hartley, C., 2000. Agricultural management of grain legumes: has it led to an increase in nitrogen fixation?. *Field Crops Research*, 65(2-3), pp.165-181.
- Vanlauwe, B., Descheemaeker, K., Giller, K.E., Huising, J., Merckx, R., Nziguheba, G., Wendt, J., Zingore, S., 2015. Integrated soil fertility management in sub-Saharan Africa: unravelling

local adaptation. SOIL 1, 491-508.

- Vucenik, I. and Shamsuddin, A.M., 2003. Cancer inhibition by inositol hexaphosphate (IP6) and inositol: from laboratory to clinic. *The Journal of nutrition*, 133(11): 3778-3784.
- Wang, Y.W.B., Liu, T., Ren, X., Li, R., Zhang, M., and Yousaf, L.L., 2014. Establishment method affects oilseed rape yield and the response to nitrogen fertilizer. *Agronomy Journal*, 106, 131-42.
- Welch, R.M., House, W.A., Beebe, S., and Cheng, Z., 2000. Genetic selection for enhanced bioavailable levels of iron in bean (*Phaseolus vulgaris* L.) seeds. *Journal of Agricultural and Food Chemistry*, 48(8), 3576-3580.
- Zimmermann, M.B., and Hurrell, R.F., 2002. Improving iron, zinc and vitamin A nutrition through plant biotechnology. *Current Opinion in Biotechnology*, 13(2), 142-145.
- Zingore, S., Murwira, H. K., Delve, R.J., Giller, K.E., 2007. Soil type, management history and current resource allocation: Three dimensions regulating variability in crop productivity on African smallholder farms. *Field Crop. Res.* 101, 296-305.