

**EFFECT OF SEED GENERATION, RHIZOBIA INOCULATION AND
PLANT DENSITY ON PRODUCTIVITY AND SEED QUALITY OF
SOYBEAN [*GLYCINE MAX* (L.) Merril] AND GROUNDNUT
[*ARACHIS HYPOGAEA* (L.)] IN DEDZA AND MACHINGA
DISTRICTS OF MALAWI**

MSc. THESIS (AGRONOMY)

JESTER PAUL KALUMBA

**LILONGWE UNIVERSITY OF AGRICULTURE AND NATURAL
RESOURCES**

NOVEMBER, 2021

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DEDZA AND MACHINGA DISTRICTS OF MALAWI**

JESTER PAUL KALUMBA

BSc. (Agriculture), Malawi

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DEGREE OF MASTER OF SCIENCE IN AGRONOMY**

LILONGWE UNIVERSITY OF AGRICULTURE AND NATURAL RESOURCES

NOVEMBER, 2021

DECLARATION

I declare that this thesis is a result of my own original effort and work, and that to the best of my knowledge, the findings have never been presented to the Lilongwe University of Agriculture and Natural Resources or elsewhere for the award of any academic qualification. All sources of information have been fully acknowledged.

Jester Paul Kalumba

Signature: _____

Date: _____

CERTIFICATE OF APPROVAL

We the undersigned, certify that this thesis is a result of the author's own work, and that to the best of our knowledge, it has not been submitted for any other academic qualification within the Lilongwe University of Agriculture and Natural Resources or elsewhere. The thesis is acceptable in form and content, and that the candidate through an oral examination demonstrated satisfactory knowledge of the field covered by the thesis held on 19th July, 2021.

Major Supervisor: Dr. Wezi G. Mhango

Signature : _____

Date : _____

Supervisor: Prof. Moses F.A. Maliro

Signature : _____

Date : _____

Supervisor: Prof. Regis Chikowo

Signature : _____

Date : _____

DEDICATION

I dedicate this piece of work to my lovely mum, Ellen John. Mum, your motivation and good will made me excel in academic circles. You always said I can make it and your faith in me was source of inspiration. I will always remember and love you. May the good Lord be with you always!

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ABSTRACT

Two on-farm, researcher designed-farmer managed trials were arranged in split-plot in a randomised complete block design (RCBD) with seed generation as main plot factor while inoculation and plant density were subplot factors for soybean and groundnut trials, respectively, in Dedza and Machinga districts in the 2017/2018 season. Following the field trials, Screen house experiments were conducted at Bunda College, Crop and Soil Sciences Students research farm to evaluate the effect of mother environment on seed quality of soybean and groundnut. This study was laid out in a completely randomised design (CRD). The overall objective of these trials was to investigate effects of seed generation, inoculation, plant density and mother environment on soybean and groundnut productivity and seed quality. In Machinga, response to inoculation for BNF and grain yield of soybean was 67 % ($p=0.002$) and 27 % ($p=0.016$) higher in recycled than certified seed, respectively. In Dedza inoculation of soybean significantly increased BNF by 74 % ($p=0.004$) and grain yield by 21 % ($p=0.029$), while planting certified soybean increased grain yield by 18 % ($p=0.031$). In Dedza, planting recycled seed significantly increased BNF by 21.4 % ($p=0.011$) and grain yield by 24.2 % ($p=0.041$) while planting groundnut in twin rows increased BNF by 38.5 % ($p=0.004$) and grain yield by 145.8 % ($p<0.001$), respectively. In Machinga, planting groundnut in twin rows increased BNF by 32.5 % ($p=0.025$) and grain yield by 87 % ($p<0.001$). Because of poor germination for certified groundnut seed, a covariate analysis controlling for plant density revealed that use of certified seed increased BNF and yield of groundnut ($p<0.001$). Results of screen house experiments showed that mother environment significantly affected seed quality for both soybean and groundnut indicating that seed production site is an important element for production of quality seed.

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LIST OF ABBREVIATIONS AND ACRONYMS

ADD	Agricultural Development Division
ATP	Adenosine triphosphate
BNF	Biological Nitrogen Fixation
DAP	Days After Planting
EPA	Extension Planning Area
FAO	Food and Agricultural Organization
GLM	Generalized Linear Model
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
ISTA	International Seed Testing Association
MoAFS	Ministry of Agriculture and Food Security
PAR	Photosynthetic Active Radiation
PEG	Polyethylene glycol
QDS	Quality Declared Seed
RISING	Research in Sustainable Intensification for the Next Generation
SOM	Soil organic matter
SSU	Seed Service Unit
SVI	Seedling Vigor Index
LSD	Least Significant Difference of means

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CHAPTER ONE

INTRODUCTION

1.1 Importance of soybean and groundnut

Soybean and groundnut are important food security crops for the rural and urban populace. They contribute to household food and nutrition, income, soil health and fertility (MoAFS, 2012). In Malawi, 25 % of the agricultural income among smallholder farmers is realized from groundnut (Diop *et al.*, 2003; Chirwa, 2007). Groundnut is used to make cooking oil, salads, and margarines; groundnut kernel contains between 45 % and 55 % oil. It contains high levels of energy, fat-soluble vitamins (A, D, E and K) and essential fatty acids (Mahamood *et al.*, 2009). Soybean contains dietary fibre, minerals, vitamins, proteins (36 %), carbohydrates (30 %) and 20 % oil (FAO, 2008). Soybean compliments carbohydrate-dominated diets such as maize (FAO, 2008).

Njira *et al.* (2012) reported 55.8 kg N ha⁻¹ fixed by sole groundnut in sand soils of Kasungu district while Mhango (2011) reported 57 kg N ha⁻¹ (2011) by sole groundnut in smallholder groundnut farms in Mzimba district. Similarly, intercropping groundnut in maize plots contributed 96 kg of N/ha at a ratio of one maize plant to four groundnut plants (Mandimba, 1995). Osunde *et al.* (2004) reported intercropping soybean contributed 40 % increase in nitrogen fixation.

1.2 Farmer saved seed

Seed recycling refers to a practice where seed from previous harvest is re-sown hence changing seed class to another class, example, is re-sowing certified class to produce

grain (ICRISAT, 2014). The difference is grain is used for food or feed while seed for production. (FAO, 2014). However, majority of smallholder farmers use own saved legume seed. For instance, ICRISAT (2014) estimated that in Malawi 90 % of smallholder farmers recycle groundnut seed. Predicting legume seed demand is difficult for seed companies because legume crops are self-pollinated hence farmers save own seed for several seasons (Abate, 2012).

Accessing quality seed at right time and place in good quantities and at better prices from the formal seed sector depends on several factors. Unlike cereals, legumes seed multiplication ratio is low. Firstly, high costs and more seasons required to breed legume seed from one generation to another (FAO, 2014). Secondly, large seed size of some legumes means more seed is required per unit area, which increases storage space and transportation costs. Thirdly, some legume seed is easily damaged in transit and storage. Fourthly, some legume seed lose quality soon after harvesting. (Sperling and McGuire, 2010).

1.3 Problem statement and justification

Yields of groundnut and soybean in Malawi are low, averaging 800 kg ha^{-1} , compared to yield of 3000 kg ha^{-1} (MoAFS, 2012). This low yield is due to unavailability of quality seed and high seed prices, which remain major hindrance to increase yield and production of legumes in Malawi (MoAFS, 2012). As a result, most smallholder farmers in Malawi opt to planting recycled seed (ICRISAT, 2014; MoAFS, 2012). Some of the seed sourced from open-air grain markets are over aged in air-dried conditions, which had undergone degree of deterioration (Butler *et al.*, 2009). Use of

such aged seed especially pulses like soybean and groundnut for planting predisposes the seed to imbibitional injury due to their hygroscopic nature which result in poor seed germination (Tilden and West, 1984). Poor germination result in low plant population and poor field establishment, which reduces productivity. Mhango *et al.* (2017), Gabisa *et al.* (2017) and Kurt *et al.* (2019) reported a linear link between groundnut plant population and pod yield.

Recycled seed is by far, low yielding compared to basic or certified seed (Minde, 2008). Clayton *et al.* (2009) reported reduced plant population, yield and oil by 17 %, 12 % and 5 gkg⁻¹, respectively, when own saved canola seed was used over certified hybrid. Edwards (2006) reported 48 % wheat yield loss resulting from recycling seed up to third generation.

High quality certified seed alone might not be enough to increase productivity. Bio fertilizers such as *Rhizobia* inoculants and *mycorrhizae* increases yield by increasing nutrient utilization.

Use of certified seed combined with integrated soil fertility management (ISFM) such as *Rhizobia* inoculation technology needed to be investigated. The extent to which planting recycled seed affect groundnut and soybean productivity in Malawi was not investigated until this study. Similarly, variation in the response of *Rhizobia* inoculation on smallholder farms was found alarming (Mhango, 2011). Absence of literature on productivity gap that exists due to use of farmer saved soybean and groundnut seed, response of soybean seed generation to *Rhizobia* and effect of groundnut seed generation on productivity plant, and lack of data on soybean and

groundnut seed quality produced in different environments and from different seed generations in Malawi prompted this study.

1.4 Main objective

To determine effect of seed generation, *Rhizobia* inoculation and plant density on productivity and seed quality of groundnut and soybean under smallholder farms in Dedza and Machinga Districts in Malawi.

1.4.1 Specific objectives

The specific objectives were to evaluate the effect of:

- i. Seed generation and inoculation on nodulation and biological nitrogen fixation of soybean
- ii. Seed generation and inoculation on yield and yield components of soybean
- iii. Seed generation and plant density on biological nitrogen fixation of groundnut
- iv. Seed generation and plant density on yield and yield components of groundnut
- v. Mother environment on seed germination, emergence and seedling vigor in soybean and groundnut

1.5 Research hypotheses

- i. Application of *Rhizobia* inoculant to certified soybean seed increases nodulation and biological nitrogen fixation of soybean more than with recycled seed
- ii. Application of *Rhizobia* inoculant to certified soybean seed increases yield and yield components more than with recycled seed

- iii. Groundnut seed planted at higher density increases yield and yield components and biological nitrogen fixation compared to groundnut seed planted at lower density
- iv. Certified groundnut seed planted at same density outperforms recycled seed on measures of yield and yield components and biological nitrogen fixation.
- v. Groundnut and soybean seed produced from Dedza and Machinga environments would be of the same quality.

CHAPTER TWO

LITERATURE REVIEW

2.1 The seed system in Malawi

Malawi seed system is categorised into formal and informal. In formal seed system main activities are controlled by public institutions identified according to regulations (Seed Act 2005 and National Seed Policy, 2018). Seed supply arrangements, which does not involve formal sector, is what is referred to informal seed system where farmers obtain seed that has not been certified by authorised institutions (Ntare *et al.*, 2009).

In Malawi, small seed quantities of few crops are produced. Unless formal seed system integrates with informal seed system, the need for more certified legume seed will forever be unmet. Consequently, crop productivity will remain low (Legumes Development Trust, 2014).

Failure to provide improved legume seed to smallholder farmers by current seed seed systems is due to many factors. Public-sector led seed production neglected the demand for new varieties, as attention is production of more commercial crops of high demand like hybrid maize. Low interest by private sector to produce legume seed because of low profitability as prefer saving seed from previous harvest and high seeding rate and low seed multiplication ratio of approximately 1:10 (ICRISAT, 2014). Legume seed subsidies tend to be inefficient. (Abate, 2012)

However, recent efforts by Malawi government recognising Quality Declared Seed (QDS) where 10 % of the produced seed should be certified by authorised institutions

before selling and at selling (National Seed Policy, 2018) need to be practically implemented with full support.

2.2 Seed classes recognised under laws of Malawi

The National Seed Policy in Malawi (2018) recognises these seed classes and their definitions.

2.2.1 Nucleus seed

This is the 100 % genetically pure seed produced by the original breeder from basic nucleus seed. The producing breeder issues a pedigree certificate.

2.2.2 Breeder seed

Seed class regulated by the originating plant breeder and institution. In Malawi, ICRISAT and National Agricultural Research Services (NARS) controls production of breeder seed. Breeder seed for groundnut varieties recently released in Malawi include; Nsinjiro, CG 7, Chitala, Kakoma and Baka (Abate, 2012) while soybean breeder seed varieties available include; Nasoko, Makwacha, Serenade and Tikolore (Kananji *et al.*, 2013)

2.2.3 Pre-basic seed

Seed class from produced from breeder seed for crops with low multiplication ratios such as groundnut.

2.2.4 Basic seed

Seed class produced from pre-basic seed with genetic purity and identity. Seed is produced under ICRISAT through contract farming with farmer organisations like National Smallholder Farmer Association of Malawi (NASFAM) and Farmer Field Schools (Abate, 2012)

2.2.5 Certified seed

Seed class produced from basic seed. Certified seed for groundnut varieties recently released in Malawi include; Nsinjiro, CG 7, Chitala, Kakoma and Baka (ICRISAT, 2014) while soybean certified seed varieties available include; Nasoko, Makwacha, Serenade and Tikolore (Kananji *et al.*, 2013). Certified seed for old groundnut varieties like Chalimbana are not available.

2.2.6 Quality Declared Seed (QDS)

Seed class where certifying institutions checks 10 % of seed produced and distributed (National Seed Policy, 2018). Some CBOs are promoting QDS for soybean and groundnut in Malawi.

2.3 Germination and seed vigour

McDonald (1975) defined germination as radicle protrusion and development into new plant when favourable conditions are available. Germination can either be hypogeal and epigeal; where hypogeal refers to the germination in which cotyledons remain in the soil while epigeal, the cotyledons provide nutrients for growing plant with cotyledons raised ground (MacDonald, 1986).

Germination percentage obtained from standard germination is used to express germination capacity of a seed lot. Germination test results provide an accurate estimate of seedling emergence in the field or greenhouse, so long conditions at planting are ideal (TeKrony and Spears, 2002). However, due to inefficiency of standard germination test (which is conducted at ideal condition unlike field conditions), vigor test is more reliable as it takes field conditions into account (TeKrony and Spears, 2002).

International Seed Testing Association (1979) defined vigour as total properties determining activity of seed lots in contrasting environments. The Association of Seed Analysts (1983) and Baalbaki *et al.* (2009), referred seed properties determining production of normal seedlings in contrasting environment. Seed lots perform differently in contrasting environments because deterioration in seed lots can occur prior to noticeable change in germination (Savage and Bassel, 2016).

2.3.1 Factors affecting seed germination and seedling vigor

Oil seed crops like groundnut and soya bean are very sensitive to harsh environmental conditions. Oil content in oil seeds readily oxidizes and this causes deterioration of the seed health while in storage (Kausar *et al.*, 2009). Planting overaged seed result in poor crop stand as they get predisposed to imbibitional injury (Tilden and West, 1984). Cell membrane permeability or rupture in aged seeds is the major factor that contributes to the loss of germination, poor seedling emergence and transmission of pathogens to the new crop (Mohammadi *et al.*, 2011).

Seed storage temperature and relative humidity decreased the seedling vigour by 18.7 % in maize and by 57.1 % and 43.2 % in soybean and sunflower, respectively and reduced vigour from 91 % in maize, 88.5 % in soybean and 89 % in sunflower when stored at 25 °C/ 75 % RH (Simic *et al.*, 2006). According to Simic *et al.* (2006), quick deterioration of oil or more fat in soybean and sunflower account for quick seeds damage in storage. Seed aging whether artificial or natural causes damage to the seed and this negatively affects the germination rate of seed especially oil seeds like soybean, groundnut and sunflower (Balesevic –Tubic *et al.*, 2010).

Casteel (2011) reported that seed has to absorb water in order to be active to activate gibberellic acid, which triggers enzymes within the seed to become functional and metabolise the stored food reserves within the seed. Shortage of soil moisture during germination, therefore, leads to seedlings uneven and spotty emergence. Germination of desert plant *Eremosparton songoricum* was severely affected by drought stress. Germination of *Eremosparton songoricum* decreased when polyethylene glycol increased (Li *et al.*, 2013). Germination decreased when PEG concentration increased, which induced moisture stress.

Rasaei *et al.* (2013) using polyethylene glycol (PEG6000) to create osmotic water potentials of 0, -3, -6, -9 and -12 pascals reported that germination percentage and other traits of germination were severely affected by drought. There was no germination at potentials of less than -3 Pascals. Seed of different cultivars of soybean did not germinate at high moisture stress indicating the importance of adequate moisture availability for seed to germinate.

Mohammadi *et al.* (2013) in the investigation of effect of salinity and moisture deficit on catnip (*Nepeta persica*), reported that drought and salinity significantly decreased emergence and catnip growth.

Snider (2014) reported strong correlation of seedling vigor and total seed oil content in cotton ($r^2 = 0.573$), and seedling vigor and seed size ($r^2 = 0.642$). On average, large seeds produce vigorous seedlings than small seeds in same or different species.

2.3.2 Seedling vigour assessment based on growth tests

These tests assume that vigorous seeds grow faster than poor vigorous seeds. High vigor seeds emerge and grow quickly. Other vigor test methods are stress and biochemical tests.

2.3.2.1 First count

Germinated normal seedlings on the first count day are counted. Number of normal seedlings indicates seed vigour. More normal seedlings indicate high vigour seeds

2.3.2.2 Seedling growth rate and dry weight

Ten seeds positioned centrally on moist towel papers in laboratory, paper then rolled and placed in germinator. Remove towel paper after 5-10 days and sample 5 seedlings, measure length hence vigorous seedlots produce taller seedlings. To assess biomass accumulation, seedlings are dried at 65 °C temperature for 48 hours, hence seeds with high vigour have more biomass.

2.3.2.3 Speed of germination

Four replicates, 100 seeds each, are germinated in substratum, which is placed in germinator at recommended temperature. Then daily count number of seedlings emerging till germination stops. High vigour seeds are shown with high germination index.

2.3.2.4 Seed vigor index (S.V.I)

Four replicates, 50 seed each, are germinated in towel papers, at final germination count, sample 5 seedlings randomly and measure their length. Then use vigor indices

II and I based on germination, seedling length and dry weights (Abdul-Baki and Anderson, 1973)

- i) *Vigour Index – I =*
standard germination (%) x seedling length (cm)
- ii) *Vigour index – II =*
Standard germination (%) x seedling dry weight (g)

Higher seed vigour index is indicative of more vigorous seed lot

2.4 Biological nitrogen fixation and cropping systems

Growing plants need nitrogen in large quantities and it is key to soil fertility. It comprises 40 % to 50 % of plant protoplasm (Dreyfus *et al.*, 1987). The nutrient is needed by the plant as an integral part of all proteins, and photosynthesis, which occurs at high rates when there is sufficient nitrogen. Aboutalebian and Malmir (2017) reported that soybean will require 30 kg of inorganic starter nitrogen per hectare for optimum yield while application of starter N doses of 15-20 kg/ha when total soil nitrogen was less than 1 % increased groundnut yield (Ghosh *et al.*, 2007). Nitrogen represents about 79 % of atmospheric gases but plants absorb it in form of ammonium (NH₄⁺) and nitrate (NO₃⁻). During mineralization, ammonia is realised for plant uptake. In addition to organic N, plants are also supplied with inorganic N fertilizer when soil nitrogen is deficient (Giller, 2001).

Legumes contribute to soil nitrogen budget through biological nitrogen fixation, a potential alternative to ever expensive inorganic fertilizers but also for more sustainable agricultural production (Boddey *et al.*, 1997; Giller *et al.*, 1997). Studies established that nitrogen from soybean was transferred to intercropped maize (Fujita

et al., 1992; Stern, 1993; Ledgard and Giller, 1995; Yusuf *et al.*, 2009). For example, Eaglesham *et al.* (1981) reported that 24.9 % of nitrogen cowpea fixed was transferred to maize. Osunde *et al.* (2004) noted that soybean BNF contributed 40 % of soil nitrogen when intercropped with maize and 30 % as sole crop. Ghosh *et al.* (2007) estimated that groundnut contributed up to 60 kg N ha⁻¹ to the following nitrogen non-fixing crop.

To get same yield, sorghum required 20 kg N ha⁻¹ following groundnut while sorghum following cowpea required 60 kg N ha⁻¹ (Bado *et al.*, 2006). Wheat following pearl millet yielded lower than wheat following groundnut (Ghosh *et al.*, 2007). Hedge and Dwivedi (1993) reported 56 kg N ha⁻¹ residual nitrogen from groundnut previous season for use by next crop. Francis (1986) reported that nitrogen content in maize intercropped with groundnut was higher than sole maize. In Malawi Njira *et al.* (2012) reported 55.8 kg N ha⁻¹ fixed by sole groundnut in Kasungu district while Mhango (2011) reported 57 kg N ha⁻¹ fixed by sole groundnut in smallholder groundnut farms, Mzimba district.

Carlsson and Huss-Danell (2003) reported BNF as best compliment of inorganic fertilizers. Efforts to breed legumes with efficient nitrogen fixation have recently scaled up (Matusso *et al.*, 2014). On short term, incorporating lignified legume residues with low carbon to nitrogen ratio increases immobilization of nitrogen. (Toomsan *et al.*, 1995).

Promiscuous soybean varieties that produce large quantities of leafy biomass fix more nitrogen than those requiring *Rhizobia* inoculant as most smallholder farmers cannot afford inoculant strains (Mpeperekwi *et al.*, 2000).

2.4.1 Factors affecting biological nitrogen fixation

Edaphic; climatic; and biotic factors affect BNF. Edaphic factors are those factors that concern the soil and include soil moisture, availability of macro and micronutrients, soil pH and soil salinity.

Both water deficit and water logged soil conditions negatively affect biological nitrogen fixation. *Rhizobia* respond to moisture deficit by undergoing a morphological change in which cells are deformed. This deformation eventually leads into reduction in infection, nodulation and a reduction in biological nitrogen fixation (Busse and Bottomley, 1989). Drought conditions reduce the number of soil *Rhizobia* bacteria and prolonged drought promotes nodule decay (FAO, 2013).

In soybean, a reduction in nodule numbers under mild moisture stress conditions and a reduction in both number and size of nodules were noted under moderate to severe moisture stress conditions (Williams and Mallorca, 1984). Devries *et al.* (1989) reported reduced biological nitrogen fixation in the crop under soil moisture deficit conditions. Waterlogged conditions and too much soil moisture impede root hair growth, nodulation and BNF by interfering with oxygen flow into root systems (FAO, 2013). *Rhizobia* fails to colonise *rhizosphere* under extremes of pH. Acidic soils have low levels of nutrients that promote nodulation (phosphorus, calcium, and molybdenum) but dominant in nutrients toxic to *Rhizobia* and legume plant (aluminium and manganese). Soils, pH > 8, usually have high concentration of sodium chloride, bicarbonates and borate, which limits nitrogen fixing (Bordeleau and Prévost, 1994).

Macro and micronutrients also affect BNF. Among the macronutrients are mineral N and P. Too much soil nitrogen causes the impairment of the recognition mechanism by nitrate, which in turn inhibits the infection process (FAO, 2013). Process of N₂ fixation is also impaired by too much soil nitrogen in the sense that photosynthates are diverted towards assimilation of nitrates (FAO, 2013). In high mineral N soil conditions, nitrogen-fixing plants favour soil nitrogen and hence low BNF. Legume productivity cannot be negatively affected but this trend exploits soil nitrogen (Peoples *et al.*, 1989). Phosphorus is a component adenosine triphosphates (ATP) which provide energy for nitrogenase to breakdown the nitrogen triple bonds (Hussein, 2009).

Zinc enhances water use efficiency and photosynthesis (Weisany *et al.*, 2013). Iron and molybdenum are components of enzyme nitrogenase that catalyses the breakdown of dinitrogen (N₂) to ammonia (FAO, 2013). Iron is a component of leghaemoglobin in the nodule that protects nitrogenase from oxygen inactivation and, cobalt facilitate nodule growth and nitrogen fixing (Weisany *et al.*, 2013).

Climatic factors that affect biological nitrogen fixation include temperature and light. Nitrogen fixation is efficient at 20–30 °C. (Bordeleau and Prévost, 1994). Light on the other hand affect biological nitrogen fixation in a sense that photosynthesis which supplies photosynthates to fixing bacteria, occurs in presence of light (FAO, 2013). Reducing the supply of light will reduce supply of photosynthates to the nodule and reduce the limit dinitrogen fixation, a process that is energy dependent (Fujita *et al.*, 1992). *Mycorrhizae* infection promotes BNF and nodulation by improving both

availability and uptake of phosphorus, an important nutrient (Redecker *et al.*, 1997; Fukutoku and Yamada, 1982).

2.4.2 Methods of determining biological nitrogen fixation

Basis of quantifying BNF by any method is differentiating the N fixed from soil nitrogen. This is technically not easy; as such no method is overly accurate. However, the methods will give an estimation of the nitrogen biologically fixed by the legume (Giller, 2001). Each of the techniques has its own unique advantages and limitations (Peoples *et al.*, 1989).

The following are some of the methods, their principles, assumption, and potential advantages and limitation as described by Unkovich *et al.* (2008).

2.4.2.1 Nitrogen balance technique

This method attributes a gain in nitrogen to BNF if other sources of nitrogen are accounted for. Assumptions are that potential inputs of N through leaves are negligible compared to BNF. This technique is simple since nitrogen from applied sources can be easily accounted including losses. However, nitrogen losses through leaching, erosion, volatilisation and denitrification is difficult to measure hence these losses need to be estimated to avoid underestimating BNF (Unkovich *et al.*, 2008).

2.4.2.2 Nitrogen difference method

This method differentiate nitrogen content of non-fixing plant from fixing plant at the same or near sites. This method assumes that non-fixing plants use soil nitrogen only while fixing plant use nitrogen from soil and fixed nitrogen. It also assumes both fixing and non-fixing plants use same amount soil mineral N. Much as this method is simple and cheap, its design requires a non-fixing plant as control. Errors emanating

from poor estimation of total nitrogen accumulated by control and fixing plant can undermine this method.

2.4.2.3 Ureide method

This method is built on the principle that there are different forms of nitrogen transported in the xylem of fixing and non-fixing plants, making fixed and soil nitrogen to be easily distinguished. It assumes that sap in the xylem and stem contains recently fixed nitrogen. Percentage of nitrogen assimilated is obtained from ureides abundance relative to other N solutes. This method does not need special design so it can also apply to plants not grown the field. Potential limitation is that it can only be used to plants, which export ureides such as soybean.

2.4.2.4 N¹⁵ isotope technique

The method uses the principle that nitrogen exist in two isotopes—¹⁴N and ¹⁵N with the former more abundant than the latter. Fixed nitrogen is determined from the difference between soil nitrogen and concentration of ¹⁵N in atmospheric nitrogen of fixing and non-fixing plants. Potential limitations of the ¹⁵N-based methodologies are that: it is expensive because it requires a high cost mass spectrometer.

2.4.2.5 Acetylene reduction method

The method uses the principle that nitrogenase which breaks dinitrogen gas to ammonia also breaks acetylene gas to ethylene. Acetylene replaces dinitrogen gas in this method where roots are exposed to acetylene in airtight containers. Gas chromatograph measures rates of acetylene accumulation. Assumption is that nitrogenase activity is not affected by substituting dinitrogen gas with acetylene gas. Advantages of this method are that: nitrogenase is very sensitive to acetylene, quick,

cheap and simple to use. The challenge with this method is that effectiveness is only during period of acetylene assay.

2.4.2.6 Hydrogen evolution method

This method is based on the principle that during BNF hydrogen gas is produced and consumes 35 % of nitrogenase activity. Nitrogenase activity is indirectly measured by detecting hydrogen gas concentration on the chromatograph. This technique does not demand more labour as acetylene reduction besides being simple and nitrogenase activity not affected. The potential limitations are that; the method requires keeping nodulated roots away from nitrogen gas in order to measure total nitrogenase activity because it reduces with long exposure to argon.

2.4.3 The need to inoculate soybean

High yielding soybean demands more nitrogen than low yielding soybean (Herridge *et al.*, 2008). Salvagiotti *et al.* (2008) reported that BNF meets 50 % – 60 % soybean nitrogen requirement. Soybean respond to inoculation when *Rhizobia* are introduced in soils with no residual *Rhizobia* (Ulzena *et al.*, 2018). biological nitrogen fixation increases with inoculation in soils with low nitrogen. However, soybean yield increase due to inoculation depends on field differences, variability in edaphic and climatic factors (Ulzena *et al.*, 2018).

Lindstrom (2010) argued that soil lack effective native strains of *Bradyrhizobium japonicum* if the land was not planted with legumes for many years. Hiltbold *et al.* (1985) established that inoculating soybean in soils where legume is planted for first time increased grain yield and nodulation. The effect of inoculating soybeans depends on site cultivation history more than on *Rhizobium* strains and legume species.

Choudhry (2012) observed more nitrogen is fixed with effective symbiosis between host and *Rhizobia* even when residual nitrogen is enough for host plant growth. Improved management practices and nutrient availability increases nitrogen fixation even with less effective symbiosis because nitrogenase adjust according host plant nitrogen demand (Mengel 1994). The amount of nitrogen fixation depends more on nitrogen demand by host plant than *Rhizobia* ability to fix nitrogen.

2.5 Plant density and groundnut productivity

Main stems per unit area refers to plant density. Kurt *et al.* (2019) noted that competition for growth resources increased with increasing plant density. Gabisa *et al.* (2017) outlined two approaches describing relationship of yield, spacing and densities. Firstly, increasing canopy during reproductive stage maximises light energy capturing hence maximum yield. Secondly, Maximum yield is obtained if plant competition is minimised through proper plant spacing

Plant density affect groundnut productivity (Yousif and Hussain, 2019). In Africa, groundnut productivity is high between 98, 000-274,000 plants ha⁻¹ (Gardner and Auma, 1989). Rasekh *et al.* (2010) reported that productivity increased when plant density increased from 3 to 8.3 plants m⁻² however, plant density beyond 14.8 plants m⁻² decreased productivity. Magagula *et al.* (2019) indicated that ideal plant density in groundnut varies depending on cultivars, environments, and growth habit. Onat *et al.* (2017) reported that high seed yield in twin over single rows was due to high pod yield and high shelling % which were contributed by high plant population which increases capturing of photonsynthetically active radiation into dry matter production.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Site description

On-farm researcher designed farmer managed experiments were conducted during the 2017/2018 growing season in Linthipe Extension Planning Area (EPA) of Dedza district; and Mtubwi EPA of Machinga district of Malawi. These on-farm trials were conducted within the framework of Africa RISING program in Malawi. These sites represent agro-ecological zones with different rainfall patterns, soil characteristics, temperatures and altitude. The sites experience unimodal rainfall pattern, with the planting season starting in November/December to April depending on the onset of effective planting rains (>30mm).

Linthipe EPA is located at 14°12' S, 34°05' E and at an altitude of 1240 m, part of mid-altitude zone (Dedza upland) about 1200 m above sea level. It has a cool climate with annual rainfall of about 800mm and temperature between 14 °C and 21 °C (David, 2003; Dedza District Assembly, 2012). The EPA covers an area of 1120 km² (26,050 ha) with an estimated population of more than 160 000 people (Lorkeere and Venema, 1991, David, 2003). The area has gently undulating plain with low rock hills commonly found, dominated by ferruginous, and weakly ferruginous soils (Brown and Young, 1965)

Mtubwi lies between 15°18' S, and 35°33' E and at an altitude of 507 m above sea level and receives average rainfall between 600-800 mm, usually distributed between November to February, and high temperatures above 35 °C (Accu Weather, 2016).

3.2 Selection of famers hosting on-farm experiments and source of recycled seed

3.2.1 Selection of farmers and plots for field experiments

Eight farmers four from each EPA were identified to host the trials based on accessibility of plot to other farmers and previous cropping history. In Mtubwi EPA, sole maize and maize-cassava intercrop were dominant practices on the sites for past three seasons prior to this trial while in Linthipe EPA sole maize and maize-pumpkin intercrop for past three seasons prior to this trial were dominant practices. CIAT (1988) reported evidence of high-level native *Rhizobia* on plots with previous soybean history. Selection also considered willingness of the farmer to host trials.

3.3 Treatments

There were three experiments, two on-farm experiments and one Screen house experiment. On-farm experiments comprised soybean and groundnut experiments. Each EPA had two sites for each on-farm experiment. The treatment structure for on-farm experiment comprised four treatments described under each below:

3.2.2 Experiment 1. Effect of seed generation and Rhizobia inoculation on productivity of soybean on smallholder farms in Dedza and Machinga districts

Two farmers in each district implemented the soybean experiment. The four treatments were:

1. Recycled soybean-inoculated
2. Recycled soybean non-inoculated

3. Certified soybean inoculated
4. Certified soybean non-inoculated

Tikolore soybean variety used in this experiment was a promiscuous variety, takes 120-140 days to mature and adapted to high, low and medium altitudes. Small seeded, brown hilum, tolerant to frogeye disease, susceptible to rust, has 37 pods per plant on average and yields up to 2500 kg/ha and it was released in the year 2011 (Mviha *et al.*, 2011; Kananji *et al.*, 2016).

Soybean experiment was laid out in a split-plot in a randomised complete block design (RCBD). There were four replications for treatments per farm. The main plot factor was seed generation and the subplot factor was inoculation was subplot factor.

3.3.1 Experiment 2. Effect of seed generation and plant density on productivity of groundnut on smallholder farms in Dedza and Machinga districts

Two farmers in each district implemented the groundnut trial. The Four treatments were:

1. Recycled groundnut planted in twin rows
2. Recycled groundnut planted in single rows
3. Certified groundnut planted in twin rows
4. Certified groundnut planted in single rows

Virginia bunch variety, CG7 of pedigree (ICGV-SM 83708) was used. CG 7 is a confectionery nut with medium seed size. It is recommended for production in all groundnut-growing areas of Malawi (MoAFS, 2012). It has a bunch growth habit and tolerates drought. It matures in 130 to 150 days. The seeds are red, uniform and

contain 48 % oil. It has a yield potential of 2500 kg per hectare (Monyo and Gowda, 2014).

Groundnut experiment was laid out in a split-plot in a randomised complete block design (RCBD). There were four replications for treatments per farm. The main plot factor was seed generation and the subplot factor was plant density.

Note: Recycled soybean and groundnut seeds were sourced from International Institute for Tropical Agriculture (IITA) farmers in Lilongwe (Chiseka EPA) and Mchinji (Kalulu EPA), respectively. Recycled soybean seed planted at both sites was from one farmer and recycled groundnut seed was also from another farmer. Both farmers were under IITA seed production project. Prior to these experiments, soybean and groundnut seeds were recycled four and five times, respectively. Records of seed recycling were obtained from IITA project records. Prior to planting certified groundnut seed was packed shelled in plastic papers while farmer saved groundnut seed was stored unshelled till time of planting.

Maize (unfertilized) was planted 5 metres away from the soybean and groundnut plots as reference crop for determination of biological nitrogen fixation to avoid affecting growth and yield of legumes. Soybean, and groundnut samples obtained at early podding and maize samples at tasselling were compared on N content. Post-harvest soil samples from legumes and maize plots were also obtained and compared for N content.

3.3.3 Experiment 3. Effect of mother environment on seed quality of soybean and groundnut.

This experiment was conducted in a Screen house at Bunda College Crop Science Students Research Farm. Treatments comprised seeds harvested from all farmers in both soybean and groundnut on-farm experiments described in sections 3.3.1 and 3.3.2

Groundnut average seed size produced from Machinga and Dedza were 51 and 54 grams, respectively, and 13.6 and 14.3 grams for Machinga and Dedza soybean, respectively. The Screen house experiment was a factorial experiment laid in completely randomized design (CRD) and replicated four times.

3.4 Seed sample and breaking dormancy for Screen house experiment

For the Screen house experiment, four hundred seeds each (both groundnut and soybean) were taken at random from the well-mixed seeds from each farmer and treatment per site. Replicates of 50 seeds per pot were used, spaced 5cm apart on the seedbed to minimize the effect of adjacent seeds on seedling development (ISTA Handbook, 2017).

ISTA (2017) provided recommendation to break dormancy before testing for germination and vigour in groundnut (*Arachis hypogea*) but not in soybean (as long as it is well sun-dried before sowing). To break dormancy, shelled groundnuts were put in an envelope and placed in oven adjusted to 40 ± 2 °C for 7 days.

Fifty (50) groundnut and fifty (50) soybean seeds were each planted in a pot containing sterilized river sand (boiled for an hour). Then 3cm deep holes were drilled uniformly on the surface of the pot where 1 seed was placed per hole. Then another

2cm layer of sterilized river sand was added on top of the seed to ensure seeds were buried 5cm deep as recommended under field conditions. Planted seeds were watered with tap water twice before emergence with ½ litre amount at each watering day per pot (ISTA Handbook, 2017). Watering after emergence was done after every two days till day of data collection. Four holes were made at the bottom of each pot to let water out and avoid humid condition, which could cause seed rotting. Soybean and groundnut were planted same day on 26th October, 2018.

3.5 Plot size and management of on-farm experiments

On-farm gross plot had 12 ridges; each ridge was 5 m long. Ridges were spaced at 0.75 m apart. The gross plot and net plot sizes were 41.25 m² and 16 m², respectively. No residues were incorporated at ridge making. Planting was done with the first effective rains on 29th and 30th November, 2017, in Mtubwi EPA, Machinga district and on 4th and 5th December, 2017, in Linthipe EPA, Dedza district.

Groundnut was planted on twin and single rows. Spacing between the two groundnuts rows on the ridge was 20 cm. One seed was sown per station, each spaced at 15 cm. Soybean was planted in double rows on the ridges. Spacing between the two soybean rows on the ridge was 20 cm. One seed was sown per station, each plant station spaced at 5 cm.

Soybean was inoculated with *Rhizobia* before planting as described by the Ministry of Agriculture and Food Security (2013). A 200 ml of 5 % sugar solution was prepared. One sachet of inoculant (50g) was mixed into the solution to form slurry. Inoculum slurry was poured over 12.5 kg seed and mixed until seed was evenly coated. Seed was left to dry in the shed and planted after an hour of inoculation. Management

activities such as weeding were done by the host farmers while researcher did supervision, inoculation and data collection among others. All host farmers were trained on soybean and groundnut best agronomic practices including pest and disease management.

3.6 Data collection

3.6.1 Soil data

Baseline soil samples were collected with a soil auger from a depth of 0-15 cm and 15-30 cm before planting and after harvest from five randomly selected points using the zigzag sampling pattern to collect representative soil sample of the plot. Five samples from same depth were thoroughly mixed together in a bucket and a composite sample of approximately 0.5 kg per plot was obtained. Soil samples were then passed through 2 mm sieve at Bunda College Soil Laboratory and a fraction of each sample was used for specific analysis.

3.6.1.1 Determination of soil texture

Air-dried 50 grams properly sieved soil was put in a beaker. To it, 50 ml of 10 % calgon and 100 ml of distilled water was added, then contents allowed to stand for 15 minutes. The contents were then poured and washed into a 1000 ml cylinder making the contents into the 1000 ml mark with distilled water. Mixing of the contents was carefully done by end to end shaking of the cylinder for 10 times, before putting each cylinder on the bench and noting the time. A Bouyoucos hydrometer was gently placed into the contents after 5 minutes of settling and gave mass in grams of silt and clay followed by insertion of a thermometer into the contents and recording the contents. Second sets of hydrometer and temperature readings were taken after 2

hours to obtain the mass in grams of clay. Readings were corrected by adding 0.3 g to hydrometer reading for every 1 °C above 19.5 °C and subtracting the weight of calgon (blank reading) from the hydrometer readings (Anderson and Ingram, 1989)

$$\% \text{ silt} + \text{clay} = \frac{5 \text{ minute corrected hydrometer reading}}{\text{Sample weight}} \times 100$$

$$\% \text{ clay} = \frac{2 \text{ hours corrected hydrometer reading}}{\text{Sample weight}} \times 100$$

$$\% \text{ silt} = \% (\text{clay} + \text{silt}) - \% \text{ clay}$$

$$\% \text{ sand} = 100 \% - (\text{clay \%} + \text{silt \%}) \text{ (Anderson and Ingram, 1989)}$$

Soil textural classes were then determined using a textural triangle.

3.6.1.2 Determination of soil pH

Soil pH was determined in water as described by Wendt (1996). Soil (10 g) was placed in 50 ml test tube and 25 ml of room temperature distilled water was added. The contents were then shaken for 10 minutes, left to stand for 30 minutes before being shaken again for 2 minutes. Solution pH was read carefully on a properly calibrated pH meter by placing the electrode of the pH meter into the solution. The pH was allowed to stabilize before taking the reading. Electrode was rinsed properly with distilled water before dipping into the next sample. To ensure accuracy of results a duplicate was made for each sample.

3.6.1.3 Determination of soil organic matter

Organic matter was determined using the Walkley – Black wet oxidation method as described by Anderson and Ingram (1989). Soil weighing 0.25 g was placed into 50 ml digestion tubes. In separate digestion tubes, 1, 2, 3, 4 and 5 ml of sucrose solution

was added for standards. Addition of 5 ml of $K_2Cr_2O_7$ solution was done to the samples and standards. To the contents, 10 ml of concentrated H_2SO_4 was added to speed up the oxidation process before capping with a rubber stopper. Digestion tubes were then swirled on a vortex mixer until the soil samples were completely dispersed. Samples and standards were then read on a spectrophotometer at a wavelength of 600 nm using a 1 cm cell.

The amount of carbon was determined from a standard curve and percent organic carbon

(OC) was calculated as follows:

$$\% OC = \frac{mg\ C}{mg\ of\ sample} \times 100$$

% Soil organic matter (SOM) was estimated by multiplying organic carbon by a factor of 1.724 (Anderson and Ingram, 1989)

3.6.1.4 Determination of total nitrogen

Determination of total soil nitrogen was done as described by Anderson and Ingram (1989). First, the digestion solution was made by placing 700 ml of 30 % hydrogen peroxide in a 2 litre volumetric flask. To the content, 28 g of lithium sulfate was added and mixed until dissolved, before putting in a large stirring bar. The flask was then placed in a plastic bucket on the large stirring bar. Ice was added until the bottom round part of the flask was covered. The stirrer was then turned on to slow speed. Slowly 840 ml concentrated sulfuric acid was added, a process that took about 1 hour. The flask was never allowed to become warm.

Digestion of soil samples was done by weighing 0.2 g of ground soil into 50 ml digestion tubes and 4.4 ml of the digestion solution was quickly added into the tube, ensuring no soil stuck to the bottom. Each tube was swirled for a few seconds immediately after adding digestion solution to ensure good mixing. To each batch of the soil, 5 blank tubes with 4.4 ml of digestion solution, without soil, were added. A funnel was put on each tube and put on a hot plate for 2 hours at 360 °C ensuring that the solution was clear. If not clear, the solution was swirled and allowed to digest for an extra hour. Solutions were allowed to cool and thereafter, were diluted to 50 ml. To the blank solutions, 0, 0.2, 0.4, 0.6 and 0.8 ml were removed and the same amount of 1000 ppm N stock solution was added and mixed well.

1000 ppm N stock solution was made by adding 4.714 g $(\text{NH}_4)_2\text{SO}_4$ and 0.439 g of KH_2PO_4 in a 100 ml volumetric flask. Both chemicals were oven-dried at 105 °C before weighing. The contents were then diluted to mark.

Total nitrogen was determined by placing 0.5 ml of samples and standards in 20 ml glass vials. To each tube, 2 drops of p-nitrophenol were added and thereafter, a drop wise addition of 5 N NaOH was done and shaken after each drop until the colour changed to yellow. Addition of 5 ml of N1 solution followed. The solution was allowed to stand for 10 minutes before adding 5 ml of N2 solution. Colorimetric determination of total nitrogen was done by reading colour at 655 nm and sample concentrations were calculated from standard curve.

N1 solution was made by dissolving 68 g sodium salicylate, 50 g sodium citrate and 50 g sodium tartarated in 1.5 litres of water. Then 0.24 ml sodium nitroprusside was added before diluting the content to 2 litres.

N2 solution was made by adding 60 g NaOH and 28 ml of 3.5 % sodium hypochlorite to 1.5 litres of water before diluting the content to 2 litres.

3.6.1.5 Determination of available phosphorus

Mehlich-3 method was used to determine available soil phosphorus as described by Anderson and Ingram (1989). First, 2.5 g of air dried soil samples were weighed into 25 ml glass vials. To each vial, 25ml of Mehlich-3 extracting solution was added and contents were shaken for 5 minutes. The samples were then centrifuged at 3500 rpm for 5 minutes to separate liquid from soil particles, and then the contents were filtered with filter paper. Five P standard solutions were prepared by adding 0, 0.5, 1.0, 1.5 and 2 ml of 100 ppm P stock solution into 50 ml centrifuge tubes and made to 20ml with Mehlich-3 solution. The solutions contained 0, 2.5, 5.0, 7.5, and 10 ppm P. The sample filtrate and standard, 1 ml were pipetted into 25 ml glass vials before 8 ml of Murphy-Riley working Solution was added. Contents were allowed to stand for 30 minutes before reading absorbance at 880 nm. Concentrations (ppm) of the samples were determined from the standard curve.

3.6.1.6 Determination of potassium, calcium and magnesium

Extracts of soil solution used in determination of phosphorus were taken to Agriculture Research and Extension Trust (ARET) laboratory for the analysis of potassium, calcium and magnesium.

3.6.2 Crop data

3.6.2.1 Germination, Emergence and seedling vigor

For on-farm experiments, stand count after emergence was recorded at 15th day after planting. The number of plants per net plot was used to compute plant population per

hectare. Germination and emergence in the Screen house experiment was determined on the 12th day after planting. The following formulae were used;

$$\text{Germination \%} = \left[\frac{I+II}{\text{Total seeds sown}} \right] \times 100 \% \text{ (ISTA, 2017)}$$

$$\text{Emergence \%} = \left[\frac{I}{\text{Total seeds sown}} \right] \times 100 \% \text{ (ISTA, 2017)}$$

Where;

- I. Number of emerged seedlings
- II. Number of un emerged seedlings but germinated with radicle \geq 4mm (ISTA, 2017)

Seedling vigour index (SVI) was determined using the formula by Abdul-Baki and Anderson, (1973) as follows;

$$\text{SVI} - \text{II} = \text{Germination \%} \times \text{mean seedling dry weight (g)}$$

Seedling vigor index II is preferred over seedling vigor index I because of its use of plant biomass, an indicator of overall plant growth and development while use of vigor index I is limited by its use of plant height which may vary due to plant response to phototropism in the Screen house not necessarily plant growth and development.

3.6.2.2 Plant biomass at early podding stage

Plant physiologists and agronomists consider between 50 % flowering and early podding as the peak biomass accumulation stages in both groundnut and soybean (Long *et al.*, 2006). At early podding stage 40 soybean and 10 groundnut plants were randomly selected for nodulation assessment and 1m ridge plants for estimation of

biomass and plant N analysis. Shoots and roots were immediately weighed to obtain fresh above ground dry matter in the field using a 0.01g precision electronic scale. All the samples were taken to Laboratory for determination of biomass. Shoot and root dry matter were taken after oven drying fresh sample for 48 hours at 70 °C as described by Jones (2001).

3.6.2.3 Plant chemical analysis for N

In preparation, for plant chemical analysis, plant shoot and roots were cut into small pieces and oven dried for 48 hours at 70 °C. Dried plant samples were then milled and analysed for plant N using the method described by Winkleman *et al.* (1985).

3.6.2.4 Plant biomass and grain yield

Plants were uprooted from the net plot (4 m x 4 m), fresh weight determined and samples oven dried for 48 hours at 70 °C and weighed to determine total dry biomass. Senesced dropped leaves in furrows were estimated per plot by collecting them from a quadrant of 4 m x 4 m and add its biomass to oven dry biomass. The net plot pods were threshed from each treatment plot; the grains were weighed on a scale. Grain samples were dried in oven at 70 °C for 48 hours and hundred seeds from each treatment were randomly selected and weighed. Soybean and groundnut grain yield were adjusted to standard storage moisture content of 12 % and 8 %, respectively (Mloza-Banda, 1992)

Harvest index (%) was calculated using the following formula:

$$HI \% = \frac{\text{Grain yield}}{\text{Total biomass}} \left(\frac{\text{kg}}{\text{ha}} \right) \times 100 \% \text{ (Mloza-Banda, 1992)}$$

3.6.2.5 Determination of biological nitrogen fixation

3.6.2.5.1 Nodulation assessment

According to Long *et al.* (2006) sampling of plants for nodulation assessment should be done between early podding for both groundnut and soybean, as was the case in these experiments. Forty soybean and ten groundnut plants were sampled from net plot for nodulation assessment. The sampled plants were dug out and uprooted carefully using a hand hoe, ensuring no any damage was caused to the roots or nodules. Soil around the roots was then gently loosened by dipping the plant in bucket of water for 20 minutes to avoid losing some nodules. The roots were then thoroughly washed with clean water and rinsed. Thereafter, plants were separated into roots and shoots by cutting from the first node, care was taken not to damage the nodules. Nodules were counted, detached from the plant and nodule colour assessed by slicing each nodule into two halves, viewed with naked eye, weighed on precision electronic scale to obtain fresh weights and then oven dried for 72 hours at 70 °C (Jones, 2001) and weighed again for nodule dry matter assessment. Plant vegetative growth was also assessed, this was important to avoid relying on nodule dry matter assessment only.

The assessment was done on the following parameters: vegetative growth (healthy, vigour, and colour of plants), nodule number per plant, and nodule colour. Healthy, vigorous and green plants are most likely to have fully effective symbioses (Zaychuk, 2009). Effective nodules generally have predominant pinkish, red or brown internal colour, while ineffective nodules have green or white internal colour when dissected (Bala *et al.*, 2010; Tiwari *et al.*, 2012; Wagner, 2012). For colour assessment, 10

nodules were randomly assessed with naked eyes. The 10 nodules were stripped off the crown and sliced into halves so as to observe the colours of the exposed centre of the nodules.

Plant growth and vigour, nodule number and nodule colour were assessed using the procedure described by Zaychuk (2009).

a) Plant growth and vigour

- Plants predominantly green and vigorous.....5
- Plants predominantly green but relatively small.....4
- Plants slightly green and relatively small.....3
- Plants slightly chlorotic (yellowing).....2
- Plants very chlorotic.....1

b) Nodule number

- Super-nodulated (>50 nodules).....5
- Abundant (>20 nodules).....4
- Moderate (>11-20 nodules).....3
- Few (5-10 nodules).....2
- Root nodule absent (0-4 nodules).....1

c) Nodule internal colour

- Predominantly red in colour.....5
- Some pink or reddish colour.....4
- Some pink or greenish colour.....3
- Some white or greenish colour.....2
- Predominantly white or greenish colour.....1

Nodulation assessment, which took into account plant growth and vigour, nodule number and nodule colour, was done using a scale of 1 to 15 to rate nodulation effectiveness (Table 3.1).

Table 3.1 Criteria for assessing nodulation in soybean

Total scores	Assessment	Comments
10-15	Effective nodulation	Good N-fixation potential
7-9.9	Nodulation less effective	Reduced fixation potential (needs probing)
1-6	Unsatisfactory nodulation	Requires evaluation of growing conditions at site

Source: Adapted from Zaychuk (2009) Bala *et al.* (2010)

3.6.2.5.2 Measurement of biological nitrogen fixation

Modified nitrogen difference method technique was used to quantify the nitrogen fixed by soybean and groundnut. Nitrogen difference method compares total N of the nitrogen fixing species with that of a neighbouring non-nitrogen fixing species, with the net gain of N in the nitrogen fixing species assumed to be due to N₂ fixation. The method assumes that the two plant species under comparison use the same amount soil mineral N. It also assumes that N accumulated by the non-nitrogen fixing control is derived only from soil N and that the nitrogen-fixing plants use the same amount of soil mineral N as the non-nitrogen fixing control. It also assumes that mineralization, leaching and denitrification are identical under each crop (Peoples *et al.*, 1989).

The method is expressed in the following equation;

$$BNF \left(\frac{kg}{ha} \right) = [N \text{ yield}(\text{legumes}) - N \text{ yield}(\text{control})] + [\text{soil N}(\text{legumes}) -$$

soil N (control)] (Peoples *et al.*, 1989), *Where;*

N yield (legume) = Nitrogen amount in soybean or groundnut plants

N yield (control) = Nitrogen amount in maize plant (reference)

Soil N (legume) – soil N (control) = Difference between soil nitrogen amount under soybean or groundnut and soil nitrogen amount under reference crop.

3.6.3 Rainfall and Temperature

Rainfall amount received was collected using rain gauges installed near each trial site.

Temperature data was accessed from the Linthipe and Mtubwi EPA offices.

3.7 Data analysis

All data were subjected to analysis of variance (ANOVA) using GenStat package (18th edition). Count data was subjected to log transformation before running the ANOVA because the original data approximately followed a log-normal distribution. Significant differences were separated by least significant differences (LSD) at 5 %. Covariate and correlation analyses between yield and yield component and between soil and some yield components were performed.

Total number of observations for all crop data is 32; this represents two farms data with 16 subplots each. Number of observations for Mtubwi and Linthipe soil data is 128 each from 4 farms with 16 subplot each, each subplot with 2 samples; sub and top soil.

The linear statistical model for the on-farm experiments is as below;

$$\gamma_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + \varepsilon_{ijk}$$

γ_{ijk} = Observation from main plot i , block j and subplot k

μ = Overall mean

α_i, i = Effect of seed generation (fixed effect),

β_j, j = Effect of j^{th} block (random effect),

γ_k = Effect of inoculation or plant density (fixed effect),

$(\alpha\beta)_{ij}$ = Random error associated with seed generation in block j ,

$(\alpha\gamma)_{ik}$ = Interaction between seed generation and inoculation or plant density,

$(\beta\gamma)_{jk}$ = Interaction between block j and subplot factor inoculation or plant density,

ε_{ijk} = Error term associated with j^{th} block and subplot factors

The linear statistical model for Screen house experiment was;

$$\gamma_{ijkl} = \mu + \alpha_j + \beta_k + \gamma_i + (\alpha\beta)_{jk} + (\alpha\gamma)_{ji} + (\beta\gamma)_{ki} + (\alpha\beta\gamma)_{jki} + \varepsilon_{ijkl} , \text{ where;}$$

γ_{ijkl} = Observation within k^{th} block, j^{th} site, k^{th} inoculation and i^{th} seed generation

μ = Overall mean

α_j = Main effect of the j^{th} site ($i= 1, 2$)

β_k = Main effect of the k^{th} inoculation ($i= 1, 2$)

γ_i = Main effect of the i^{th} seed generation ($i= 1, 2$)

$(\alpha\beta)_{jk}$ = Interaction effect of j^{th} site and k^{th} inoculation

$(\alpha\gamma)_{ji}$ = Interaction effect of j^{th} site and i^{th} seed generation

$(\beta\gamma)_{ki}$ = Interaction effect of i^{th} seed generation and k^{th} inoculation

$(\alpha\beta\gamma)_{jki}$ = Interaction effect of j^{th} site, k^{th} inoculation and i^{th} seed generation

ε_{ijkl} = Error term associated with i^{th} , j^{th} and k^{th} effects

CHAPER FOUR

RESULTS AND DISCUSSION

4.1 Soil characterisation

Table 4.1 shows results on soil characteristics of Linthipe and Mtubwi. The soils are predominantly sandy clay loams with a pH of 5.0-5.1. Much as soybean and groundnut tolerate wide range of textural classes, soybean and groundnut do well in sandy loam to sandy clay loam soils and pH range of 6 to 8 (Nicol *et al.*, 2008). Groundnut pod and seed quality tend to decline when grown in soils with pH below 5 due to calcium fixation. Calcium levels for both sites was adequate for production of both soybean and groundnut. Soybean and groundnut do well when soil calcium is above 2 cmol_c/kg. The average SOM for top soils were 2.14 ±1.37 % and 1.27 ±0.57 % for Linthipe and Mtubwi sites, respectively. High variation of SOM in Linthipe might have accounted for high variation in soybean and groundnut BNF and grain yield. Low SOM in Mtubwi might have accounted for low BNF and grain yield. Groundnut and soybean do well in soils with minimum of 1.5 % organic matter. SOM reduces P fixation, leaching of nutrients and decreases toxicities of Al and Mn (Baligar *et al.*, 2001).

Inorganic nitrogen, available phosphorus, potassium, magnesium and calcium for Linthipe were all above critical levels for soybean and groundnut growth and development. Soil test results in Mtubwi revealed that only magnesium and potassium were above critical levels for groundnut and soybean growth and development. These crops grow well in soils with more than 25-ppm available phosphorus, 0.1 % inorganic nitrogen, 0.2-ppm potassium, 2 cmol_c/kg calcium and above 0.24 cmol_c/kg

magnesium (Mekki, 2015). Low availability of most nutrients in Mtubwi EPA could be attributed to low SOM and high sand content (75 % to 84 %). Mean N, available P, K, Ca and Mg for Linthipe top soils were; 0.15 %, 33.18 ppm, 0.68 ppm, 17.11 cmol_c/kg and 3.4 cmol_c/kg; respectively. In Mtubwi top soil test results were 0.08 %, 10.8 ppm, 0.61 ppm, 9.14 cmol_c/kg and 1.9 cmol_c/kg for N, P, K, Ca and Mg; respectively.

Table 4.1 Baseline soil properties of Linthipe and Mtubwi EPAs at 0-15cm and 15-30cm

Soil variable	Critical value	Linthipe EPA N=128				Mtubwi EPA N=128			
		0-15 cm		15-30 cm		0-15 cm		15-30 cm	
		Mean	Std dev	Mean	Std dev	Mean	Std dev	Mean	Std dev
pH (water)	6-8	5.1	0.41	5.0	0.40	5.0	0.70	5.0	0.63
% OM	1.50	2.14	1.37	1.64	0.77	1.27	0.57	1.12	0.50
Total N (%)	0.1	0.15	0.04	0.07	0.03	0.08	0.02	0.05	0.03
Available P(ppm)	25	33.18	8.01	27.55	6.81	10.80	4.48	8.91	4.32
K (ppm)	0.20	0.68	0.17	0.52	0.14	0.61	0.64	0.44	0.09
Ca (cmol_c/kg)	2.0	17.11	2.93	10.25	2.36	9.14	2.22	6.01	1.31
Mg (cmol_c/kg)	0.24	3.4	0.58	2.6	0.56	1.9	1.35	1.3	0.51
% Sand	-	71.7	2.49	67.5	2.94	82.5	2.61	77.7	2.67
% Clay	-	21.1	2.30	24.2	2.33	11.9	1.64	15.5	1.64
Textural class	SL	SCL	-	SCL	-	LS	-	SL	-

Std. Dev = standard deviation of means; Critical value = A value below which implies low amount; n = number of observations; LS = Loamy sand; SL = Sandy loam; and SCL = Sandy clay loam

Critical values based on Snapp (1998), Nicol *et al.* (2008) and Mekki (2015)

4.2 Rainfall and Temperature, 2017/2018 cropping season

4.2.1 Rainfall distribution

Figure 4.1 details monthly distribution of rainfall for Mtubwi and Linthipe sites in 2017/18 growing season. Mtubwi received annual rainfall of 706 mm in 38 rainy days while Linthipe received 631 mm rainfall in 58 rainy days. The annual rainfall received at both sites falls within required amount for Tikolore soybean (Kananji *et al*; 2013) and slightly below rainfall required for (medium maturing CG7) groundnut (Ntare *et al.*, 2008). However, rainfall distribution in Machinga was very poor as it rained only 38 days compared to 58 days in Dedza. Consequently, both crops were negatively affected from early podding stage (Mid-February) to harvest period (early May) where it only received less rainfall in Machinga

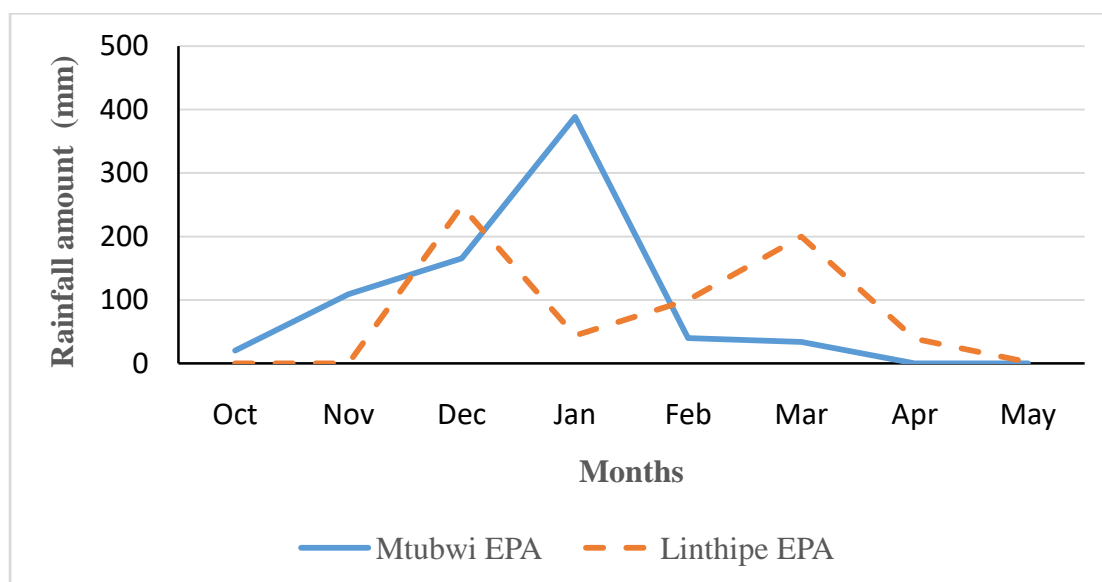


Figure 4.1 Monthly rainfall for Mtubwi and Linthipe EPAs during the 2017/2018 cropping season

4.2.2 Temperature

Lowest mean temperatures were experienced in the month of April of the 2017/2018 growing season in all the two sites (Figure 4.2). Mean monthly temperature in Machinga at planting of 27 °C in November was high for soybean and groundnut germination (25 °C) (Khalil, *et al.*, 2010, ISTA, 2017). In Dedza, at planting in December mean temperature of 24 °C was lower than 25 °C. Mean temperature of 21 °C between month of February and April in Dedza was within recommended for soybean seed setting and development but mean temperature of 23 °C between February and April in Machinga (Mtubwi) was slightly above recommended temperature for soybean seed setting and development (Khalil *et al.*, 2010).

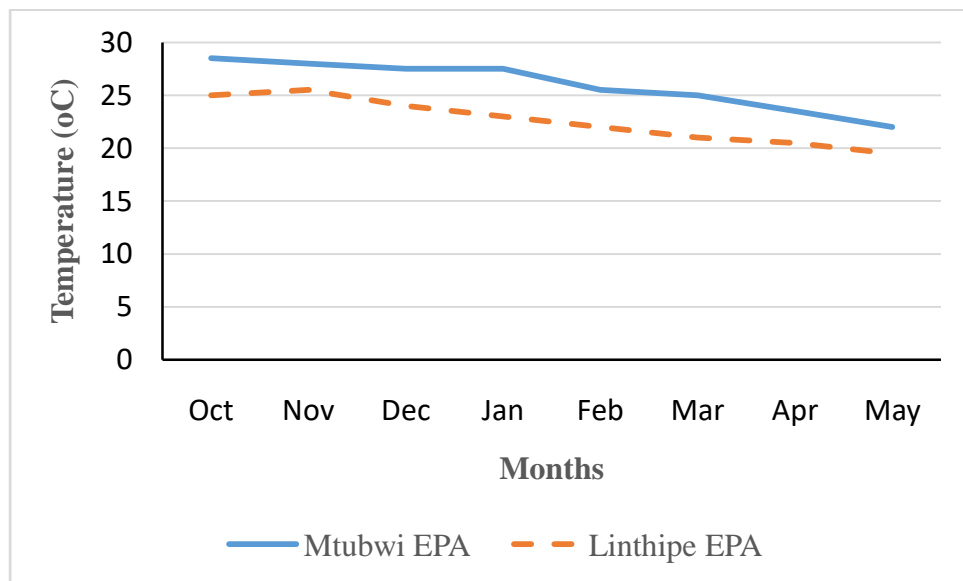


Figure 4.2 Mean monthly temperature for Mtubwi and Linthipe EPAs during the 2017/2018 cropping season

4.3 Effect of seed generation and *Rhizobia* inoculation on soybean nodulation and biological nitrogen fixation

Two generations of Tikolore soybean were evaluated for their response to *Rhizobia* inoculation in Machinga and Dedza districts. The two generations of soybean were farmer-saved seed (recycled for 5 generations) and certified seed. The results of the effect of seed generation and *Rhizobia* inoculation on nodulation and biological nitrogen fixation, presented in this section, respond to first objective of soybean experiment.

4.3.1 Germination and biomass of soybean at early podding stage

Seed germination was not significantly affected by seed generation at both sites (Table 4.2) but was significantly affected by inoculation in Machinga ($p=0.01$) unlike in Dedza. Low soil moisture in Machinga could account for inoculation effect on seedling emergence because inoculated seeds imbibed some water during inoculation process unlike uninoculated dry seeds. Casteel (2011) reported that seed has to absorb enough water in order to be active to activate gibberellic acid, which triggers enzymes within the seed to become functional and metabolise the stored food reserves for the seed to germinate. Adequate soil moisture in Dedza ensured seeds suffered no imbibition stress to trigger germination and seedling emergence. Despite planting in moist soils at both sites, it was observed that it did not rain for four days in Machinga after planting and that account for extremely low seedling emergence of 63 % against 85 % reported in Dedza.

Seed generation affected plant biomass at early podding in Machinga ($p=0.04$) and no significant differences were observed in Dedza. In Machinga, certified and recycled

plants accumulated dry matter of 7.6 g and 7.2 g per plant, respectively (Table 4.2). Under stressful conditions, vigorous plants tend to compete and put in biomass unlike low vigour plants (Abdul-Baki and Anderson, 1973). This agrees with many findings that certified or basic seed tends to be vigorous than farmer saved seed in general (Clyton *et al.*, 2009). *Rhizobia* inoculation increased plant biomass at early podding at both sites by 14 % and 35 % for Machinga and Dedza, respectively. Inoculation increased root growth hence increasing exploration area for nutrients and water, which supported plant growth and biomass accumulation. Sogut (2006) reported that inoculation increased soybean vegetative dry matter accumulation. The interaction of seed generation and *Rhizobia* inoculation was not significant to affect seedling emergence and plant biomass at both sites.

Correlation analysis (Table 4.11 and 4.12) with grain yield showed that plant population at harvest significantly increased grain yield in Machinga ($r = 0.65$, $p < 0.001$) and Dedza ($r = 0.73$, $p < 0.001$).

Table 4.2 Effect of seed generation and Rhizobia inoculation on germination % and biomass at early podding stage of soybean

Treatment	Germination (%)		Biomass (gplant ⁻¹)	
	Machinga	Dedza	Machinga	Dedza
Seed generation				
Certified	66	86	7.6	9.5
Recycled	60	84	7.2	9.2
F. Prob	0.092	0.277	0.04	0.28
LSD	7.8	6.8	0.32	0.79
Inoculation				
Inoculated	68	89	7.9	10.6
Un-inoculated	57	83	6.9	8.0
F. Prob.	0.01	0.376	0.01	0.004
LSD.	7.9	3.5	0.37	1.39

4.3.2 Effect of seed generation and *Rhizobia* inoculation on nodule number per plant in soybean

Results in Table 4.3 show that for nodule numbers per plant, the response to inoculation was dependent on seed generation at both sites ($p < 0.05$). In Machinga, inoculation increased nodule numbers per plant in both recycled and certified seed with higher increase under recycled seed than for certified seed. Recycled seed might have evolved and formed effective symbiosis with introduced bacteria while certified seed, genetically promiscuous, might have failed to form effective symbiosis with

introduced bacteria. Overall, in Machinga, average number of nodules per plant (5) is lower than (20) (Bala *et al.*, 2010). Poor nodule formation in Machinga could be due to dry spells as *Rhizobia* responsible for nodule formation tend to be inactive or die under dry conditions and low available soil phosphorus which play vital role in nodule formation and development (Kumaga and Ofori, 2004; Aduloju *et al.*, 2009, Tahir *et al.*,2009). In Dedza, inoculation increased nodule numbers in recycled soybean seed only with no responses in certified seed. Recycled seed might have evolved and formed effective symbiosis with introduced bacteria while certified seed, genetically promiscuous, might have failed to form effective symbiosis with introduced bacteria. Average nodule number per plant (21) in Dedza is within average nodule number in soybean (Bala *et al.*, 2010). However, in both sites, for the uninoculated seed, higher number of nodules were observed from certified seed than recycled seed probably due to differences in seed quality

Table 4.3 Nodule number per plant in soybean as affected by seed generation and Rhizobia inoculation

Seed generation	Machinga		Dedza	
	Inoculated	Uninoculated	Inoculated	Uninoculated
Certified	0.72 (5)bA	0.52 (3)aB	1.30 (20)aA	1.28 (19)aB
Recycled	0.75 (6)bA	0.39 (2)aA	1.32 (21)bA	1.21 (16)aA
F. Prob (SG)	0.09		0.08	
F. Prob (Inoc)	0.001		0.01	
F. Prob (SG x Inoculation)	0.002		0.011	
LSD (SG x Inoculation)	0.017		0.034	

NB: Nodule numbers are log-transformed; in parenthesis are original nodule numbers
For each location, the number in a row or column followed by the same lower or upper case letters are not significant at $p=0.05$

4.3.3 Effect of seed generation and *Rhizobia* inoculation on nodulation and N content in soybean plant tissue at early podding

Results in Table 4.4 show that seed generation ($p=0.011$) and inoculation ($p=0.001$) significantly increased nodulation for soybean in Dedza. For effective nodulation, response to depended on inoculation in Machinga ($p=0.001$). Nodulation was effective in Dedza but unsatisfactory in Machinga. Nodulation assessment score comprised combined analysis of nodule number, nodule weight, nodule effectiveness

(by colour observation with naked eyes) and vegetative growth (Table 3.1). Nodulation score of 10-15 signifies effective nodulation; 7-9.9 means nodulation less effective and a score of 1-6 means unsatisfactory nodulation (Zaychuk, 2009; Bale *et al.*, 2010). Zaychuk (2009) reported that healthy, vigorous and green plants are most likely to have fully effective symbioses. Clayton (2009) observed that certified seeds produce more vigorous plants compared to farmer saved seeds. Unsatisfactory nodulation in Machinga due to low soil moisture and drought conditions, which led to the reduction in nodule number as *Rhizobia* bacteria responsible for nodulation tend to die or inactivated (Table 4.3). Nodule cell wall lose form, leading to nodule senescence and death after the exposure to drought (Ramos *et al.*, 2003)

Seed generation and inoculation did not significantly affect N content in plant tissue in Machinga but both significantly affected N content in plant tissue in Dedza ($p < 0.05$) (Table 4.4). Variation in cumulative rainfall and some soil factors including soil nitrogen, phosphorus, soil organic matter, pH and texture between these sites might account for varying response (Fig 4.1 and 4.2 and Table 4.1). Ulzena *et al.* (2018) reported that these factors explained about 42–79 % variation in soybean nodulation and grain yield. Certified seed increased N accumulation in plants from 2.9 % to 3.2 % representing 10 % benefit in Dedza. Vigorous growth characteristics associated with certified seed coupled with high photosynthesis rates might account for high N accumulation in certified seed over recycled seed. The soybean mean nitrogen content of 3.5 % in Dedza is higher than values reported by Rahman and Sampa (2012) and Njira *et al.* (2012) but in the same range as the values reported by Ponsakul and Jensen, (1991) and Campbell, (2000)

Table 4.4 Effect of seed generation and *Rhizobia* inoculation on soybean nodulation and plant tissue nitrogen at early podding stage

Treatment	Nodulation score		% N in plant tissue	
	Machinga	Dedza	Machinga	Dedza
Seed generation				
Certified	0.48 (3)	0.98 (10)	1.8	3.2
Recycled	0.55 (4)	1.12 (13)	1.4	2.9
F. Prob	0.05	0.011	0.36	0.017
LSD	0.021	0.022	1.25	0.2
Inoculation				
Inoculated	0.65 (5)	1.18 (15)	1.9	3.6
Un-inoculated	0.36 (2)	0.92 (8)	1.3	2.5
F. Prob	0.001	0.001	0.102	0.001
LSD	0.039	0.063	0.081	0.039

NB: Nodulation scores are log-transformed data; in parenthesis are original nodulation scores and SG is seed generation

4.3.4 Effect of seed generation and *Rhizobia* inoculation on nodule weight and biological nitrogen fixation

Results in Tables 4.5 shows that nodule weight response to inoculation depended on seed generation in Dedza ($p=0.012$) where inoculation increased nodule weight in both recycled and certified soybean treatments. However, no effects observed in Machinga. There were no differences in nodule weight of soybean between the certified and recycled seed within each seed generation.

Table 4.6 shows results on biological nitrogen fixation in soybean. Inoculation increased biological nitrogen fixation of soybean by 74 % in Dedza ($p < 0.004$). In Machinga, biological nitrogen fixation response to inoculation depended on seed generation ($p = 0.002$). Inoculation increased biological nitrogen more in recycled seed than certified seed probably recycled seed might have involved and introduced bacteria formed effective symbiosis with recycled seed than certified seed. Effective inoculation increases infection rate and nodule formation and development, which affect rate of nitrogen fixation. Mohamed and Hassan (2015), Mzumara (2016), and Siyeni (2016) all reported increased nodule weight and BNF of inoculated soybean over un-inoculated soybean.

Results of this study in Dedza agree with Woomer *et al.* (2012) and Njira *et al.* (2012) who reported N fixation ranges of 13.4 kg Nha^{-1} to 35.8 kg Nha^{-1} , per season in Malawi, against estimated 103 kg Nha^{-1} to 313 kg Nha^{-1} per season in Austria (Peoples *et al.*, 1995). Low soil nutrients especially P and low organic matter (Table 4.1) and prolonged drought (Figure 4.1) accounted for low nitrogen fixation in Machinga. Organic matter increases availability of plant nutrients by reducing P fixation, leaching of nutrients and decreasing toxicities of Al and Mn (Baligar *et al.*, 2001). Innocent *et al.* (1995) reported that incorporating organic matter into acid and saline soils increased nodulation and BNF.

Correlation analysis (Table 4.11 and 4.12) showed that fixed nitrogen increased soybean grain yield in Machinga ($r = 0.56$, $p < 0.001$), hence practices aimed at maximising BNF would also maximise grain yield

Table 4.5 Effect of seed generation and inoculation on soybean nodule weight

Seed generation	Dedza		Machinga	
	Nodule weight (mg nodule⁻¹)		Nodule weight (mg nodule⁻¹)	
	Inoculated	Uninoculated	Inoculated	Uninoculated
Certified	75.8b	70.5a	38.2	37.3
Recycled	78.3b	72.1a	38.4	36.3
F. Prob (SG x Inoc)	0.012		0.281	
LSD	3.38		1.49	

Table 4.6 Effect of seed generation and Rhizobia inoculation on nitrogen fixation (kg/ha) in soybean in Machinga and Dedza

	Machinga			Dedza		
	Inoculation			Inoculation		
Seed generation (SG)	Inoculated	Un inoculated	mean	Inoculated	Un inoculated	Mean
Certified	12	18	15	71	37	54
Recycled	20b	8a	14	61	39	50
Mean	16	14		66	38	
F. Prob. SG	0.547			0.689		
F. Prob. inoculation	0.086			0.004		
F. Prob. SG x Inoculation	0.002			0.402		
LSD (inoculation)	5.6			15.4		
LSD (SG x inoculation)	11.1			26.9		

Numbers in a row followed by the same letter are not different at $p < 0.05$

4.4 Effect of seed generation and Rhizobia inoculation on soybean yield and yield components

In this experiment, two generations of Tikolore soybean variety were planted with or without *Rhizobia* inoculant in Machinga and Dedza districts. This section responds to second objective of soybean experiment where the study investigated effect of seed generation and *Rhizobia* inoculation on yield and yield components.

4.4.1 Effect of seed generation and Rhizobia inoculation on pod and seed number per plant

Results in Table 4.7 show that in Machinga, inoculation increased pod and seed number per plant but there was no observed significant effect from seed generation. Inoculation increased number of pods/plant from 14 to 19 representing by 36 % increase and seed number per plant from 19 to 30, representing 58 % increase. Inoculation increases N fixation hence meeting nitrogen demand required for high pod and seed production in low nitrogen environment without application of starter N doses (Ulzena *et al.*, 2018). This agrees with other studies by Abdel-Fattah *et al.* (2011) and Bhuiyan *et al.* (2008) who reported that inoculating mung bean and soybean with *Bradyrhizobium* significantly increased pod number and seed number plant per plant. However, number of pods per plant reported in this study for Tikolore variety were below 37 reported by Mviha *et al.* (2011).

In Dedza, for pod and seed number per plant, the response to inoculation depended on seed generation ($p < 0.05$) (Table 4.8). Seed inoculation increased number of pods and seeds in recycled seed more than in certified seed. The effective symbiosis between

recycled seed and *rhizobia* inoculant resulted in more N fixation, which later was assimilated to pod and seed.

Low pod and seed number per plant in Machinga could be attributed to high temperatures and dry spells during podding and seed setting. Sadeghipour (2012) reported that high temperature above 24 °C and dry spells decreased soybean pods and seed number per plant.

Correlation analysis (Table 4.15 and 4.16) showed that as seed number per plant increased so was grain yield in Machinga ($p < 0.01$) and Dedza ($p < 0.001$). Increase in pod number per plant also increased grain yield in Dedza ($p < 0.001$) but not in Machinga probably some pods might have aborted seed setting due to dry spells.

Table 4.7 Soybean pod and seed number per plant as affected by seed generation and Rhizobia inoculation in Machinga

Treatment	Pod number plant⁻¹	Seed number plant⁻¹
Seed generation		
Certified	1.25 (18)	1.41 (26)
Recycled	1.19 (15)	1.34 (22)
F. Prob	0.114	0.205
LSD	0.084	0.139
Inoculation		
Inoculated	1.29 (20)	1.47 (30)
Un-inoculated	1.15 (14)	1.28 (19)
F. Prob	0.024	0.014
LSD	0.114	0.138

Pod and seed numbers are log-transformed; in parenthesis are original pod and seed numbers and SG is seed generation

Table 4.8 Soybean pod and seed number as affected by seed generation x *Rhizobia* inoculation in Dedza

Seed generation	Pod number plant ⁻¹		Seed number plant ⁻¹	
	Inoculated	Uninoculated	Inoculated	Uninoculated
Certified	1.50 (32)	1.46 (29)	1.89 (78)	1.84 (69)
Recycled	1.54 (35)	1.38 (23)	1.95 (89)	1.74 (55)
F. Prob (SG x Inoc)	0.003		0.004	
LSD (SG x Inoc)	0.009		0.047	

Pod and seed numbers are log transformed; in parenthesis are original pod and seed numbers

4.4.2 Effect of seed generation and *Rhizobia* inoculation on soybean grain yield per plant and seed size

Results in Table 4.9 show that in Machinga inoculation significantly increased grain yield per plant while in Dedza increasing grain yield per plant through inoculation depended on seed generation ($p=0.021$). Inoculation increased seed yield per plant from 3.5 g to 4.6 g, representing 31 %. Inoculation increases N fixation hence meeting nitrogen demand required for high seed production in low nitrogen environment without application of starter N doses. Ulzena *et al.* (2018) reported that biological nitrogen fixation meets 70 % to 80 % of soybean N demand and that soybean N requirement increases for each ton of grain yield. Abdel-Fattah *et al.* (2011), Bhuiyan *et al.* (2008) and Barsum and Abd-El-Gawad (1990) also reported that inoculating soybean with *Rhizobia* significantly increased seed yield per plant. Seed generation and *Rhizobia* inoculation significantly increased seed size in Dedza (Table 4.9).

Table 4.9 Grain yield per plant and seed size as affected by seed generation and inoculation

Treatment	Grain yield plant ⁻¹ (g)		100 seed weight (g)	
	Machinga	Dedza	Machinga	Dedza
Seed generation				
Certified	4.2	12.0	13.6	15.9
Recycled	3.8	10.9	14.5	15.4
F. Prob	0.243	0.091	0.247	0.026
LSD	0.89	1.42	1.76	0.44
Inoculation				
Inoculated	4.6	12.7	13.8	16.4
Un-inoculated	3.5	10.3	14.4	14.9
F. Prob	0.014	0.001	0.056	0.001
LSD	0.80	0.57	2.02	0.63

SG is seed generation

4.4.3 Effect of seed generation and *Rhizobia* inoculation on biological yield, grain yield and harvest index of soybean

Results in Table 4.10 show that in Machinga, biomass production response to inoculation depended on seed generation ($p=0.018$). In Dedza, seed generation ($p=0.013$) and inoculation ($p=0.001$) significantly affected biological yield. Planting certified seed increased biological yield by 12 % while inoculation increased biological yield by 19 %. Certified seed tend to produce vigorous tall and more branched plants than recycled seed (Clyton *et al.*, 2009, Edwards, 2006) while

inoculation improves nitrogen nutrition to the plant by increasing nitrogen availability through BNF. Inoculation effect on biological yield in Dedza agree with Solomon *et al.* (2012) who reported that inoculating soybean with *Rhizobia* strains significantly increased biological yield at mid-flowering to early podding stages.

Correlation analysis (Table 4.15 and 4.16) showed that total biomass increased grain yield in Machinga ($p < 0.001$) and Dedza ($p < 0.001$). Again step-wise regression analysis picked out total biomass, as the strongest individual grain yield predictor with the highest coefficient determination of 90.2 % when tested in simple linear regression, hence efforts to maximise grain yield must focus on increasing biomass yield first.

Results in Table 4.11 show that in terms of grain yield, response to inoculation depended on seed generation in Machinga ($p = 0.016$). Inoculation increased soybean grain yield more for recycled seed than for certified seed. In Dedza, both seed generation and inoculation increased grain yield ($p < 0.001$) (Table 4.12). Grain yield of 1709 kg ha^{-1} and 1453 kg ha^{-1} were obtained from certified and recycled seed, respectively, representing 18 % benefit of certified seed over recycled seed. Certified seed has high yield potential since it grows vigorously and form more branches for pod and seed production than recycled seed. Edwards (2006) reported 48 % increase in wheat grain yield when certified seed was planted over 5th generation farmer saved seed. Clyton *et al.* (2009) reported 12 % reduction in canola grain yield when farmer saved seed was planted over certified seed. Grain yield of 1733 kg ha^{-1} and 1429 kg ha^{-1} were obtained from inoculated and un-inoculated seed, respectively, representing 21 % effect of inoculation in Dedza. Inoculation increases N fixation hence meeting

nitrogen demand required for high grain production in low nitrogen environments without application of starter N doses. Ulzen *et al.* (2018) reported that biological nitrogen fixation meets 70 % to 80 % of soybean N demand and that soybean N requirement increases for each ton of grain yield.

The yield potential of Tikolore soybean is 2500 kg ha^{-1} (Mviha *et al.*, 2011) and reported average grain yield under smallholder farms in Malawi is 1000 kg ha^{-1} (MoAFS, 2012). The maximum grain yield of 1733 kg ha^{-1} obtained in this study from Dedza site is 73 % higher than yield reported under smallholder farms in Malawi but comparable to 1732 kg ha^{-1} reported by Mzumara (2016) and 1385 kg ha^{-1} by Siyeni (2016) under on-farm trials in Malawi.

Seed generation did not significantly affect harvest index at both sites. Inoculation increased harvest index in Machinga only from 0.27 to 0.32, representing 19 % ($p=0.02$) (Table 4.8). Inoculation increases nitrogen accumulation, which affect seed production. Ali (2017) reported that seed inoculation with *Rhizobia* increased soybean harvest index by 9 % over uninoculated i.e. from 35.5 % to 38.8 %. Overall, 30 % of total biomass in Machinga was converted to grain yield while in Dedza 37 % of total biomass was assimilated to grain yield.

Correlation analysis showed that increase in harvest index led to increase in grain yield in both sites (Table 4.15 and 4.16) hence efforts to maximise grain yield must also consider practices that would optimise harvest index.

Table 4.10 Biological yield and harvest index of soybean as affected by seed generation and inoculation

Treatment	Biological yield (kg ha ⁻¹)		Harvest Index (%)	
	Machinga	Dedza	Machinga	Dedza
Seed generation				
Certified	1621	4589	30	37
Recycled	1455	4080	29	36
F. Prob	0.218	0.013	0.837	0.133
LSD	330.6	306.5	6.6	2.5
Inoculation				
Inoculated	1482	4708	32	37
Un-inoculated	1593	3961	27	36
F. Prob	0.403	0.001	0.02	0.168
LSD	302.0	135.8	4.2	1.2

SG is seed generation

Table 4.11 Soybean grain yield (kgha⁻¹) as affected by seed generation x inoculation in Machinga and Dedza districts

	Machinga			Dedza		
	Inoculation			Inoculation		
Seed generation (SG)	Inoculated	Un inoculated	Mean	Inoculated	Un inoculated	Mean
Certified	429	561	495	1876	1543	1710
Recycled	543	346	445	1590	1316	1453
Mean	486	454		1733	1430	
F. Prob. SG	0.405			0.031		
F. Prob. inoculation	0.533			0.029		
F. Prob SG x Inoculation	0.016			0.216		
LSD (inoculation)	120.5			53.3		
LSD (SG x inoculation)	167.5			54.8		

4.5 Correlation analysis for studied parameters

4.5.1 Correlation of soil and soybean parameters

Results in Table 4.12 show correlation analysis of 7 key soil properties with soybean growth, yield and yield components in Machinga, which show varied significant correlations with root and shoot biomass, % N at early podding stage, total biomass, biological nitrogen fixation (BNF) and grain yield. Soil organic matter, residual nitrogen, available P and magnesium showed significant correlation with all growth, yield and yield components parameters studied ($p < 0.05$), calcium showed significant correlation with all except BNF. Soil pH and K showed significant correlation with all except % plant N and BNF ($p < 0.05$). Correlation analysis of soil properties with soybean growth, yield and yield components in Dedza (Table 4.13) showed that soil organic matter, calcium, residual nitrogen, available P and magnesium had significant correlation with all growth, yield and yield components parameters studied ($p < 0.05$). Soil pH and K showed significant correlation with all except % plant N, BNF and grain yield ($p < 0.05$).

In Machinga Soil pH and available P were below critical levels, however calcium was above critical level required for soybean production soybean production (Table 4.1). In Dedza soil organic matter %, available K and magnesium were above critical levels for soybean production (Table 4.1). Low calcium impair nitrogen fixation because attachment of *Rhizobia* bacteria to root hairs is affected (Smith *et al.*, 1992) and nodule effectiveness and development (Alava *et al.*, 1991). As natural stress, pH has far-reaching effects on the growth and nutrient uptake by plants and therefore grain yield. Phosphorus requirement for nitrogen fixation might be higher than for root or shoot growth in soybean. Nodules themselves are

strong sinks for P and nodulation and N₂ fixation are strongly influenced by P availability (Singleton *et al.*, 1985)

Table 4.12 Correlation of studied soil properties with various studied soybean parameters for Machinga district

	Root DM	Shoot DM	% plant N	Biomass	BNF	Grain yield
pH	0.69***	0.50**	0.24	0.53**	0.20	0.52**
OM	0.50**	0.78***	0.67***	0.65***	0.60***	0.64***
P	0.63***	0.69***	0.66**	0.70***	0.76***	0.65***
% soil N	0.40*	0.56**	0.65***	0.73***	0.47**	0.60***
Mg	0.53**	0.70***	0.57**	0.78***	0.64***	0.76***
K	0.46**	0.40*	0.18	0.48**	0.25	0.17
Ca	0.39*	0.34*	0.48**	0.78***	0.22	0.70***

*, ** and *** correlation is significant at <0.05, <0.01 and <0.001 levels of probability, respectively and coefficients without marked asterisk (*) are not significant at these levels of probability. Root DM= Root dry matter, Shoot DM= Shoot dry matter, Biomass and grain yield are in kg/ha

Table 4.13 Correlation of studied soil properties with various studied soybean parameters for Dedza district

	Root DM	Shoot DM	% plant N	Biomass	BNF	Grain yield
pH	0.40*	0.65***	0.12	0.59**	0.28	0.43*
OM	0.67***	0.85***	0.80***	0.72***	0.70***	0.69***
P	0.75***	0.70***	0.71**	0.84***	0.86***	0.67***
% soil N	0.38*	0.68**	0.60***	0.70***	0.34*	0.69***
Mg	0.63***	0.75***	0.70***	0.80***	0.72***	0.66***
K	0.49**	0.36*	0.23	0.48**	0.11	0.42*
Ca	0.42*	0.53**	0.40*	0.67***	0.65***	0.38*

*, ** and *** correlation is significant at <0.05, <0.01 and <0.001 levels of probability, respectively and coefficients without marked asterisk (*) are not significant at these levels of probability. Root DM= Root dry matter, Shoot DM= Shoot dry matter, Biomass and grain yield are in kg/ha

4.5.2 Correlation of soybean parameters with grain yield

In Machinga, five soybean parameters showed significant correlation with grain yield. Plant population at harvest ($r = 0.65$, $p < 0.001$), total biomass ($r = 0.95$, $p < 0.001$), BNF ($r = 0.56$, $p < 0.001$), seed number per plant ($r = 0.46$, $p < 0.01$) and harvest index ($r = 0.60$, $p < 0.001$) (Table 4.14). In Dedza, five soybean parameters showed significant correlation with grain yield. Plant population at harvest ($r = 0.73$, $p < 0.001$), total biomass ($r = 0.85$, $p < 0.001$), seeds per plant ($r = 0.74$, $p < 0.001$), pods per plant ($r = 0.69$, $p < 0.001$) and harvest index ($r = 0.47$, $p < 0.01$) (Table 4.15). High plant population, pod and seed number per plant at optimum plant density increases grain yield because of the additive effects these parameters have (Abdel-

Fattah *et al.*, 2011, Phakamas *et al.*, 2008). Plant biomass is a function of plant population (400,000 to 450, 000 plants/ha) and is the same biomass assimilated to grain production. Efficient plants/ environment convert great deal of biomass by partitioning it to grain production. (Zimmer *et al.*, 2016, Ali, 2017, Ulzen *et al.*, 2018) reported high soybean grain yield from high plant biomass and harvest index plots.

When these parameters were subjected to stepwise regression analysis, total biomass was chosen as strongest single predictor of the 5 potential predictors of grain yield. Simple linear regression showed that biomass yield accounted for 90.2 % variation in grain yield with a standard error of 60 (lowest value among the 5 predictors). General linear mixed model analysis of the factors used in the study showed compelling ‘site’, ‘seed generation’ and ‘inoculation’ effect on soybean grain yield (Table 4.16).

Table 4.14 Correlation of matrix among various studied parameters of Tikolore soybean variety in Machinga district

	% plant N	BNF	Bio yield	PP	Grain yld	H.I	Seeds plant⁻¹	Pods plant⁻¹
% plant N	-							
BNF	-0.26	-						
Bio yield	-0.01	0.57***	-					
PP	0.08	0.47**	0.77***	-				
Grain yld	-0.07	0.56***	0.95***	0.65***	-			
H.I	-0.16	0.25	0.35*	-0.04	0.60***	-		
Seeds plant⁻¹	-0.25	0.27	0.39*	-0.02	0.46**	0.50**	-	
Pods plant⁻¹	-0.26	0.24	0.24	-0.04*	0.32	0.40*	0.93***	-

*, ** and *** correlation is significant at <0.05, <0.01 and <0.001 levels of probability, respectively; coefficients without marked asterisk (*) are not significant at these levels of probability; PP = Plant population at harvest, Bio yield = Biological yield and H.I = Harvest index

Table 4.15 Correlation of matrix among various studied parameters of Tikolore soybean variety in Dedza district

	% plant N	BNF	Bio yield	PP	Grain yld	H.I	Seeds plant⁻¹	Pods plant⁻¹
% plant N	-							
BNF	-0.26	-						
Bio yield	0.18	-0.20	-					
PP	-0.04	-0.37*	0.45**	-				
Grain yld	0.29	-0.11	0.85***	0.73***	-			
H.I	0.21	0.14	-0.06	-0.09	0.47**	-		
Seeds plant⁻¹	0.36*	-0.06	0.77***	0.22	0.74***	0.11	-	
Pods plant⁻¹	0.30	-0.02	0.73***	0.19	0.69***	0.08	0.99***	-

*, ** and *** correlation is significant at <0.05, <0.01 and <0.001 levels of probability, respectively; coefficients without marked asterisk (*) are not significant at these levels of probability; PP = Plant population at harvest, Bio yield = Biological yield and H.I = Harvest index

Table 4.16 Summary results of the GLM model for explaining grain yield variability of soybean in the on-farm trials in Machinga and Dedza districts

Model	F-statistic	P-value	Df
Site	508.85	< 0.001	1
Seed generation	218.84	< 0.001	1
Inoculation	252.49	< 0.001	1
Site x seed generation	13.36	0.011	1
Site x inoculation	2.22	0.013	1
Inoculation x seed generation	4.27	0.037	1
Site x seed generation x inoculation	1.27	0.281	1

4.6 Effect of seed generation and plant density on biological nitrogen fixation and yield of groundnut

Performance of two generations of groundnut variety ICVG-SM 83708, locally known as CG7, were evaluated at two plant densities in Machinga and Dedza districts. The two generations were farmer-saved seed (recycled for 6 generations) and certified seed produced same season. The two plant densities were 89 000 and 178 000 plants/ha, for single and twin row planting, respectively.

4.6.1 Groundnut biomass at early podding stage

Results in Table 4.17 show that seed generation significantly affected germination % in both sites ($p < 0.05$). In Dedza, the mean plant density after germination for certified seed under single and twin rows were 35,200 plants/ha and 102,800 plants/ha, respectively; while for recycled seed were 67,600 plants/ha and 142,800 plants/ha for

single and twin rows, respectively. This means that certified seed under single and twin rows reduced plant population by 60.4 % and 42.2 %, respectively; while for recycled seed reduction was 24.0 % and 19.8 % for single and twin rows, respectively. In Machinga, the mean plant density after germination for certified seed under single and twin rows were 53,000 plants/ha and 135,298 plants/ha, respectively; while for recycled seed were 61,099 plants/ha and 145,195 plants/ha for single and twin rows, respectively. This means that certified seed under single and twin rows reduced plant population by 40.5 % and 24.0 %, respectively; while for recycled seed reduction was 31.4 % and 18.4 % for single and twin rows, respectively. The reason recycled seed germinated better could be that unshelled pods protected seed before shelling for planting while certified seed was packaged shelled in plastic packets six months prior to planting. Yousif and Hussein (2019) reported that groundnut seed should be kept in the shell until near time to planting since viability declines rapidly after shelling. Certified seed germinated better in Machinga than Dedza because after planting seed in Dedza on moist soil it rained once followed by a 6 days dry spell while in Machinga after planting on moist soil it rained 3 days later. Casteel (2011) reported that seed has to absorb enough water to activate gibberellic acid, which triggers the enzymes within the seed to metabolise the stored food reserves for the seed to germinate. Studies have established that plant population affect grain yield and biological nitrogen fixation in groundnut. Rasekh *et al.* (2010) pointed out that pod yield increased with increasing plant density from 3.0-8.3 plants m⁻² but increasing plant density to 14.8 plants m⁻² pod yield decreased.

Results in Table 4.17 show that in Machinga the effect of seed generation to affect biomass per plant depended on plant density (p=0.013) while seed generation

significantly increased plant biomass per plant at early podding ($p=0.012$) in Dedza. High plant biomass in certified and single rows could be attributed to vigorous and competitive growth habit exhibited by certified seed (Clyton *et al.*, 2009, Edwards 2006). Rasekh *et al.* (2010) reported reduced biomass yield per plant as plant density increased from 8.3 plants/m² to 14.8 plants/m² as was the case in this trial.

Correlation analysis (Table 4.26 and 4.27) revealed strong relationship of plant population at harvest with grain yield in Dedza ($r =0.95$, $p<0.001$) and Machinga ($r =0.93$, $p<0.001$).

Table 4.17 Germination % and biomass of groundnut at early podding stage

Treatment	Germination %		Biomass plant ⁻¹ (g)	
	Machinga	Dedza	Machinga	Dedza
Seed generation				
Certified	67.8	48.7	44.2	55.3
Recycled	75.1	78.1	41.5	53.6
F. Prob	0.048	0.011	0.018	0.012
LSD	7.23	16.61	1.84	1.01
Plant density				
Twin row	78.8	69	37.0	54.6
Single row	64.1	57.8	48.7	55.3
F. Prob	0.006	0.025	0.003	0.149
LSD	3.46	9.24	2.14	0.42

NB: SG is seed generation and PD is plant density

4.6.2 Effect of seed generation and plant density on nodule number and tissue nitrogen in groundnut at early podding stage

Results in Table 4.18 show that seed generation had no significant effect on nodule number per plant at both sites. However, in Machinga, plant density significantly affected number of nodules per plant ($p=0.03$). Nodule number per plant for single and twin row were 100 and 110, respectively, representing 10 % increase, which could be due to active photosynthesis. Wanger (2012) reported that actively photosynthesizing plants fix more nitrogen for its own growth and that of resident bacteria, which affect nodule formation and biological nitrogen fixation. More plants in twin rows than single rows might have increased moisture retention, moisture is a limiting factor for photosynthesis and low plant population in single rows might have encouraged evaporation due to direct radiation, hence reducing photosynthetic rates as plants close stomata to cut on water loss thereby reducing carbon dioxide assimilation.

Seed generation and plant density did not significantly affect nitrogen content in groundnut at early podding stage at both sites. The average N content in plant tissue was 1.6 % and 2.8 % for Machinga and Dedza, respectively.

Table 4.18 Nodule number and tissue nitrogen at early podding as affected by seed generation and plant density

Treatment	Nodule number plant ⁻¹		Tissue nitrogen (%)	
	Machinga	Dedza	Machinga	Dedza
Seed generation				
Certified	2.01 (102)	2.12 (134)	1.6	2.7
Recycled	2.02 (105)	2.13 (135)	1.6	2.8
F. Prob	0.609	0.30	0.809	0.679
LSD	0.032	0.039	0.86	0.82
Plant density				
Twin row	2.04 (110)	2.14 (138)	1.8	2.6
Single row	2.00 (100)	2.11 (128)	1.4	2.9
F. Prob	0.04	0.114	0.109	0.394
LSD	0.009	0.039	0.55	0.87

NB: Nodule numbers are log transformed; in parenthesis are original nodule numbers, SG is seed generation and PD is plant density

4.6.3 Effect of seed generation and plant density on biological nitrogen fixation in groundnut

Results in Table 4.19 show that seed generation significantly affected biological nitrogen fixation in Dedza ($p=0.011$) but not in Machinga. In Dedza, recycled and certified seed fixed 68 kg Nha⁻¹ and 56 kg Nha⁻¹, respectively; representing 21.4 % recycled seed superiority over certified seed. Plant density significantly affected BNF at both sites ($p<0.05$). In Machinga, twin and single rows fixed 53 kg Nha⁻¹ and 40 kg Nha⁻¹, respectively, representing 32.5 % twin row advantage. In Dedza, twin and single

rows fixed 72 kg Nha⁻¹ and 52 kg Nha⁻¹, respectively, representing 38.5 % advantage of planting in twin rows.

Since biological nitrogen fixation in groundnut is a function of plant population and biomass yield (Rasekh *et al.*, 2010; Mhango *et al.*, 2017). Appendix 5 is a covariance analysis, which showed that increased BNF in recycled seed was due to high plant population. The reason for high nitrogen fixation by recycled seed and twin rows was due to more biomass production per unit area achieved by high plant population than certified seed and single rows, which had low plant population since BNF is calculated directly from plant biomass.

In this study reported 72 kg Nha⁻¹ under twin rows in Dedza is higher than 55.8 kg Nha⁻¹ reported by Njira *et al.* (2012) on sole groundnut trials in Kasungu district and 57 kg Nha⁻¹ reported by Mhango (2011) on sole groundnut trials in Mzimba district. This means that planting in twin rows is important in order to increase nitrogen fixation.

Correlation analysis (Table 4.25 and 4.26) revealed that as BNF increased grain yield also increased in Dedza ($r = 0.91$, $p < 0.001$) and Machinga ($r = 0.43$, $p < 0.05$). More nitrogen fixed is used to support photosynthesis resulting in more grain production.

Table 4.19 Biological nitrogen fixation in groundnut as affected by seed generation and plant density in Machinga and Dedza

Treatment	BNF (kg ha ⁻¹)	
	Machinga	Dedza
Seed generation		
Certified	46	56
Recycled	47	68
F. Prob	0.767	0.011
LSD	16.0	4.9
Plant density		
Twin row	53	72
Single row	40	52
F. Prob	0.025	0.004
LSD	10.5	4.4

NB: SG is seed generation and PD is plant density

4.7 Effect of seed generation and plant density on yield and yield components of groundnut

This section responds to second objective of on-farm groundnut experiment where this study investigated effect of seed generation and plant density on yield and yield components. Treatments in this section were same as those of the first objective (section 4.5).

4.7.1 Effect of seed generation and plant density on number of pods/plant, number of seeds/plant and grain yield per plant in groundnut

Results in Table 4.20 show that in Dedza, seed generation significantly affected pod yield per plant ($p=0.043$) but not pod number per plant, while plant density affected both ($p<0.05$). Pod yield per plant for certified seed was 21.16g and for recycled seed was 17.38g, this represents 21.7 % increase. Per plant pod number for twin rows was 19 and for single row was 28, this represents 47 % increase, while twin row and single row pod yield per plant was 17.96g and 21.59g, respectively, this represents 20.2 % increase. Seed generation x plant density interaction did not significantly affect pod number and yield per plant.

Results also showed that seed generation significantly affected seed yield per plant ($p=0.015$) but not seed number per plant. Certified seed yield per plant was 15.40g and recycled seed yield was 12.10g, this represents 27.3 % increase. Plant density significantly affected both seed number and yield per plant ($p<0.05$). Per plant seed number for twin rows was 32 and for single rows was 46, this represents 43.8 % increase, while twin row and single row seed yield per plant was 12g and 15.51g, respectively, this represents 29.3 % increase.

Significant low per plant pod and seed yield for recycled seed reducing yield potential could be due to minimum cross pollination that still occurs in self-pollinated groundnut. Clayton *et al.* (2009) reported 12 % canola yield reduction in recycled over certified seed and Edwards (2006) reported 48 % wheat yield reduction when third generation seed was recycled. Low per plant yield in twin rows could be due to competition for growth resources such as nutrients, water and light as number of

plants per unit area increased. Gabisa *et al.* (2017), Onat *et al.* (2017), Howlader *et al.* (2009) and Magagula *et al.* (2019) reported highest and lowest number of matured pods and yield per plant at lowest and highest plant densities, respectively.

Results in Table 4.21 show that in Machinga, pod and seed number per plant and grain yield per plant depended on seed generation x plant density interaction ($p < 0.05$). Highest pod and seed number per plant and grain yield per plant were obtained from certified x single row plots and low pod and seed number and grain yield per plant from recycled x twin row plots. High yielding potential of certified seed and reduced competition in single rows accounted for the observed trend. Clayton *et al.* (2009) and Edwards (2006) reported 12 % and 48 % high yield for certified canola and wheat over recycled seed, respectively. Konlan *et al.* (2013) reported that pod yield and number per plant increased at low plant density in groundnut since reduced competition allows more branches and pegs to form horizontally.

Correlation analysis (Table 4.26) revealed that as pod number ($r = -0.97$, $p < 0.001$) and seed number ($r = -0.94$, $p < 0.001$) per plant increased grain yield per unit area decreased in Machinga. The reason is that pod and seed number per plant increases as plant population decreases hence cumulative yield is low under low plant density. This inverse relationship was not significant in Dedza this could mean that competition for growth resources was not maximum in Dedza, particularly owing to its good edaphic and climatic conditions.

Table 4.20 Pod and seed number per plant and grain yield per plant affected by seed generation and plant density in Dedza

Treatment	Pod number plant ⁻¹	Pod yield plant ⁻¹ (g)	Seed number plant ⁻¹	Seed yield plant ⁻¹ (g)
Seed generation				
Certified	1.39 (25)	21.2	1.61 (41)	15.4
Recycled	1.35 (22)	17.4	1.57 (37)	12.1
F. Prob	0.06	0.043	0.114	0.015
LSD	0.047	0.74	0.068	0.19
Plant density				
Twin row	1.29 (19)	18.0	1.51 (32)	12.0
Single row	1.44 (28)	21.6	1.66 (46)	15.5
F. Prob _{PD}	0.001	0.014	0.001	0.002
LSD	0.036	0.48	0.035	0.25

NB: Pod and seed numbers are log transformed and values in parenthesis are original pod and seed numbers, SG is seed generation and PD is plant density.

Table 4.21 Pod and seed number per plant and grain yield per plant affected by seed generation and plant density in Machinga

	Pod number plant ⁻¹		Pod yield plant ⁻¹ (g)		Seed number plant ⁻¹		Seed yield plant ⁻¹ (g)	
	Twin row	Single Row	Twin row	Single row	Twin row	Single row	Twin row	Single row
Seed generation								
Certified	1.21 (17)	1.39 (25)	15.38	18.04	1.40 (25)	1.59 (39)	8.9	9.6
Recycled	1.14 (13)	1.30 (19)	13.41	17.80	1.31 (20)	1.53 (34)	8.1	9.1
F. Prob	0.039		0.009		0.044		0.011	
LSD (SG x PD)	0.032		0.81		0.035		0.61	

Note: Pod and seed numbers are log-transformed; in parenthesis are original pod and seed numbers.

4.7.2 Effect of seed generation and plant density on plant biomass, grain yield, harvest index, shelling percent and seed size

Table 4.22 show results on total biomass, grain yield, harvest index, shelling percent and seed size. Seed generation significantly affected total biomass in Dedza ($p=0.013$) but not Machinga. Recycled and certified seed total biomass were 5645 kg ha^{-1} and 3811 kg ha^{-1} , respectively, representing 48 % increase. Plant density significantly affected total biomass at both sites ($p<0.001$). In Dedza, total biomass for twin and single rows were 5682 kg ha^{-1} and 3773 kg ha^{-1} , respectively, representing 50.6 % increase, while in Machinga, total biomass for twin and single rows were 3965 kg ha^{-1} and 2509 kg ha^{-1} , respectively, representing 58 % increase.

Correlation analysis (Table 4.25 and 4.26) revealed that as total biomass increased grain yield also increased in Dedza ($r = 0.96$, $p<0.001$) and Machinga ($r = 0.97$, $p<0.001$). Again, stepwise regression analysis picked it as the single and strongest predictor of grain yield with a coefficient determination of 90.9 %. Therefore, practices aimed at increasing grain yield must focus on biomass productivity.

Seed generation significantly affected grain yield in Dedza ($p=0.041$). Grain yield from certified and recycled seed were 1101 kg ha^{-1} and 1368 kg ha^{-1} , respectively, representing 23 % lower yield than from recycled seed. Poor certified seed germination in this study lowered plant population and account for low grain yield (Table 4.17). Grain yield in groundnut is a function of plant population (Mhango *et al.*, 2017; Onat *et al.*, 2017; Zhao *et al.*, 2017). Appendix 5 is a covariance analysis, which showed that increased grain yield for recycled seed was due to high plant population.

Plant density significantly affected grain yield at both sites ($p < 0.001$). In Dedza, planting groundnut at high density in twin rows (122,820 plants/ha) increased grain yield from 714 kg ha^{-1} obtained at low density in single rows (51,442 plants/ha) to 1755 kg ha^{-1} , representing 146 %. In Machinga, grain yield for high density twin rows (140,264 plants/ha) and low density single rows (57,672 plants/ha) were 818 kg ha^{-1} and 437 kg ha^{-1} respectively, representing 87 % increase. High grain yield at optimum density in twin rows could be due to more plants per unit area, which improved efficient utilization of growth resources such as moisture, light and nutrients while at low plant density, reduced competition for growth resources favours vegetative growth at the expense of grain assimilation. Magagula *et al.* (2019) reported that differences in seed yield of groundnuts might be due to efficient partitioning of assimilates into the grain rather than the pod in the higher plant density (88,889 plants/ha) and more luxurious growth in the lower plant density (29,630 plants/ha) favoured more pod formation than seed yield. Gabisa *et al.* (2017) reported highest grain yield per hectare at high plant density (250, 000 plants/ha) and low pod and grain yield at low plant density (142, 857 plants/ha). Rasekh *et al.* (2010), Onat *et al.* (2017), Kurt *et al.* (2017), Zhao *et al.* (2017) and Magagula *et al.* (2019) support these findings.

Grain yield obtained in this study in single rows, 714 kg ha^{-1} in Dedza and 437 kg ha^{-1} in Machinga is below national average of 600 kg ha^{-1} (MoAFS, 2012) and within 521 kg ha^{-1} reported by Mhango (2011). However, this study found that planting in twin rows is viable option to increase grain yield (192.5 %) above current national average at $1,755 \text{ kg ha}^{-1}$

Plant density significantly increased harvest index at both sites ($p < 0.05$) but seed generation did not increase at both sites. In Machinga, harvest index for twin and single rows were 37 % and 33 %, respectively, this represents 12 % increase. In Dedza, harvest index for twin and single rows were 42.1 % and 34.8 %, respectively, this represents 21 % increase. High plant population in twin rows might have increased efficient utilization of growth resources including soil moisture, light energy and nutrients towards reproductive organs while luxury growth in single rows might have promoted vegetative growth at the expense of grain filling. Howlader *et al.* (2009) reported that decrease in plant density favors huge vegetative growth at the expense of reproductive function hence lowering harvest index. Gabisa *et al.* (2017) reported highest harvest index (36.5%) at 250 000 plants ha⁻¹ and lowest harvest index (15.17%) at lowest plant density of 142 000 plants ha⁻¹.

In Machinga, effect of seed generation to increase groundnut shelling % and seed size depended on plant density ($p < 0.05$) Seed generation did not significantly affect shelling % at both sites ($p > 0.05$). In Dedza, seed generation and plant density did not significantly increase seed size and shelling %. While investigating effect of plant density on groundnut seed size in Turkey, Kurt *et al.* (2017) reported non-significant difference of seed size of the same cultivars. Howlader *et al.* (2009) reported that plant population had no effect on shelling % and seed size as they were genetically controlled.

Table 4.22 Total biomass, grain yield, harvest index, shelling percent and 100 seed weight of groundnut as affected by seed generation and plant density

Treatment	Total biomass (kgha ⁻¹)		Grain yield (kgha ⁻¹)		Harvest index %		Shelling %		100 seed weight (g)	
	Machinga	Dedza	Machinga	Dedza	Machinga	Dedza	Machinga	Dedza	Machinga	Dedza
Seed generation										
Certified	3105	3811	588	1101	34	38.8	55.1	68.6	52.5	54.3
Recycled	3369	5645	667	1368	35	38.1	56.4	69.7	50.5	52.8
F. Prob	0.144	0.013	0.084	0.041	0.69	0.285	0.155	0.165	0.053	0.073
LSD	427.4	1098.1	99.0	246.6	0.5	1.30	2.21	0.60	1.96	0.87
Plant density										
Twin row	3965	5682	818	1755	37	42.1	59.0	70.1	50.9	52.0
Single row	2509	3773	437	714	33	34.8	52.4	68.1	52.1	55.2
F. Prob	<0.001	<0.001	<0.001	<0.001	0.043	0.006	0.038	0.102	0.057	0.903
LSD	296.3	549.9	62.1	106.3	0.4	3.37	0.90	0.68	1.33	0.41

4.8 Correlation analysis for studied parameters

4.8.1 Correlation of soil with groundnut yield and yield components

Results in Table 4.23 and 4.24 show correlation analysis of 7 key soil properties with groundnut BNF, yield and yield components, which show varied significant correlations with BNF, pod and seed number plant⁻¹, total biomass, harvest index, pod and seed yield (kg ha⁻¹) and shelling %. Inorganic soil N, available P, Ca, and % sand and clay showed significant correlation with all selected parameters mentioned. However, % sand negatively correlated with the selected parameters. Soil pH and magnesium did not significantly correlate with any of the selected parameters. Soil organic matter %, significantly correlated with harvest index, seed yield and seeds plant⁻¹. The effect of available P and soil mineral N was significant in all parameters presented. One possible reason for negative correlation of % sand with all parameters could be associative effects such as low nutrient availability, low organic matter, increased bulk density and poor water holding capacity. Low % clay and high % sand in Machinga partly explains why the study found low BNF, pod and seed yield despite the site having high plant population compared to Dedza.

4.8.2 Correlation of groundnut parameters with grain yield

Results in Table 4.25 and 4.26 show that in a correlation matrix of 7 studied parameters with grain yield, 6 parameters were significant on grain yield which were BNF, total biomass, plant population at harvest, pod number per plant, seed number per plant, seed size and harvest index ($p < 0.05$). In all sites; seed size, seeds and pods per plant negatively correlated with grain yield while plant population at harvest, BNF and total biomass positively correlated with grain yield.

Plant population affect biomass yield as well as BNF and high biomass yield is achieved at high plant density. Konlan *et al.* (2013) and Rakesh *et al.* 2010) reported linear relationship between plant biomass and pod yield and that grain yield is calculated from pod yield multiplied by shelling %. High grain yield is reported at high plant population while high harvest index reported at both high plant population and high grain yield. At low plant densities seed size, pods and seeds per plant increase but the cumulative effect on per unit area reduces grain yield. Onat *et al.* (2017), Gabisa *et al.* (2017) and Magagula *et al.* (2019) reported low grain yield at low plant densities (below 100,000 plants/ha)

When these parameters were subjected to stepwise regression analysis, total biomass was chosen as single strongest predictor of the 6 potential predictors of grain yield. Simple linear regression showed that total biomass accounted for 90.9 % variation in grain yield with a standard error of 159 (lowest value among the 6 predictors). Evidence of direct and strong relationship of total biomass with grain yield is critical to improve management practices and breeding programs that optimise groundnut biomass to increase grain yield. In this study, it was found that increasing grain yield depended on seed generation while the response of grain yield to plant density depended on site (Table 4.27).

Table 4.23 Correlation of studied soil properties with various studied groundnut parameters for Dedza district

	BNF	Biomass	Pod yld	H.I	Seed yld	Shelling %
pH	0.40*	0.25	0.49**	0.36*	0.40*	0.30
SOM %	0.56**	0.76***	0.46**	0.19	0.42*	0.43*
P	0.80***	0.70***	0.50**	0.62***	0.55**	0.68***
% N	0.38*	0.68***	0.50**	0.55**	0.66***	0.67***
Mg	0.72***	0.75***	0.69***	0.54**	0.57**	0.44*
K	0.22	0.39*	0.58**	0.54**	0.62***	0.26
Ca	0.38*	0.43*	0.77***	0.55**	0.65***	0.65***

*, ** and *** correlation is significant at <0.05, <0.01 and <0.001 levels of probability, respectively and coefficients without marked asterisk (*) are not significant at these levels of probability. BNF, biomass, pod yield and seed yield are in kg/ha.

Table 4.24 Correlation of studied soil properties with various studied groundnut parameters for Dedza district

	BNF	Biomass	Pod yld	H.I	Seed yld	Shelling %
pH	0.49**	0.25	0.43*	0.38*	0.41*	0.30
OM	0.42*	0.46**	0.49**	0.28	0.44*	0.40*
P	0.73***	0.63***	0.52**	0.60***	0.56**	0.65***
% N	0.42*	0.65***	0.62***	0.55**	0.63***	0.70***
Mg	0.68***	0.73***	0.65***	0.54**	0.47**	0.39*
K	0.25	0.36*	0.68***	0.48**	0.60***	0.22
Ca	0.34*	0.31*	0.65***	0.43*	0.50**	0.43*

*, ** and *** correlation is significant at <0.05, <0.01 and <0.001 levels of probability, respectively and coefficients without marked asterisk (*) are not significant at these levels of probability. BNF, biomass, pod yield and seed yield are in kg/ha, H.I= Harvest index

Table 4.25 Correlation of studied groundnut parameters in Dedza district

	PP	Grain yld	Plant N	Seeds/plant	Pods/plant	H.I	Biomass	BNF	100-SW
PP	-								
Grain yield	0.95***	-							
Plant N	-0.17	0.15	-						
Seeds/plant	-0.85***	-0.84***	0.12	-					
Pods/plant	-0.90***	-0.90***	0.14	0.95***	-				
H.I	0.82***	0.91***	-0.11	-0.83***	-0.85***	-			
Biomass	0.97***	0.96***	-0.46*	-0.87***	-0.81***	0.79***	-		
BNF	0.95***	0.91***	0.78***	-0.40*	-0.38*	0.60**	0.94***	-	
100-SW	-0.43*	-0.40*	-0.10	-0.43*	-0.36*	-0.58**	-0.33	-0.49**	-

*, ** and *** correlation is significant at <0.05, <0.01 and <0.001 levels of probability, respectively; coefficients without marked asterisk (*) are not significant at these levels of probability; PP = Plant population, Biomass= (kg/ha) and H.I = Harvest index, SW = Seed weight

Table 4.26 Correlation of studied groundnut parameters in Machinga district

	PP	Grain yld	Plant N	Seeds/plant	Pods/plant	H.I	Biomass	BNF	100-SW
PP	-								
Grain yield	0.93***	-							
Plant N	0.27	0.26	-						
Seeds/plant	-0.93***	-0.92***	-0.35	-					
Pods/plant	-0.96***	-0.96***	-0.32	0.97***	-				
H.I	0.96***	0.95***	-0.23	-0.92***	-0.95***	-			
Biomass	0.96***	0.97***	-0.42*	-0.93***	-0.97***	0.92***	-		
BNF	0.86***	0.80***	0.86***	-0.50**	-0.46**	0.42*	0.43*	-	
100-SW	-0.58**	-0.48**	-0.28	-0.47*	-0.45**	-0.47**	-0.45**	-0.21	-

*, ** and *** correlation is significant at <0.05, <0.01 and <0.001 levels of probability, respectively; coefficients without marked asterisk (*) are not significant at these levels of probability; PP = Plant population, Biomass= (kg/ha), SW= Seed weight and H.I = Harvest index.

Table 4.27 Summary results of the GLM model-explaining groundnut grain yield variability in the on-farm trials in Machinga and Dedza districts

Model	F-statistic	P-value	df
Seed generation	15.55	0.008	1
Density	803.31	<0.001	1
Site	40.36	0.008	1
Seed generation x density	0.01	0.921	1
Site x seed generation	476	0.072	1
Site x density	161.62	<0.001	1
Site x seed generation x density	2.37	0.150	1

4.9 Effect of mother environment on soybean and groundnut seed quality

The objective of the Screen house experiment was to assess quality of soybean and groundnut seed produced in Dedza and Machinga under on-farm experiments above. It took 103 and 124 days from the day of planting to harvest soybean and groundnut, respectively, from the on-farm experiments in Machinga while in Dedza it took 125 and 143 days from the day of planting to harvest soybean and groundnut, respectively. Briefly, three months after harvesting these crops, seeds were sown in the Screen house at Bunda College Crop Science Students Research Farm. Prior to planting, seed dormancy in groundnut was broken following ISTA rules (2017) as described in section 3.5. Seed viability test using in between paper method was conducted before full experiment to test if soybean and groundnut from on-farm experiments were viable. Seed quality properties tested herein determine potential crop establishment in

the field and hence decide plant population, which affect total biomass and grain yield as shown in the two on-farm experiments above.

Treatments shown in the tables of results are those received by the parent plants in the mother environment. Quality assessment on soybean and groundnut was done 12 days after planting (DAP) based on ISTA rules (2017). This section present results of effect of mother environment on seed quality.

4.9.1 Effect of mother environment on soybean germination and seedling emergence 12 days after planting

Results in Table 4.28 show that mother environment ($p=0.002$) and seed generation ($p=0.004$) significantly affected seed germination of soybean. Germination for seed produced from Dedza and Machinga were 87 % and 76 %, respectively. 1st and 6th recycled seed germination were 84 % and 79 %, respectively. Seed production in Machinga was done under temperature above 26 °C, which is high for soybean seed (Khali *et al.*, 2010). Li *et al.* (2017) reported that seed produced under high temperature and low elevation tend to have low longevity and high electrical conductivity of leachate, therefore it could be because of this that seed from Machinga, on low elevation, had low germination. Such seed with low germination if planted in the field reduces plant population and biomass and consequently grain yield. Germination for seed produced from Dedza and 1st recycled seed were within 80 % recommendation by ISTA (2017) and Seed Service Unit in Malawi (2009)

Results in Table 4.29 show that mother environment significantly affected seedling emergence ($p<0.001$) of soybean. Seedling emergence of seed produced from Dedza and Machinga were 82 % and 67 %, respectively. Seed generation significantly

affected seedling emergence ($p=0.044$) with average values of 77 % and 72 % for 1st and 6th recycled seed, respectively.

High seedling emergence of seed produced from Dedza than Machinga can be attributed to low temperature in Dedza. Low temperature slows seed maturation process and this increases duration for nutrient translocation to seed resulting in large seed with high nutrient density. Canavar and Kaynak (2013) reported that delayed groundnut maturity increased seed size and oleic and protein content. Khalil *et al.* (2010) reported that soybean seed harvested from mother plant exposed to 18/10 °C day and night temperature resulted in large seed with higher germination and emergence than seed harvested from mother plant grown at 25/15 °C day and night temperature. Mean temperature for Machinga was above 26 °C day and night temperature during seed set and maturation in February and March (Fig 4.1)

Table 4.28 Effect of mother environment on soybean germination 12 days after planting

	Germination %, N=32				Mean
	T1	T2	T3	T4	
Mother environment					
Machinga	75.0	77.5	69.0	82.5	76
Dedza	86.0	85.5	84.0	91.8	87
Mean	81	82	77	87	
	F. Prob	LSD			
Mother environment	0.002	3.35			
Seed generation	0.004	3.74			
Inoculation	0.016	4.55			
E x seed generation	0.202	4.20			
E x inoculation	0.748	4.98			
SG x inoculation	0.704	5.44			
E x SG x inoculation	0.541	7.29			

Note: T1=6th recycled soybean inoculated, T2=1st recycled soybean un-inoculated, T3=6th recycled soybean un-inoculated, T4=1st recycled soybean inoculated, LSD=seed generation and E=mother environment

Table 4.29 Effect of mother environment on soybean seedling emergence 12 days after planting

	Seedling emergence %, N=32				Mean
	T1	T2	T3	T4	
Mother environment					
Machinga	66.0	68.2	60.2	72.5	67
Dedza	80.5	81.8	79.5	87.2	82
Mean	73	75	70	80	
	F. Prob	LSD			
Mother environment	<0.001	2.92			
Seed generation	0.044	1.97			
Inoculation	0.088	4.85			
E x seed generation	0.139	2.87			
E x inoculation	0.701	5.14			
SG x inoculation	0.742	5.05			
E x SG x inoculation	0.513	7.18			

Note: T1=6th recycled soybean inoculated, T2=1st recycled soybean un-inoculated, T3=6th recycled soybean un-inoculated, T4=1st recycled soybean inoculated, SG= seed generation and E= mother environment

4.9.2 Effect of mother environment on soybean seedling biomass and vigour 12 days after planting

Results in Table 4.30 show that for high seedling biomass, effect of seed generation depended on mother environment ($p=0.003$). Seedlings with more biomass were observed from Dedza x 1st recycled seed pots. Delayed maturity due to low temperature in Dedza could have led to production of relatively large seed with high nutrient density. Li *et al.* (2017) reported that the best environment for the production

of high quality *Vicia sativa* seed is one where temperature is relatively low during seed development.

Results in Table 4.31 show that mother environment, seed generation and inoculation significantly affected seedling vigor ($p < 0.05$). Seedling vigor for Machinga and Dedza were 429 and 513, respectively, for 1st and 6th recycled seed were 500 and 442, respectively.

1st recycled seed outperforming 6th recycled seed might be due to inherent vigor. Edwards (2006) and Clyton *et al.* (2009) reported that 1st recycled wheat and canola seed outperformed 3rd recycled seed on measures of germination, vigour and growth rate. High biomass and vigor seedlings from Dedza might be due to low temperature during seed development, adequate soil moisture and nutrients hence prolonged growing period, which allowed more nutrient like NPK and Ca translocation to the seed compared to short growing duration in Machinga.

High quality soybean seed produced in Dedza than Machinga could be due to variability in terms of growth resources such as residual nutrients. Correlation analysis Table 4.13 show that residual soil nutrients affected soybean productivity including grain production.

Table 4.30 Effect of mother environment on soybean seedling biomass 12days after planting

Seedling dry weight (g), N=32					Mean
Mother environment	T1	T2	T3	T4	
Machinga	5.78	5.58	5.57	5.65	5.7
Dedza	5.71	6.00	5.48	6.41	5.9
Mean	5.8	5.8	5.5	6.0	
	F. Prob	LSD			
Mother environment	0.180	0.57			
Seed generation	0.011	0.24			
Inoculation	0.031	0.19			
E x seed generation	0.003	0.53			
E x inoculation	0.312	0.53			
SG x inoculation	0.906	0.28			
E x SG x inoculation	0.389	0.54			

Note: T1=6th recycled soybean inoculated, T2=1st recycled soybean un-inoculated, T3=6th recycled soybean un-inoculated, T4=1st recycled soybean inoculated, SG=seed generation and E= mother environment

Table 4.31 Effect of mother environment on soybean seedling vigour 12 days after planting

Mother environment	Seedling vigor (Index II), N=32				Mean
	T1	T2	T3	T4	
Machinga	434	432	383	466	429
Dedza	491	513	460	589	513
Mean	463	473	422	528	
	F. Prob.	LSD			
Mother environment	<0.001	59.1			
Seed generation	<0.001	32.1			
Inoculation	0.001	29.1			
E x seed generation	0.229	55.7			
E x inoculation	0.682	55.0			
SG x inoculation	0.633	39.7			
E x SG x inoculation	0.266	63.5			

Note: T1=6th recycled soybean inoculated, T2=1st recycled soybean un-inoculated, T3=6th recycled soybean un-inoculated, T4=1st recycled soybean inoculated, SG=.seed generation and E= mother environment.

4.9.3 Effect of mother environment on groundnut germination and seedling emergence 12 days after planting

Results Table 4.32 and 4.33 show that seed generation, and mother environment x plant density interaction increased seed germination and seedling emergence ($p < 0.05$). High germination % was observed in Dedza x single row produced seed and high emergence in Dedza x twin row produced seed. Germination for 1st and 7th recycled seed were 85 % and 78 %, respectively, while germination for Dedza x

single row produced seed was 84 %. According to ISTA (2017) and Seed service Unit in Malawi (2009) germination % for groundnut should be above 80 %. Seedling emergence for 1st and 7th recycled seed were 76 % and 66 %, respectively, while germination % for Dedza x twin row produced seed was 74 %. High germination % in Dedza x single row produced seed could be due to adequate seed reserves in the large seed produced, which was due to low temperature and reduced competition for growth resources like soil moisture and nutrients like NPK and calcium, which were above critical levels. Dedza and Machinga seed sizes were 53.8 and 51.5 grams, respectively. Increased competition for growth resources in Dedza in twin rows might have led to production of marginally small seed on average than in single rows that gave high emergence. Hundred (100) seed weight for single and twin rows produced in Dedza were 55.2 and 52 grams, respectively. Balesevic-Tubic *et al.* (2005) reported that small seeds emerge faster than large seed because of reduced resistance to growing media.

Table 4.32 Effect of mother environment on groundnut germination 12 days after planting

Mother environment	Seed germination %, N=32				Mean
	T1	T2	T3	T4	
Machinga	71.2	85.2	80.0	79.8	79
Dedza	78.2	87.2	82.5	87.2	84
Mean	75	86	81	84	
	F. Prob	LSD			
Mother environment	0.040	6.13			
Seed generation	0.004	5.09			
Plant density	0.274	3.68			
E x density	0.041	5.89			
E x seed generation	0.854	6.49			
SG x plant density	0.885	5.77			
E x SG x plant density	0.289	7.79			

Note: T1= 6th recycled groundnut-twin rows, T2= 1st recycled groundnut-single rows, T3= 6th recycled groundnut-twin rows, T4= 1st recycled groundnut-twin rows, SG= seed generation and E= mother environment.

Table 4.33 Effect of mother environment on groundnut seedling emergence 12 days after planting

	Seedling emergence %, N=32				Mean
	T1	T2	T3	T4	
Mother environment					
Machinga	51.0	72.8	69.8	70.8	66
Dedza	75.2	79.5	67.8	79.0	75
Mean	63	76	69	75	
	F. Prob	LSD			
Mother environment	0.002	8.29			
Seed generation	0.002	5.61			
Plant density	0.243	5.94			
E x plant density	0.038	8.39			
E x seed generation	0.459	5.14			
SG x plant density	0.438	7.50			
E x SG x plant density	0.052	10.86			

Note: T1= 6th recycled groundnut-twin rows, T2= 1st recycled groundnut-single rows, T3= 6th recycled groundnut-twin rows, T4= 1st recycled groundnut-twin rows, SG= seed generation and E= mother environment.

4.9.4 Effect of mother environment on groundnut seedling biomass and vigour 12 days after planting

Results in Table 4.34 and 4.35 show that seed generation and mother environment x plant density interaction significantly affected seedling biomass and vigour ($p < 0.05$). Seedlings with more biomass and vigour were observed in Dedza x single row produced seed. Seedling biomass for 1st and 7th recycled seed were 10.5 and 9.1 grams, respectively, while for Dedza x single row produced seed was 10.5 grams compared to Dedza twin row produced seed biomass of 9.8 grams. Seedling vigour

for 1st and 7th recycled seed were 892 and 746, respectively, while for Dedza x single row produced seed was 869 compared to Dedza x twin row produced seed vigour index of 781.

Seedlings with more biomass and vigour produced from Dedza x single row and 1st recycled seed could be due to large seed produced, which tends to have high nutrient density required to nourish young plant at early stage. Seeds produced from single rows were marginally larger than those produced in twin rows and that could account for more biomass and vigorous seedlings. Li *et al.* (2017) and Canavar and Kaynak (2013) reported that seedlings developed from small seeds have low biomass and vigour. High germination rates and biomass of 1st recycled and Dedza x single row produced seed accounted for production of more vigorous seedlings. According to Abdul-Baki and Anderson (1973), seedling vigour is a product of germination rate and seedling biomass; therefore, seedlot with high vigour is the one that germinate well and produce more biomass seedlings. Consequently, seedlots with high vigour will likely maximise grain yield as the two on-farm experiments above showed that plant biomass accounted for large part of grain yield variability. For both soybean and groundnut.

High quality groundnut seed produced in Dedza than Machinga could be due to variability in terms of growth resources such as residual nutrients. Correlation analysis Table 4.24 show that residual soil nutrients affected groundnut productivity including grain production.

Table 4.34 Effect of mother environment on groundnut biomass 12 days after planting

Seedling dry weight (g), N=32					Mean
Mother environment	T1	T2	T3	T4	
Machinga	8.30	10.58	9.36	9.08	9.3
Dedza	9.25	10.44	9.26	11.64	10.1
Mean	8.8	10.5	9.3	10.4	
	F. Prob	LSD			
Mother environment	0.04	1.57			
Seed generation	0.027	0.56			
Plant density	0.554	0.72			
E x density	0.009	1.45			
E x seed generation	0.331	1.47			
SG x plant density	0.886	0.81			
E x SG x plant density	0.413	1.55			

Note: T1= 6th recycled groundnut-twin rows, T2= 1st recycled groundnut-single rows, T3= 6th recycled groundnut-twin rows, T4= 1st recycled groundnut-twin rows, SG= seed generation and E= mother environment.

Table 4.35 Effect of mother environment on groundnut seedling vigour 12 days after planting

Seedling vigor (index-II), N=32					Mean
Mother environment	T1	T2	T3	T4	
Machinga	599	902	749	723	743
Dedza	811	950	825	990	894
Mean	705	926	787	857	
	F. Prob	LSD			
Mother environment	<0.001	180.4			
Seed generation	<0.001	72.3			
Plant density	0.312	76.8			
E x plant density	<0.001	166.5			
E x seed generation	0.363	167.8			
SG x plant density	0.907	96.8			
E x SG x plant density	0.780	179.3			

Note: T1= 6th recycled groundnut-twin rows, T2= 1st recycled groundnut-single rows, T3= 6th recycled groundnut-twin rows, T4= 1st recycled groundnut-twin rows, SG= seed generation and E=mother environment

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

In Machinga, biological nitrogen fixation and soybean grain yield response to inoculation was high for recycled seed than certified seed. In Dedza, inoculating soybean increased BNF while inoculation or seed generation increased grain yield.

Planting in twin rows increased groundnut nitrogen fixation and grain yield in Machinga while in Dedza planting in twin rows and planting recycled seed increased nitrogen fixation and grain yield. Poor germination for certified seed was the cause for low nitrogen fixation and grain yield.

High quality soybean and groundnut seed were produced in high potential area, Dedza than low potential area, Machinga.

5.2 Recommendations

This study recommends inoculating recycled soybean seed for high productivity in Mtubwi EPA and planting certified soybean seed or inoculating in Linthipe EPA for high productivity. Planting certified groundnut seed and in twin rows must be encouraged in all sites only that there is need to intensify seed handling practices (after harvest) that prevents seed quality deterioration like proper drying and keeping the seed in unshelled pods until near time to planting. Seed production must be restricted to high potential areas like Linthipe EPA because seed quality strongly depended on maternal environment where seed was produced. Economic analysis on the use of inputs used in these experiments must be evaluated to provide practical recommendations on smallholder farms.

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APPENDICES

Appendix 1 Soil characteristics of two soybean plots in Mtubwi EPA

Soil variable	Critical value	Plot 1 n=16				Plot 2 n=16			
		0-15 cm		15-30 cm		0-15 cm		15-30 cm	
		Mean	Std dev	Mean	Std dev	Mean	Std dev	Mean	Std dev
pH (water)	5-7	4.2	0.22	4.4	0.32	5.4	0.67	5.4	0.69
Total N (%)	0.1	0.08	0.02	0.06	0.03	0.08	0.02	0.06	0.02
Inorganic P (ppm)	25	8.01	3.65	6.23	3.60	14.77	3.91	12.57	3.93
K (ppm)	0.20	0.44	0.07	0.35	0.04	0.89	1.24	0.49	0.04
Ca (cmol_c/kg)	2.0	4.53	1.40	3.35	1.03	5.46	1.12	4.54	0.95
Mg (cmol_c/kg)	0.24	1.03	0.47	0.69	0.32	0.93	0.16	0.62	0.16
% OM	1.70	0.83	0.48	0.62	0.32	0.93	0.41	0.65	0.24
% Sand	-	84.8	1.11	79.7	1.63	81.4	1.95	76.9	1.12
% Clay	-	10.5	0.59	14.5	0.61	12.3	1.15	15.6	1.89
Textural class	SL	LS	-	LS	-	LS	-	SL	-

Std. Dev = standard deviation of means; Critical value = A value below which implies low amount; n = number of observations; LS = Loamy sand; SL = Sandy loam; and SCL = Sandy clay loam

Critical values based on Snapp (1998), Nicol *et al.* (2008) and Mekki (2015)

Appendix 2 Soil characteristics for two soybean plots in Linthipe EPA

Soil variable	Critical value	Farm 1 n=16				Farm 2 n=16			
		0-15 cm		15-30 cm		0-15 cm		15-30 cm	
		Mean	Std dev	Mean	Std dev	Mean	Std dev	Mean	Std dev
pH (water)	5-7	5.0	0.23	5.0	0.19	4.8	0.34	4.7	0.38
Total N (%)	0.1	0.14	0.03	0.08	0.03	0.10	0.01	0.04	0.02
P_Av. (ppm)	25	31.65	6.44	26.60	5.95	35.09	9.18	27.96	7.47
K (ppm)	0.20	0.68	0.24	0.52	0.15	0.63	0.07	0.49	0.12
Ca (cmol_c/kg)	2.0	9.56	1.46	7.93	1.15	7.78	1.44	6.34	1.23
Mg (cmol_c/kg)	0.24	2.77	0.45	2.34	0.45	2.52	0.62	2.05	0.45
% OM	1.70	1.50	0.77	1.30	0.53	1.60	0.45	1.31	0.62
% Sand	-	72.3	2.16	67.8	2.30	72.6	2.41	68.8	2.79
% Clay	-	20.1	2.26	23.4	2.15	20.6	1.43	23.7	1.22
Textural class	SL	SCL	-	SCL	-	SCL	-	SCL	-

Std. Dev = standard deviation of means; Critical value = A value below which implies low amount; n = number of observations; LS = Loamy sand; SL = Sandy loam; and SCL = Sandy clay loam

Critical values based on Snapp (1998), Nicol *et al.* (2008) and Mekki (2015)

Appendix 3 Soil characteristics of two groundnut plots in Mtubwi EPA

Soil variable	Critical value	Farm 1 n=16				Farm 2 n=16			
		0-15 cm		15-30 cm		0-15 cm		15-30 cm	
		Mean	Std dev	Mean	Std dev	Mean	Std dev	Mean	Std dev
pH (water)	5-7	5.0	0.43	4.9	0.49	5.6	0.47	5.3	0.38
Total N (%)	0.1	0.08	0.02	0.06	0.04	0.08	0.01	0.05	0.02
P_Av. (ppm)	25	8.39	2.00	6.82	1.60	12.0	4.40	10.02	4.40
K (ppm)	0.20	0.47	0.07	0.38	0.05	0.66	0.09	0.55	0.05
Ca (cmol_c/kg)	2.0	5.05	1.26	4.14	0.64	4.72	0.85	4.00	0.57
Mg (cmol_c/kg)	0.24	3.17	1.76	1.42	0.18	1.06	0.43	0.66	0.27
% OM	1.70	1.53	0.42	1.39	0.36	1.80	0.34	1.72	0.31
% Sand	-	84.3	0.95	79.1	1.56	79.5	1.59	75.0	2.01
% Clay	-	10.8	0.56	14.8	0.79	14.0	1.17	17.2	1.49
Textural class	SL	LS	-	LS	-	LS	-	SL	

Std. Dev = standard deviation of means; Critical value = A value below which implies low amount; n = number of observations; LS = Loamy sand; SL = Sandy loam; and SCL = Sandy clay loam

Critical values based on Snapp (1998), Nicol *et al.* (2008) and Mekki (2015)

Appendix 4 Soil characteristics of two groundnut plots in Linthipe EPA

Soil variable	Critical value	Farm 1 n=16				Farm 2 n=16			
		0-15 cm		15-30 cm		0-15 cm		15-30 cm	
		Mean	Std dev	Mean	Std dev	Mean	Std dev	Mean	Std dev
pH (water)	5-7	5.1	0.20	5.1	0.17	5.5	0.44	5.4	0.46
Total N (%)	0.1	0.19	0.02	0.08	0.03	0.17	0.03	0.07	0.02
P_Av. (ppm)	25	36.56	7.78	29.86	7.30	29.43	7.03	25.78	6.34
K (ppm)	0.20	0.69	0.20	0.51	0.16	0.74	0.11	0.57	0.11
Ca (cmol_c/kg)	2.0	12.88	2.34	10.35	1.83	10.61	2.20	8.86	1.21
Mg (cmol_c/kg)	0.24	3.09	0.16	2.58	0.35	2.28	0.54	1.84	0.56
% OM	1.70	2.82	2.20	1.96	0.99	2.68	0.80	1.98	0.61
% Sand	-	69.9	2.46	65.5	3.05	72.0	2.12	68.0	2.72
% Clay	-	23.0	2.44	26.2	2.80	20.5	1.89	23.7	1.93
Textural class	SL	SCL	-	SCL	-	SCL	-	SCL	-

Std. Dev = standard deviation of means; Critical value = A value below which implies low amount; n = number of observations; LS = Loamy sand; SL = Sandy loam; and SCL = Sandy clay loam

Critical values based on Snapp (1998), Nicol *et al.* (2008) and Mekki (2015)

Appendix 5 Analysis of variance of groundnut biological nitrogen fixation as affected by seed generation adjusted for covariate plant density, in Machinga and Dedza

Source of variation	d.f.	s.s.	m.s.	v.r.	cov.ef.	F pr.
Rep stratum	3	53.5	17.8	0.13		
Rep.*Units* stratum						
Seed generation	1	410.1	410.1	3.08	1.00	0.178
Plant density	1	4096	4096	73.16	1.00	<.001
SG x PD	1	22.6	22.6	0.40	1.00	0.549
Covariates	1	2992.9	2992.9	19.19		<.001
Residual	47	7329.1	155.9		1.38	
Total	54	15639.8				

Appendix 6 Analysis of variance of groundnut grain yield as affected by seed generation adjusted for covariate plant density, in Machinga and Dedza

Source of variation	d.f.	s.s.	m.s.	v.r.	cov.ef.	F pr.
Rep stratum	3	30069	10023	0.57		
Rep.*Units* stratum						
Seed generation	1	481028	481028	27.24	1.00	0.014
Plant density	1	8082885	8082885	1473.25	1.00	<.001
SG x PD	1	1577	1577	0.26	1.00	0.630
Covariates	1	3557072	3557072	32.33		<.001
Residual	47	5171044	110022		1.65	
Total	54					