GROWTH, NODULATION AND YIELD RESPONSE OF PROMISCUOUS AND NON-PROMISCUOUS SOYBEAN CULTIVARS TO INOCULATION IN DIFFERENT SOIL TYPES UNDER GLASSHOUSE AND FIELD CONDITIONS

by

TSAKANI MARIA MAPHOSA

MINI-DISSERTATION

Submitted in partial fulfilment of the requirements for the degree of

Master of Science

in

Agriculture (Agronomy)

in the

FACULTY OF SCIENCE AND AGRICULTURE

(School of Agricultural and Environmental Sciences)

at the

UNIVERSITY OF LIMPOPO

Supervisor: Prof I.K. Mariga

Co-supervisor: Prof F.R. Kutu

(North-West University, Mafikeng Campus)

MARCH 2015

DECLARATION

I declare that the mini-dissertation hereby submitted to the University of Limpopo, for the degree of Master of Science in Agriculture (Agronomy) has not previously been submitted by me for a degree at this or any other university; that it is my work in design and in execution, and that all material contained herein has been duly acknowledged.

Maphosa T.M (Ms) 25 March 2015

Surname, Initials (title) **Date**

DEDICATION

This work is dedicated to my adorable two nephews Rifumo Maphosa and Ntoto Chabalala, and to my dearly loved parents Mr Ronald Godfrey and Mrs Rhulani Margreth Maphosa; I have witnessed their love in providing for my needs every step of the way.

ACKNOWLEDGEMENTS

I thank my helper and protector the almighty God, for protecting me throughout the study and for providing me with strength to complete this research. I could not have made it if it was not for Him.

I appreciate my supervisor Prof I.K Mariga and my co-supervisor Prof F.R Kutu for all their patience, motivation and support throughout the study; without their contributions this study would not have been successful. Thanks to the Departmental technicians Mr FH Nndwambi and Mrs PM Kgopa for their assistance, and all the lecturers who contributed to this study. I appreciate with thanks, the Department of Agriculture Forestry and Fisheries (DAFF) for providing me with financial assistance that made this study possible. Prof Felix Dakora of Tshwane University of Technology (TUT) for providing the promiscuous soybean seed used in this study.

Special thanks go to my parents, for teaching me the importance of working hard and for giving me the opportunity to study at this institution. Thanks to TJ Mokase, P.S Lediga and N Netshithuthuni for their help with field and laboratory work. May God bless them at all times. I also thank my sisters Tlharihani and Xongile Maphosa, my brothers Tiyani, Matimba and Winners Maphosa, my cousins, as well as other family members for their assistance. Thanks to all my friends, for always being there for me at the time I needed them most. Special gratitude goes to my best man M.I Magomani for his support throughout the study.

ABSTRACT

Soybean (*Glycine max* (L.) Merrill) is considered to be an important grain legume and an oil crop. It is also important in livestock feeding and improvement of soil fertility through biological nitrogen fixation (BNF). Until recently, soybean was not widely grown by smallholder (SH) farmers in Africa. This has led to breeding of promiscuous varieties to ensure wide adoption of the crop by SH farmers, without the use of inoculants or expensive nitrogen fertilizers. Field and glasshouse experiments were conducted during 2012/2013 growing season. One commercial (specific) variety Dundee and three naturally-nodulating (promiscuous) soybean varieties (TGx-1937-1F, TGx-1740-2F, TGx-1835-10E) were evaluated in a field trial for their growth, nodulation and yield response to *B. japonicum* strain WB74 inoculation. Seed inoculation in the field enhanced chlorophyll content, number of nodules, nodule dry weight, and the percentage of active nodules, number of pods, hundred seed weight, shelling percentage and grain yield. Varietal differences exerted significant ($P \leq 0.05$) effect on all field parameters evaluated except on nodule number and percentage of active nodules. TGx-1937-1F achieved the highest number of nodules (28 per plant) while the highest percentage of active nodules (69%) was achieved by TGx-1740-2F. Huge effect of inoculation was observed on Dundee variety, and resulted in significant grain yield increases (237.8%) while smaller gain increases were observed in TGx-1740-2F (43.9%) and TGx-1835-10E (38.7%). The yield of TGx-1937-1F did not respond to inoculation.

Two promiscuous (TGx-1937-1F and TGx-1740-2F) varieties and one commercial (Dundee) variety were evaluated in a glasshouse trial for their growth and nodulation response to inoculation in different soil types (sandy clay loam, sandy loam, loamy sand) of Limpopo Province. In the glasshouse inoculation showed effect on chlorophyll content only, and effect of soybean variety was found to be significant on days to flowering, chlorophyll content, plant height, number of nodules and root dry weight. Soil type showed significant effect on all parameters evaluated in the glasshouse study except on nodule dry weight. Loamy sand soil from Ga-Molepo gave tallest plants and highest nodule number at 61 cm and 29 nodules/plant compared to other soils. All soils evaluated in the study resulted in percent active nodules ranging from 74.5% to 77.4% showing possibility of presence of cowpea-type rhizobia in Limpopo soils capable of fixing atmospheric nitrogen. Inoculation x variety interaction was significant on days to flowering, plant height and chlorophyll content. Inoculant application in TGx-1740-2F variety reduced the number of days it took to flowering from 61 to 54 days and increased its plant height by 57% from 44.8 to 67.9 cm. Eighty three percent (83%) increase on chlorophyll content of variety Dundee was observed due to effect of inoculation. Inoculation x soil type interaction had significant effect on plant height and dried plant biomass. Varity x soil type interaction influenced chlorophyll content, while the interactive effects of inoculation x variety x soil type were significant on chlorophyll content only. The study showed that it is beneficial to inoculate the soybean varieties studied, especially the commercial variety Dundee, in order to enhance their growth, nodulation and yield.

Key words: Promiscuous soybean, *B. japonicum*, Inoculation, Soil type, Nodulation, Grain yield.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background

Soybean (*Glycine max* (L.) Merrill) is an important summer annual crop classified both as a grain legume and an oil crop. It is also important for livestock feed and it is grown as a commercial crop in many African countries. Soybean seed contains about 40% protein, 20% oil and 30% carbohydrate (Tefera, 2011). According to Singh and Singh (2010), soybean is important for the production of biodiesel, and biodiesel is considered to be a clean and renewable fuel which becomes the best source to substitute diesel fuel. Soybean plants can fix up to 200 kg N/ha per year thereby reducing the need for expensive and environmentally harmful nitrogen fertilizers (Javaid and Mahmood, 2010). In South Africa, Mpumalanga is the main area for growing soybean followed by KwaZulu-Natal and the Free State, while in Limpopo Province soybean is produced in Waterberg and Sekhukhune districts (DAFF, 2010).

There are promiscuous (naturally-nodulating) and specifically nodulating (non-promiscuous) soybean cultivars. The non-promiscuous soybean is usually inoculated with compatible commercial inoculants of rhizobia type (*Bradyrhizobium japonicum)* before planting in order for them to form effective nodules, while promiscuous ones form effective nodules with cowpea-type rhizobial strains or the indigenous rhizobia (Gwata *et al.,* 2004). New cultivars of soybean are continuously being developed (Akande *et al*., 2007). It is therefore important to evaluate these cultivars so as to determine their performance in terms of yield and adaptation to different ecologies.

Nitrogen is one of the most important plant nutrients needed for proper plant growth and development. Rhizobia are bacteria capable of fixing nitrogen in association with the plant. According to Sessitsch *et al*. (2002), rhizobia covers a range of bacterial genera, including *Rhizobium, Bradyrhizobium, Sinorhizobium, Mesorhizobium, Allorhizobium* and *Azorhizobium*. These bacterial genera are capable of establishing a symbiotic association with leguminous plants, and bring out the formation of nodules on roots of their hosts and reduce atmospheric nitrogen making it available to plants in these nodules (Sessitsch *et al*., 2002).

Inoculation is the process of adding effective rhizobia to the seed or soil before planting. According to Balesevic-Tubic *et al*. (2011), inoculation with a proper strain of nitrogen fixing bacteria is the best way to ensure good nodulation for increased yield in an environmentally safe manner. Shiferaw *et al*. (2004) mentioned that biological nitrogen fixation (BNF) is less costly and more sustainable. Most of the soils in Africa are characterized by low levels of BNF activities and usually do not support high yields of soybean without adding the chemical nitrogen fertilizers or the external application of soybean rhizhobia (Abaidoo *et al*., 2007).

In order to avoid the use of inoculants and chemical fertilizers, the International Institute of Tropical Agriculture (IITA) developed the Tropical *Glycine* crosses (TGx), also known as promiscuous cultivars of soybean (Sanginga *et al.,* 1996), so as to encourage widespread adoption and cultivation of soybean by smallholder (SH) farmers. The input costs of producing soybean could be reduced as promiscuous soybean form functional nodules with the indigenous rhizobia. This implies that resource-poor SH farmers only need access to the seed to be able to grow soybean, which brings multiple benefits in improved household nutrition from high protein, oil content and inputs of nitrogen thereby enhancing soil fertility and also contributing to sustainability of the cropping system (Mpepereki *et al*., 2000). Promiscuous varieties were reported to nodulate poorly in some locations (Abaidoo *et al*., 2000).

According to Odhiambo (2003), Limpopo Province has diverse soils. However, only five major associations have been identified and they include Dystrophic, red and yellow, well drained clayed soils, Red, yellow and grey soils, Black and red clay soils, Duplex and paraduplex soils and Weakly developed soils on rock.

1.2 Problem statement

Promiscuous soybean cultivars nodulate effectively with cowpea-type rhizobia (Gwata *et al.,* 2004), but not all soils contain such bacteria. The performance of promiscuous soybean cultivars has however not been evaluated in Limpopo Province while the distribution of cowpea-type rhizobia compatible with promiscuous soybean cultivars in soils of Limpopo Province is also not known. It is therefore important that promiscuous soybean cultivars be evaluated in areas with the compatible cowpea-type rhizobia where they can grow well for possible introduction to SH farmers.

1.3 Motivation of the study

Soybean is a drought tolerant crop, which makes it suitable for cultivation in Limpopo Province since the province is characterised by low rainfall. The inclusion of promiscuous soybean among crops grown by resource-poor SH farmers has potential to enhance the sustainability of their low input production systems as soil fertility is a major crop production constraint that affects both crop yield and harvest quality. The SH farmers in Africa are resource-poor and cannot afford the expensive nitrogen fertilizer inputs that are required to increase the yield of crops on infertile soil (Maingi *et al.,* 2006). This often leads to low yields and food insecurity. Most SH farmers are not aware of inoculation technology while access to commercial inoculants in most rural locations is a challenge. Promiscuous soybean cultivars benefit cropping systems because they fix atmospheric nitrogen which helps in reducing the problem of low nitrogen in soils. As a food crop, soybean is a primary source of cheap protein and mineral nutrients; and is currently underutilised by rural households in South Africa. Introduction of soybean in the SH farming systems has potential to improve nutrition of these resource-poor households by providing them with cheap plant protein. There is need to verify if Limpopo soils contain the right type of cowpea rhizobia that nodulate improved promiscuous soybean varieties developed at IITA, as well as to evaluate their potential under Limpopo conditions. This necessitated a field trial testing performance of the promiscuous varieties and their responses to inoculation, as well as a glasshouse study focussing on evaluating the nodulation of the promiscuous varieties in different soils.

1.4 Purpose of the study

1.4.1 Aim

The aim of the study was to explore the possibility of introducing promiscuous soybean into the SH farming systems of Limpopo Province.

1.4.2 Objectives

1.4.2.1 Field experiment

The objectives of the field experiment study were to determine:

i. the effect of variety on growth, nodulation and yield of soybean.

ii. effect of inoculation on growth, nodulation and yield of soybean.

iii. the interaction effect of variety and inoculation on growth, nodulation and yield of soybean.

1.4.2.2 Glasshouse experiment

The objectives of the glasshouse experiment were to determine:

i. the effect of variety on growth and nodulation of soybean.

ii. effect of inoculation on growth and nodulation of soybean.

iii. soil type effect on growth and nodulation of soybean.

iv. effect of variety × inoculation interaction on growth and nodulation of soybean.

v. effect of soil type × inoculation interaction on growth and nodulation of soybean.

vi. soybean variety × soil type interaction effect on growth and nodulation of soybean.

vii. the interaction effect of variety, inoculation and soil type on growth and nodulation of soybean.

1.5 Hypotheses

1.5.1 Field experiment

i. Variety has no effect on growth, nodulation and yield of soybean.

ii. Inoculation has no effect on growth, nodulation and yield of soybean.

iii. There is no interaction effect of cultivar and inoculation on growth, nodulation and yield of soybean.

1.5.2 Glasshouse experiment

i. Variety has no effect on growth and nodulation of soybean.

ii. Inoculation has no effect on growth and nodulation of soybean.

iii. Soil type has no effect on growth and nodulation of soybean.

iv. Variety x inoculation interaction has no effect on growth and nodulation of soybean.

v. Soil type x inoculation interaction has no effect on growth and nodulation of soybean.

vi. Variety x soil type interaction has no effect on growth and nodulation of soybean.

vii. There is no interaction effect of variety, inoculation and soil type on growth and nodulation of soybean.

CHAPTER 2

LITERATURE REVIEW

2.1 Soybean origin, description and uses

Soybean (*Glycine max (L*) Merr.) is indigenous to Manchuria, in China. It is an annual grain legume that belongs to the family Fabaceae. According to DAFF (2010), soybean is a warm temperature and short-day plant, which normally has bushy or upright growth habit. The plant usually varies from 40 to 100 cm in height and is much branched with well-developed roots, and each plant produces a number of small pods containing one to four seeds. Soybean seed can be consumed as fresh green vegetable or dried beans; and its secondary products include soy flour, soy milk, and soy sauce. Singh (2005) mentioned that soybean is useful in preparing bread, biscuits, cakes and soups, and its oil is recommended for stomach diseases, diabetes, and the oil is also used as a raw material for producing drying oil, soaps, plastics, strong glues and adhesives.

USB (2008) indicated that soybeans are especially an important component because they are the highest natural source of dietary fibre and a complete protein, which contains all eight essential amino acids needed for human nutrition. Its oil is relatively low in harmful saturated fats, and high in poly and monounsaturated fats (USB, 2008). Soybean oil is the major source of Omega-3 fatty acids in the United States diet and the primary commercial source of vitamin E whose industrial uses also include the production of biodiesel (USB, 2008).

2.2 Production level in South Africa

According to DAFF (2010), soybean production in South Africa at present ranges from 450 000 to 500 000 tons per annum at an average yield of 2.5 to 3.0 t/ha under dryland conditions. Of all provinces in South Africa, Mpumalanga produces the largest quantity of soybeans (42%), followed by the Free State (22%), KwaZulu-Natal (15%), Limpopo 8%, the North West produce (5%) and Gauteng (2%). Waterberg and Sekhukhune districts are the main production areas in Limpopo Province. According to DAFF (2011), the Western and Eastern Cape provinces of South Africa have been the lowest producers of soybeans with the Western Cape

Province going out of production from 2007 and 2009 production seasons. Very few SH farmers are involved in soybean production throughout the country.

In the year 2006, a project was started to increase the soybean yield for biodiesel production in Limpopo Province, whereby Mapfura-Makhura Incubator (MMI), which is in partnership with Limpopo Department of Agriculture (LDA), assisted 76 farmers in Sekhukhune district. The farmers were assisted with production inputs such as seeds, fertilizers and herbicides and trained them in business and technical skills related to sunflower and soybean production (Maluleke, 2008).

2.3 Effect of *B. japonicum* inoculation on soybean crop

Rhizobium inoculation is a cheaper and usually more effective agronomic practice that ensures adequate nitrogen nutrition of legumes, compared with the application of nitrogen fertiliser (Tran, 2004). According to Singh (2005), the application of *B. japonicum* is found to be very useful in soybean cultivation for producing high yield and for keeping the soil fertile for the following crop. The nitrogen demand of soybean can be supplied through BNF by inoculation with selected *B. japonicum* strains (Javaid and Mahmood, 2010). Singh (2005) further reported that inoculation of seeds with *Bradyrhizobiurn* culture gave significantly taller plants with more nodules, pods/plant, grains/pod and seed weight than untreated seeds. Yield of soybean increased due to inoculation. Yield increases of 40% have been observed in soybean inoculated with *B. japonicum* compared to non-inoculated in fields new to soybean production (Schulz *et al.,* 2005). Javaid and Mahmood (2010) reported that *B. Japonicum* inoculation had significant effect on plant growth, nodulation and yield of soybean.

2.4 Promiscuous soybean advantage to SH farmers

According to Mpepereki *et al*. (2000), a major advantage to SH farmers of the promiscuous varieties, such as Magoye and Hernon 147, is their indeterminate growth habit and the relatively low grain (and N) harvest index as these varieties produce a significantly greater biomass that can provide both fodder for livestock and an organic amendment for soil fertility improvement. The indeterminate growth habit means promiscuous varieties can withstand mid-season drought common in areas with low rainfall and then begin flowering again when rains return (Mpepereki *et al.*, 2000). They benefit the cropping system because of their ways of enhancing BNF (Tefera, 2011).

2.5 Effect of soil types on soybean growth and nodulation

The types of soil, rhizobia and nutrient components of the soil have long been known to affect the productivity and growth of nodule-forming legumes (Uaboi-Egbenni *et al.*, 2010). Uaboi-Egbenni *et al.* (2010) conducted a study to determine the effect of soil types (loamy, sandy, humus, clay soil), and their mixtures on nodulation of some leguminous plants (five bean cultivars and two groundnut cultivars). They also determined if there were any significant differences among soil types in nodule formation in legumes based on the number of nodules formed and size of nodules. From the statistical analysis of their data clay and sandy soils were poor in encouraging nodule formation.

2.6 Benefits of BNF

Lindemann and Glover (2003) defined BNF as the process whereby atmospheric nitrogen (N_2) is reduced to ammonia by living microorganisms e.g. rhizobia in the presence of nitrogenase. According to Javaid and Mahmood (2010), the soybean plants can fix up to 200 kg N/ha per year thus reducing the need for expensive and environmentally harmful nitrogen fertilizers. According to Silva and Uchida (2000), important economic benefit of BNF includes the reduced costs of production while the environmental benefit includes avoiding the contamination problems of water resources from runoff and leaching of excess use of chemical fertilizers. The use of inoculants to enhance BNF is not a threat to natural resource management. Silva and Uchida (2000) further indicated other benefits of BNF such as increase of yield, improvement of soil fertility and the fact that treating the seed with the inoculants is simple as compared to applying fertilizers on the field.

2.7 Factors affecting BNF

Soil factors such as pH, moisture content, and temperature can affect nitrogen fixation in legumes (Mohammadi *et al.*, 2012). Review by Mabrouk and Belhadj (2012) stated that Rhizobium-legume symbiosis strongly depends on the physiological state of the host plant, therefore a competitive and persistant rhizobial strain is not expected to express its full N_2 fixation activity if there are factors that impose limitations on the growth and vigour of the host legume. According to Havlin *et al.* (2005), soil acidity can limit the survival and growth of *Rhizhobia* in soil and can also affect the process of nodulation and N_2 fixation. Soil pH, generally at values less than 5.5 to 6.0, can drastically affect rhizobial infection, root growth, and legume productivity (Havlin *et al*., 2005). The pH range between 6.0 and 7.0 is considered to be suitable for rhizobial growth (Hungria and Vargas, 2000).

According to Mohammadi *et al*. (2012), soil water influences the growth of soil microorganisms through the processes of diffusion, mass flow and nutrient concentration. It directly influences the growth of rhizosphere microorganisms, like rhizobia by decreasing water activity below critical tolerance limits. Mohammadi *et al*. (2012) further indicated that during the dry season poor nodulation of legumes in arid soils is likely due to decrease in population levels of rhizobia. According to Zahran (1999), high temperature affects both free-living and symbiotic life of rhizobia in arid regions and critical temperatures ranging from 35°C to 40°C for soybean and peanuts have been reported. Drought can affect nodule formation and longevity, lowers the populations of rhizobia and can reduce nitrogen fixation (Hungria and Vargas, 2000).

2.8 Effect of nodule number and mass on BNF

Nodule dry mass and nodule number are nodulation parameters that are usually measured. Bala *et al.* (2010) stated that the measurement of nodule dry mass is problematic as it is difficult to completely clean nodules from adhering soil and such measurements of nodule mass rarely give significant differences between treatments. Gwata *et al.* (2004) stated that nodule dry weight is not valid as an indicator of N_2 Fixation, since nodule dry weight includes also the non functional nodules.

2.9 Rhizobial factors

Rhizobia include bacterial genera such as *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium, Mesorhizobium, Allorhizobium and Azorhizobium* which have the ability to establish nodules on roots and stems of their host legumes and reduce atmospheric nitrogen and make it available to plants (Sessitsch *et al*., 2002). *Rhizobium* species are fast growing, acid producing, nitrogen fixing bacteria associated with many temperate pasture legumes such as alfafa (*Medicago sativa*), and clovers (*Trifolium*). *Bradyrhizobium* species are slow growing, alkaline-producing bacteria associated with soybean and many tropical legumes such as cowpeas (Adjei *et al.,* 2002). If the well-suited indigenous soil bacteria are absent, rhizobial strains are introduced through the process of inoculation.

2.10 Soybean as a cover crop and its biomass

Soybean can be used as a cover crop. According to Fageria *et al*. (2005), cover crops have desirable attributes such as the ability to provide sufficient soil cover, fix atmospheric nitrogen, and produce organic matter with low-residue carbon/nitrogen ratio and absence of allelopathic effects on the following crop. Mpepereki *et al*. (2000) reported that soybean crop has a large leaf biomass which gives soil fertility benefit to the next crops. The large leaf biomass of soybean leaves can also suppress weed growth.

2.11 Regional research on promiscuous soybean for SH farming sector

According to Mpepereki *et al*. (2000), promiscuous soybean varieties which are more appropriate for SH farmers have been developed which fix nitrogen and nodulate well in farmers' fields without history of inoculation in Zimbabwe, Zambia and Malawi. In 1998/1999 season Zimbabwean SH farmers increased the area under promiscuous soybean as a result of increase in price of commercial soybean seed and basal fertilizer which SH farmers could not afford (Mpepereki *et al*., 2000). However promiscuous seed shortage has limited the rate of this extension and adoption by farmers. In 1997 a farmer in Mkushi district of Zambia reported that uninoculated soybean Hernon 147 variety formed effective nodules in soils with no history of soybean production (Mpepereki *et al*., 2000). In 1989 promiscuous variety Magoye was introduced in Malawi and the SH farmers readily adopted the variety, but due to its yield potential which was much less than the non-promiscuous variety when grown with rhizobial inoculant, there was resistance to the approval of Magoye variety (Mpepereki *et al*., 2000). There is paucity of published information on the performance of TGx varieties in South Africa.

2.12 Limpopo Province soils

According to Odhiambo (2003), different soils occur in Limpopo Province. However, only five major associations have been identified. These include Dystrophic, red and yellow, well drained clayed soils, Red, yellow and grey soils in caternary association, Black and red clay soils, Duplex and paraduplex soils and weakly developed soils on rock. Dystrophic, red and yellow, well drained clayed soils are found in the high rainfall areas of Drakensberg and Soutpansberg ranges. The soils are rocky and generally have low fertility status; these soils therefore have limited arable land and are highly leached, clay-like acidic soils. Red, yellow and grey soils in caternary association generally occur in the low rainfall areas west and north of Thabazimbi, Vaalwater, Liphalale and Polokwane. The soils generally have a wide range of textures, which usually vary from sandy and loamy soils and are suitable for arable farming. They are characterized by Hutton, Clovely and Griffin soil forms.

Black and red clay soils are clayey soils with varying amounts of rock and lithosol. They are found in a narrow strip parallel to the eastern border, the Springbok Flats (Bohlabela, Settlers and Roedtan) and the southwestern boundary near Dwaalpooort and Derdepoort. The soils are highly erodible and they are utilized extensively for dryland crops such as cotton and winter cereals production. Duplex and paraduplex soils are characterized by topsoil that is different from sub-soil with regard to texture, consistency and structure. These soils occur in Sekhukhune, south to southwest of Liphalale in Waterberg district, between Louis Trichardt and Tshipise, and sections of Vhembe District near the eastern border. These soils generally are not utilized as arable land due to high erodibility. Weakly developed soils on rock consist of topsoil overlying rock or weathered rock. They are found to the east of the Drakensberg, including a large section of Mopani District, and east and west of Musina. These soils tend to be rocky, with shallow soils and therefore generally unsuitable for arable farming (Odhiambo, 2003).

CHAPTER 3

FIELD STUDY

3.1 Introduction

This chapter outlines the details of the field experiment which tested the effect of soybean variety, inoculation and their interaction on growth, nodulation and yield of soybean, in order to evaluate the performance of soybean under field conditions of Limpopo Province.

3.2 Materials and methods

3.2.1 Study site description

The field trial was conducted during 2012/2013 growing season at the University of Limpopo experimental farm, Syferkuil (23° 49' S, 29° 41' E, and 1261.6 m altitude), near Mankweng in Capricorn district of Limpopo Province. The area is characterized by hot dry summers and cool dry winters. The area receives mean annual rainfall of 500 mm (Mpangane *et al.*, 2004), that falls predominantly in summer. The mean average day temperatures vary from 28 to 30°C, the soil at Syferkuil farm is sandy loam with pH values around neutrality and alkalinity and the soil is of the Hutton form in the Glenrosa family (Moshia, 2005)

3.2.2 Experimental design, procedures and treatments

The field trial was planted on 30th November 2012. The experiment was laid out as a split-plot in a randomised complete block design (RCBD) with four replications. The main plot factor was inoculation at two levels (uninoculated and inoculated). Prior to planting *Bradyrhizobium japonicum* strain WB74 obtained from Soygro Pty Ltd was used to inoculate the soybean seeds. Soybean seeds were thoroughly mixed with the inoculant and mollyflo sticker to ensure that all seeds were thoroughly treated. The inoculated seeds were left under the shade to dry, and then planted immediately in fine moist seedbed prepared by mouldboard ploughing followed by discing to a fine tilth. The treated seeds were covered with soil immediately to avoid direct sunlight contact.

The subplot factor studied included three naturally-nodulating soybean varieties (TGx-1937-1F, TGx-1740-2F and TGx-1835-10E), the seeds were obtained from

Prof F Dakora of Tshwane University of Technology and one commercial variety (Dundee) obtained from ARC- Grain Crops Institute was used as the control. Each plot consisted of 4 rows with inter-row spacing of 60 cm and intra-row spacing of 15 cm, the rows were 3 m long. The gross plot size was 4 rows × 0.6 m × 3 m = 7.2 m^2 and the net plot was 2 rows × 0.6 m × 3 m = 3.6 m^2. Weeding was done manually by hand hoeing when required and to avoid contamination, the uninoculated plots were always weeded first. No fertilizers and pesticides were used in the study and the experiment was conducted under supplementary irrigation.

3.2.3 Soil sampling

Prior to planting and post-harvest, soil samples were collected randomly from each plot in three different places using a soil auger at a depth of 0 - 15 cm to determine soil chemical properties such as soil pH, organic carbon, phosphorus and mineral nitrogen. The three soil samples were mixed to form a representative soil sample. The soil samples were then taken to the laboratory for analysis. Organic carbon was determined following Walkley-Black method (Walkley and Black, 1943), Mineral N (NO_3^- and NH_4^+) was determined using Colorimetric method (Okalebo *et al.,* 2002) and Bray 1 method was used to determine P (Bray and Kurtz, 1945).

3.2.4 Data collection

3.2.4.1 Phenological data

Phenological data namely: days to 50% flowering and days to physiological maturity, were monitored and recorded. Days to physiological maturity of soybean were recorded when pods turned brown and seeds shook loose.

3.2.4.2 Plant biomass and nodulation data

For nodulation performance, four plants were randomly selected per plot at flowering to early podding. Soybean plants were carefully uprooted with the use of a spade, placed in brown bags and quickly taken to the laboratory for processing. The plants were then placed in a plastic bucket filled with water to loosen the soil with the sieve to catch detached nodules. Plants were then separated into shoots and roots followed by washing the roots with tap water. Thereafter, the nodules were carefully picked by hand from the roots and the following were recorded: (a) number of nodules/plant, (b) number of effective nodules/plant, (c) nodule dry weight and (d) shoot dry weight/plant. To identify the number of effective nodules, fresh nodules were dissected to see the colour inside the nodules, the pink to reddish colour represented effective nodules. For biomass, soybean shoots were oven dried to a constant weight at a temperature of 65°C and weight recorded. For nodule dry weight, nodules were then oven dried at 65°C for 24 hours, and weighed using an electronic weighing balance. Leaf chlorophyll content was measured using CCM-200 plus chlorophyll content meter on fully developed intact top leaves (4 plants/plot) during flowering to early podding.

3.2.4.3 Yield and yield components data

The yield components were determined at harvest maturity. Number of primary branches/plant, number of pods/plant and plant height were determined from six randomly selected plants from two centre rows of each plot. Plant height was measured with the help of ruler. Number of primary branches and number of pods/plant were counted after harvesting, wherein pods were picked from the plants and then counted. Unshelled weight was then determined using an electronic weighing balance. The pods of all plants harvested were threshed by hand, shelled weight was thereafter determined with an electronic weighing balance. Grain yield was determined from the net plot area of (2 middle rows x 0.6 m x 2.4 m =2.88 m^2). Hundred (100) seed mass was determined by weighing 2 samples of 100 seeds per plot. Shelling percentage was then calculated using the formula: Shelling % = (shelled grain weight/ unshelled pod weight) ×100.

3.2.5 Biochemical analysis

Harvested soybean seeds were sent to KwaZulu-Natal Department of Agriculture and Environmental Affairs laboratory for commercial biochemical analysis. The seeds were analysed for: Moisture, fat, crude protein, non-saturated carbohydrates (NSC), and mineral composition such as calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), phosphorus (P), zinc (Zn), copper (Cu), manganese (Mn) and iron (Fe) (Riekert and Bainbridge, 1998).

3.2.6 Data analysis

Obtained growth, nodulation and yield data for the field experiment were subjected to Analysis of Variance (ANOVA) using Statistix 9.0 and mean separation test was done using Least Significant Difference (LSD) at $P \leq 0.05$.

3.3 Results and Discussion

3.3.1 Soil analysis results

The chemical properties (pH, organic carbon, phosphorus and mineral N) of soil for pre planting and at harvest soil samples collected at a depth of (0-15 cm) are outlined in Table 3.1. The soil pH for both pre-planting and at harvest samples was slightly alkaline soil, with pH ranging from 7.88-8.76 (Table 3.1). The soil pH values for all treatments at harvest were above 8.0 (Table 3.1). The slightly alkaline pH is good for survival of rhizobia.

Organic carbon (OC) ranged between 0.14-0.73%. The OC for treatments V_2I_1, V_3I_0, V_4I_0 and V_4I_1 were lower than that of pre-planting (0.42%) at 0.38, 0.14, 0.24 and 0.14% respectively. The soil P before planting was 11.64 mg/kg and it declined in all treatments at harvest except in V_2I_0 and V_3I_0 with P values of 13.63 and 14.53 mg/kg, respectively. The decrease in soil P may have been influenced by the uptake of P by the plant roots from the soil solution during the growing season. The low P levels of soil for the experimental area of this study might have been the reason for limited nodulation. According to FSSA (2007), phosphorus is linked with cell division, root growth, flowering and ripening. These results suggest the need to apply P, such as through application of inorganic P fertilizer or manure during cultivation of promiscuous soybean on low P soils.

The mineral N of the soil used for the field study at harvest ranged between 14.73 to 64.15 mg/kg, while the mineral N for pre planting was 51.18 mg/kg. There was 25% increase in mineral N of V_4I_0 at harvest compared to that of pre planting (Table 3.1). The mineral N of all the other treatments at harvest was lower than that of the pre planting, whereby V_2I_1 and V_4I_1 had the lowest levels of mineral N at 21.84 and 14.73 mg/kg respectively (Table 3.1). These results suggest that most of the treatments tested extracted more N than they fixed and perhaps could only maintain or increase soil residual N through the incorporation of residues.

Table 3.1 Pre-planting and at harvest soil analysis results at a depth of 0-15 cm

Treatments	pH (H_2O 1:2.5)	Organic Carbon (%)	Phosphorus (mg/kg)	Mineral N (mg/kg)
Pre-planting	7.88	0.42	11.64	51.18
V_1I_0	8.58	0.59	4.11	50.53
V_1I_1	8.67	0.45	4.01	42.46
V_2I_0	8.76	0.42	13.63	40.98
V_2I_1	8.22	0.38	6.64	21.84
V_3I_0	8.64	0.14	14.53	49.45
V_3I_1	8.59	0.73	3.98	49.42
V_4I_0	8.15	0.24	4.00	64.15
V_4I_1	8.60	0.14	6.72	14.73

V_1= Dundee, V_2= TGx-1937-1F, V_3=TGx-1740-2F, V_4=TGx-1835-10E I_0=Uninoculated I_1=inoculated

3.3.2 Effect of inoculation with *B. japonicum* strain WB74, soybean variety and their interaction on:

3.3.2.1 Phenological development

Inoculation had no significant effect on the number of days to 50% flowering and days to physiological maturity (PM) (Table 3.2), thus inoculation did not affect phenological development of the soybean varieties tested. Soybean varieties significantly differed in days to 50% flowering and PM ($P \leq 0.01$). The number of days to 50% flowering ranged from 51 to 95, while days to PM ranged from 122 to 168 days. Dundee reached its 50% flowering and PM earlier than the promiscuous varieties at 51 and 122 days respectively (Table 3.2). Of the three promiscuous varieties tested TGx-1740-2F was the earliest to reach flowering (53 days) and PM (124 days) earlier after Dundee. TGx-1937-1F was the last to mature at 168 days and its growth duration was significantly different from those of the other evaluated varieties. This suggests that TGx-1937-1F is unlikely to mature successfully under the local dryland conditions. It may require supplementary irrigation towards the end of the season. TGx-1835-10E was intermediate among the promiscuous varieties in terms of phenological development.

Table 3.2: Effect of inoculation with *B. japonicum* strain WB74 and soybean variety on phenological development of soybean

Factor	Days to 50% flowering	Days to physiological maturity
Inoculation		
Uninoculated	68.5a	139.5a
Inoculated	68.4a	139.4a
Significance	ns	ns
$LSD_{0.05}$	-	-
Variety		
Dundee	50.7d	121.7d
TGx-1937-1F	95.0a	168.0a
TGx-1740-2F	53.0c	124.0c
TGx-1835-10E	75.0b	144.0b
Significance	**	**
$LSD_{0.05}$	0.57	0.57

Means in the same column followed by the same letter are not significantly different at P≤0.05. LSD= Least significant difference, ns= non significant at P≤0.05,**= significant at P≤0.01

3.3.2.2 Chlorophyll content

Inoculation with *B.japonicum* strain WB74 significantly (P≤0.01) increased leaf chlorophyll content, as higher chlorophyll content was achieved by inoculated plants than the uninoculated ones (Table 3.3). Inoculation increased leaf chlorophyll content by 46.4%. Varieties Dundee, TGx-1740-2F and TGx-1937-1F achieved high levels of leaf chlorophyll content which were significantly higher than that of TGx-1835-10E. Increase in leaf chlorophyll content implies increased growth vigour through enhanced photosynthesis that could translate into larger leaf area and higher grain yield. Bejandi *et al.* (2012) reported high chlorophyll content of chickpea (*Cicer arietinum)* to be linked with taller plants, more pod number, high seed protein and higher yield.

Table 3.3: Effect of inoculation with *B. japonicum* strain WB74 and soybean variety on nodulation of soybean at flowering stage

Factor	Chlorophyll content (CCI)	Dried plant biomass/plant (g)	Number of nodules/plant	Nodule dry weight/plant (g)	Percentage no of active nodules/plant
Inoculation					
Uninoculated	17.9b	67.8a	7.4b	0.1b	36.8b
Inoculated	26.2a	58.0a	25.2a	1.4a	77.9a
Significance	**	ns	**	**	*
$LSD_{0.05}$	2.71	-	3.42	0.75	37.25
Variety					
Dundee	23.4a	33.4b	13.8a	1.4a	60.3a
TGx-1937-1F	23.6a	141.8a	27.8a	0.6bc	60.1a
TGx-1740-2F	23.2a	32.4b	20.5a	0.9ab	68.8a
TGx-1835-10E	17.9b	44.1b	3.3a	0.1c	40.3a
Significance	**	**	ns	**	ns
$LSD_{0.05}$	2.22	26.99	-	1.07	-

Means in the same column followed by the same letter are not significantly different at P≤ 0.05. LSD= Least significant difference, ns= non significant at P≤ 0.05, **= significant at P≤0.01,*=Significant at P≤0.05

3.3.2.3 Dried plant biomass

Statistical analysis showed no significant differences in dried plant biomass due to *B. japonicum* inoculation, although the uninoculated plants had larger biomass (Table 3.3). Of the four soybean varieties evaluated, TGx-1937-1F accumulated higher biomass (141.80 g per plant), which was significantly (P≤0.01) higher than those of Dundee, TGx-1740-2F and TGx-1845-10E (Table 3.3). Obvious differences in biomass among the four tested varieties were observed in the field. These results suggest that TGx-1937-1F is the best candidate for soil fertility amelioration due to its higher biomass as suggested by Mpepereki *et al*. (2000).

3.3.2.4 Number of nodules

Inoculation with *B. japonicum* strain WB74 significantly increased number of nodules per plant (P≤0.01) (Table 3.3). Statistical analysis showed the number of nodules to be similar among soybean varieties (P≤0.05), however TGx-1937-1F achieved highest number of nodules of 28 (Table 3.3) while TGx-1835-10E had considerably fewer nodules per plant. The number of nodules however does not mean that all nodules are active. Mohammadi *et al.* (2012) indicated that poor nodulation is likely due to decrease in the rhizobia population during dry season. Bekere *et al.* (2012) reported high number of nodules (22) to be linked with taller soybean plants. Pule-Meulenberg *et al.* (2011) reported highest mean nodule number of 517 for TGx-1740-2F in the study conducted at Wa, Ghana in 2006, and the study showed this high nodule number to be linked with more biomass in shoots and whole plants. In this study this variety had much lower numbers of nodules suggesting lower populations of compatible rhizobia.

3.3.2.5 Nodule dry weight

Nodule dry weight was significantly influenced by application of the inoculant, as the inoculated plants produced nodules with higher dry weight (1.38 g) compared to the uninoculated plants (0.14 g) (Table 3.3). Nodule dry weight similarly differed significantly (P≤0.01) among the four soybean varieties with Dundee having large nodule mass of 1.44 g per plant as compared to the promiscuous varieties possibly due to its large nodule size (Table 3.3). Dundee had the lowest number of nodules per plant and yet it had the highest nodule dry matter per plant, thus indicating much bigger nodules than the promiscuous varieties (Table 3.3). Osunde *et al.* (2003a) reported nodule dry weight of about 1.13 and 0.60 g/plant for TGx-1456-2E and TGx-1660-19F respectively.

3.3.2.6 Percent of active nodules

Inoculation with *B. japonicum* strain WB74 significantly (P≤0.05) increased the percentage number of active nodules/plant. The inoculated plants had high percentage of active nodules/plant at 78%, while the uninoculated plants had about 37% (Table 3.3). There was however no significant difference in the percentage of active nodules among evaluated varieties; TGx-1740-2F none the less, gave higher percentage of active nodules/plant of about 68.9%. The difference might have been caused by the early senescence and some of the nodules were never active. More detailed monitoring of the active life of nodules could shed more light on this aspect.

Figure 3.1 shows the effect of inoculation x soybean interaction variety on chlorophyll content (A) and nodule dry weight/plant (B), wherein inoculation increased chlorophyll content of varieties Dundee, TGx-1740-2F and TGx-1835-10E by 82%, 61% and 40% respectively. High influence of inoculation was observed on nodule dry weight of Dundee and TGx-1740-2F (Figure 3.1B). Inoculation did not influence the nodule mass of variety TGx-1835-10E (Figure 3.1B). Zhang *et al*. (2002) reported an increase in nodule dry weight due to *B. japonicum* strains inoculation and variations in nodule dry weight due to varieties tested (Marple Glen and Bayfield varieties) were also reported.

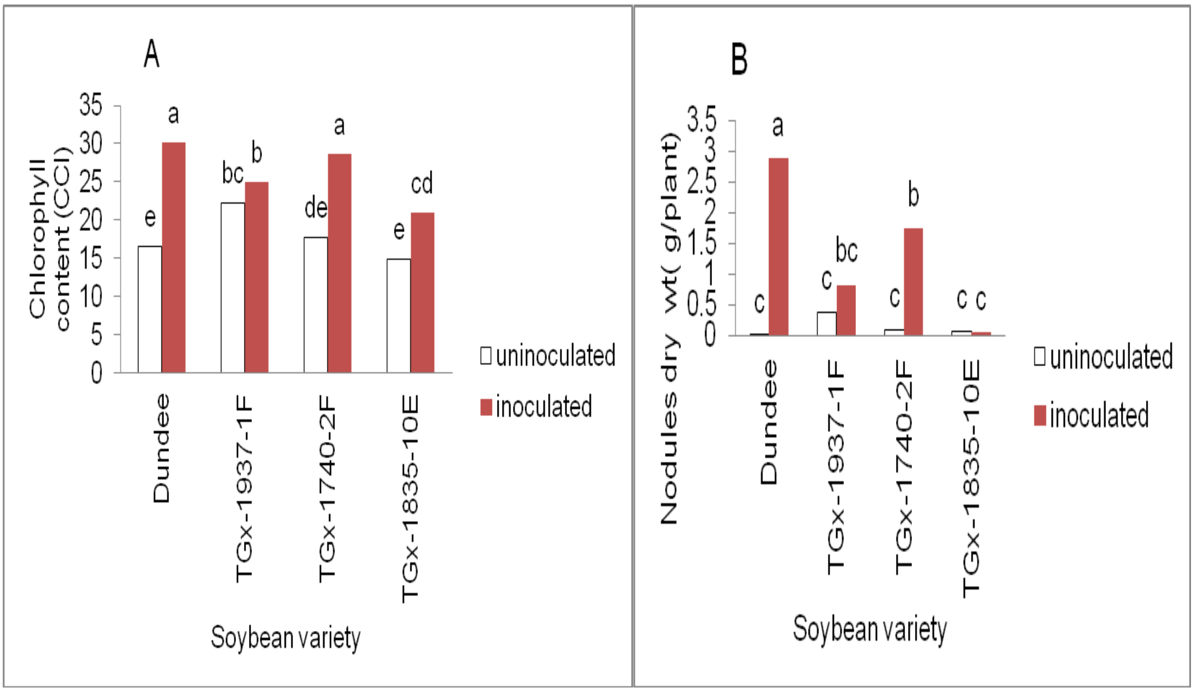

Figure 3.1: Interactive effects of inoculation with *B. japonicum* strain WB74 and soybean variety on chlorophyll content (A) and nodule dry weight (B). Bars followed by the same letter are not significantly different at P≤0.05

3.3.3 Effect of inoculation, soybean variety and their interaction on:

3.3.3.1 Plant height

Inoculation had no significant effect on plant height, although inoculated plants were taller (Table 3.4). The results are in agreement with previous findings of Bekere *et al.* (2012) who reported a similar trend of results where the plant height of inoculated and uninoculated plants did not differ. Soybean variety had highly significant effect on plant height (P≤0.01). TGx-1937-1F was the tallest (82.3 cm) and was significantly taller than Dundee (67.9 cm) and TGx-1845-10E (57.3 cm). This suggests that not all promiscuous varieties are taller than the specific nodulating varieties as would be expected from a biomass consideration as suggested by Mpepereki *et al.,* (2000).

Table 3.4: Effect of *B. japonicum* inoculation and soybean variety on vegetative growth, yield and yield components of soybean at harvest maturity

Factor	Plant height (cm)	Number of primary branches/ plant	Number of Pods/plant	Hundred seed weight (g)	Shelling percentage	Grain yield (kg/ha)
Inoculation						
Uninoculated	67.5a	8.1a	136.8b	12.8b	60.7a	3325b
Inoculated	73.2a	8.4a	186.4a	14.4a	65.6a	4994a
Significance	ns	ns	**	*	ns	**
$LSD_{0.05}$	-	-	23.80	1.14	-	794.32
Variety						
Dundee	67.9b	8.0b	116.3c	17.6a	59.0b	4010b
TGx-1937-1F	82.3a	9.0a	238.6a	10.6c	65.8a	5582a
TGx-1740-2F	73.8ab	6.0c	124.0c	15.2b	65.4a	3377b
TGx-1835-10E	57.3c	9.9a	167.5b	11.0c	62.3ab	3668b
Significance	**	**	**	**	**	**
$LSD_{0.05}$	9.43	0.95	40.74	1.17	4.15	1031.0

Means in the same column followed by the same letter are not significantly different at P≤0.05. LSD= Least significant difference, ns= non significant, **= significant at P≤0.01, *= significant at P≤0.05

3.3.3.2 Number of primary branches/plant

Inoculation showed no significant effect on number of primary branches, while soybean variety showed a highly significant (P≤0.01) effect. TGx-1835-10E and TGx-1937-1F achieved higher number of primary branches, 10 and 9 branches/plant, respectively and were significantly higher than those of Dundee and TGx-1740-2F

(Table 3.4). The number of primary branches is mainly determined by the growth and development of the plant. It may differ depending on whether the variety has upright or bushy growth habit. It is likely to be a genetic trait although most grain legumes tend to compensate for low plant populations through increased plant growth and branching. It was observed in this study that short plants seemed to have produced fewer primary branches compared to taller plants.

3.3.3.3 Number of pods/plant

Inoculation with *B. japonicum* strain WB74 showed significant differences ($P≤0.05$) in number of pods per plant, whereby the inoculated plants produced higher number of pods compared to uninoculated plants (Table 3.4). Inoculation increased the number of pods/plant by 36%. Soybean varieties showed highly significant ($P≤0.01$) difference in number of pods.TGx-1937-1F achieved highest number of pods of about 238 pods, which were significantly higher than the other varieties, however TGx-1937-1F was followed by TGx-1835-10E with 167 pods, and the lowest number of pods were produced by variety Dundee (Table 3.4). Number of pods can be affected by the number of primary branches a plant produces. Bejandi *et al.* (2012) reported higher grain yield in chickpea to be linked with higher number of pods/plant. Ikeogu and Nwofia (2013) also reported pods number to influence grain yield of a soybean crop, wherein fewer pods number resulted in lower grain yield, while higher pods number was linked with higher yield.

3.3.3.4 Hundred seed weight

Inoculation showed significant differences in 100 seed weight ($P≤0.05$), thus inoculated plants developed larger seeds compared to uninoculated ones (Table 3.4). Of the four varieties Dundee achieved a significantly ($P≤0.01$) higher 100-seed weight at 17.6 g than the promiscuous varieties (e.g TGx-1937-1F (10.6 g) TGx-1740-2F (15.2 g), TGx-1835-10E (11.0 g). Ikeogu and Nwofia (2013) reported variation in mean seed size of five promiscuous soybean varieties ranging from 9.3 to 11.5 g. Those promiscuous varieties had much smaller seed size than that of Dundee reported in this study. Smaller seed size of promiscuous varieties may be an advantage as plants can fill them easier under marginal environmental conditions. Lower seed rates can also be required for these varieties. Figure 3.2 shows larger seeds of Dundee variety as compared to those of promiscuous soybean varieties.

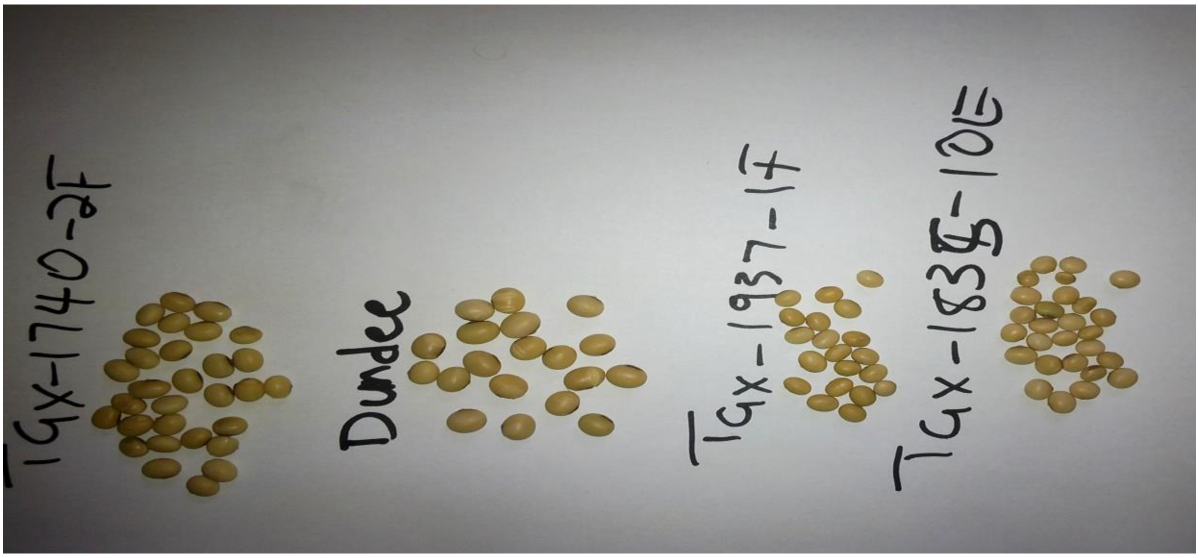

Figure 3.2: Comparison of seeds among the studied soybean varieties.

3.3.3.5 Shelling percentage

There were no significant differences (P≤0.05) in shelling percentage between the inoculated and uninoculted plants, meaning inoculation did not influence shelling percentage (Table 3.4). Shelling percentage was influenced significantly (P≤0.01) by soybean variety. The shelling percentage of Dundee was low (59%) and differed significantly with those of promiscuous varieties. Shelling percentage can be affected by the unshelled and shelled seed weight, whether the pods consist of empty pods or not and also by losing some of the seeds while shelling the pods. This result is rather surprising given that specific nodulating varieties, such as Dundee, are expected to have higher harvest index than promiscuous varieties. Shelling percentage contributes to the harvest index.

3.3.3.6 Grain yield

Inoculation significantly (P≤0.01) influenced soybean grain yield as much higher yield was achieved in inoculated plants relative to the uninoculated ones (Table 3.4). Inoculation increased grain yield by 50.2%. TGx-1937-1F had the highest grain yield of about 5582 kg/ha. Two of the promiscuous varieties (TGx-1740-2F and TGx-1835-10E) achieved lower, but comparable grain yield to Dundee. The large seed size of Dundee contributed to its increase in grain yield that is why even though it had lowest pod number per plant its yield was slightly higher than those of TGx-1740-2F and TGx-1835-10E. This shows that seed weight exerts positive effect on grain yield.

Hundred seed weight, shelling percentage and grain yield were influenced by the interaction between inoculation and soybean variety. Inoculation increased 100 seed weight of variety Dundee only (Figure 3.3A). Shelling percentage of varieties Dundee and TGx-1740-2F was also influenced by inoculation. This implies that pods of variety Dundee partitioned more assimilates to the seed when inoculated (Figure 3.3B). Inoculation x soybean variety interaction indicated greater effect of inoculation on grain yield of Dundee whereby its yield significantly ($P \leq 0.01$) increased from 1832 to 6189 kg/ha (+237.8%), while lower increases were observed in TGx-1740-2F, from 2769 to 3984 kg/ha (+43.9%), and TGx-1835-10E from 3073 to 4262 kg/ha (+38.7%). The yield of TGx-1937-1F did not respond to inoculation (Figure 3.3C). The grain yields for the TGx varieties in this study were much higher compared to some studies conducted in Nigeria where Ikeogu and Nwofia (2013) reported much lower grain yields of 1128 kg/ha and 597 kg/ha for TGx-1835-10E variety at Umudike and Amakama locations, respectively.

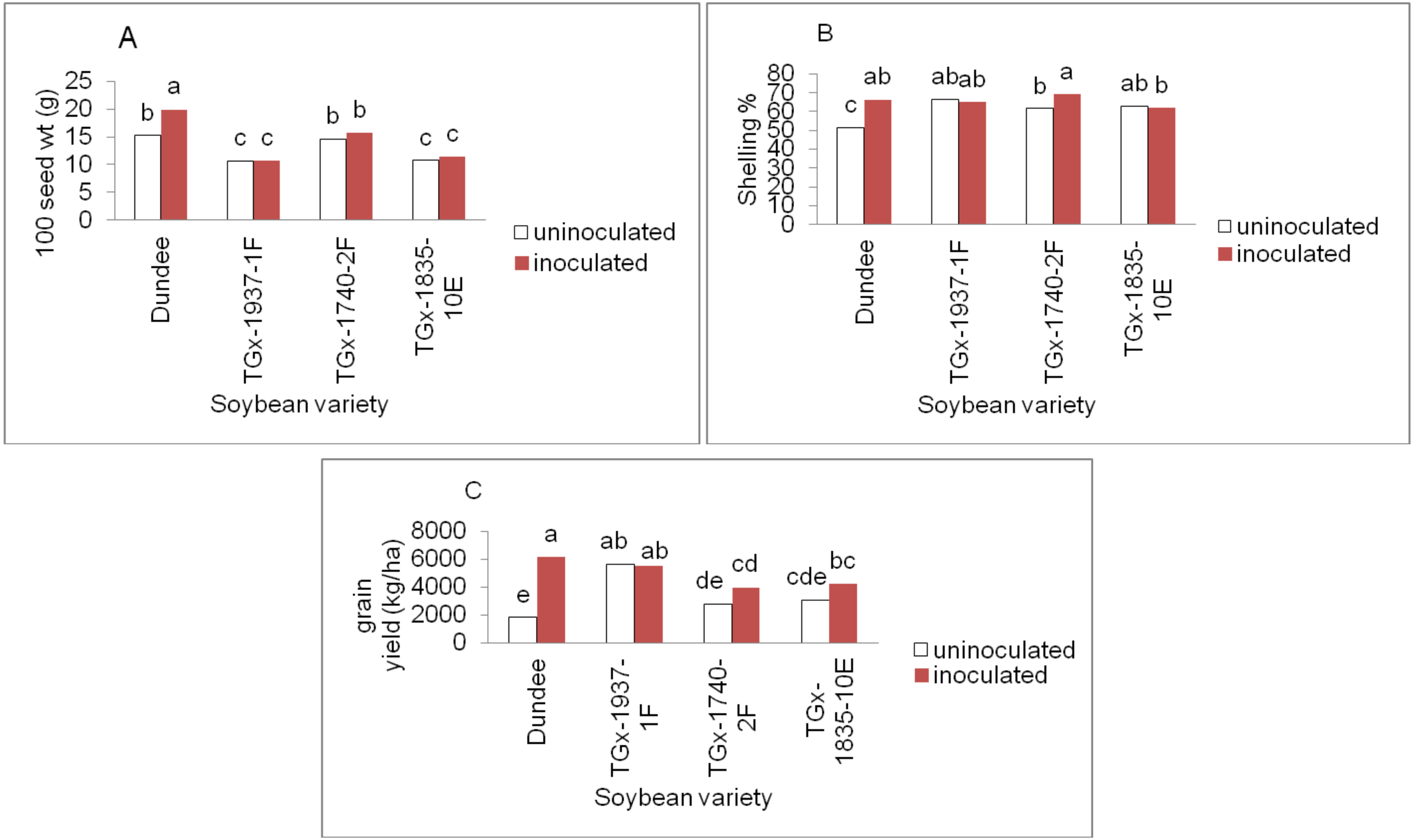

Figure 3.3: Interactive effects of inoculation with *B. japonicum* strain WB74 and soybean variety on 100 seed weight (A), shelling percentage (B) and grain yield (C). Bars followed by the same letter are not significantly different at P≤0.05

These results clearly show that successful production of Dundee is unlikely without inoculation. Varieties TGx-1740-2F and TGx-1835-10E both had mild but meaningful gains following inoculation. These results confirmed the promiscuous nature of the TGx varieties tested in this study. The results also suggest that inoculation can benefit some promiscuous soybean cultivars hence the need to always evaluate them for response to inoculation in South Africa (SA), as information of studies based on promiscuous varieties in SA is very limited. It appears that the *Bradyrhizobium* strain WB74 used in this study was effective in significantly raising the yield levels of two of the three promiscuous varieties, by more than a tonne per hectare. This is against the R147 cost of one sachet of commercial inoculum. The response to inoculation reported for TGx-1740-2F and TGx-1835-10E in this study suggests that the *Bradyrhizobium* strain WB74 used in this study does compete well against some of the indigenous rhizobia in the soil.

Ikeogu and Nwofia (2013) similarly reported the lowest mean performance for seed yield of TGx-1835-10E, the least seed size and number of pods when compared to other TGx genotypes such as TGx-1910-1D and TGx-1440 1E in Nigeria. In their study, yield parameters and stability of soybean was influenced by phosphorus. TGx-1740-2F during 2009/2010 season yielded all the new types of varieties under testing by giving 2248 kg/ha which exceeded the grain variety *Nasoko* and widely grown promiscuous variety *Magoye* that were used as checks by 15% and 38%, respectively (CBU, 2011).

3.3.4 Effect of inoculation with *B. japonicum* strain WB74, soybean variety and their interaction on nutritional quality parameters of soybean grain

3.3.4.1 Soybean seed moisture, fat, crude protein and non-saturated carbohydrates

There were no significant differences in both moisture and non saturated carbohydrates due to application of the inoculant (Table 3.5). The four soybean varieties had similar levels of seed moisture content, which can be affected by the relative humidity of the surrounding atmosphere at time of harvest and storage. However, significant differences occurred in both fat and crude protein content, with the inoculated plants showing lower fat but higher crude protein content.

Soybean varieties differed significantly ($P \leq 0.01$) in seed fat and crude protein content. The seeds of Dundee had the highest percentage fat content (16.88%). Crude protein content among the four soybean varieties tested ranged between 34.06% and 38.42% with the seeds of promiscuous varieties containing significantly higher crude protein relative to the commercial variety. The results showed high levels of crude protein content in the different varieties, indicating that soybean crop is a rich source of protein and that it would bring important benefits to poor households who cannot afford protein from animal sources. According to Olatunji *et al.* (2011), proteins play an important role in body building and maintenance of health and it remains a very crucial element in the diet of man. Non-saturated carbohydrates differed significantly ($P \leq 0.05$) and ranged between 10.75 and 12.02%. The seeds of TGx-1937-1F achieved highest content (12.02%) of non-saturated carbohydrates, but this was not significantly different from that of Dundee and TGx-1835-10E. Carbohydrate is a major source of energy in the human diet.

Table 3.5: Effect of inoculation with *B. japonicum* strain WB74 and soybean variety on seed moisture, fat, crude protein and non-saturated carbohydrates

Factor	Moisture %	Fat %	Crude protein%	Non- saturated carbohydrates%
Inoculation				
Uninoculated	5.05a	14.15a	35.03b	11.68a
Inoculated	5.72a	12.54b	38.69a	11.48a
Significance	ns	*	**	ns
$LSD_{0.05}$	-	0.87	1.37	-
Variety				
Dundee	5.34a	16.88a	34.06c	11.63ab
TGx-1937-1F	5.91a	11.14c	38.42a	12.02a
TGx-1740-2F	5.28a	12.88b	38.32ab	10.75b
TGx-1835-10E	5.02a	12.49b	36.64b	11.93a
Significance	ns	**	**	*
$LSD_{0.05}$	-	1.12	1.69	0.88

Means in the same column followed by the same letter are not significantly different at P≤0.05. LSD= Least significant difference, ns= non significant at P≤0.05, **= significant at P≤0.01,*=Significant at P≤0.05

Significant (P≤0.05) inoculation x soybean variety interaction effect on moisture content, crude protein and non saturated carbohydrates was observed. The moisture content of the harvested seeds from inoculated Dundee, TGx-1937-1F and TGx-1740-2F were marginally higher than those of their uninoculated seeds (Figure 3.4A). Inoculation increased seed crude protein of Dundee, TGx-17402F and TGx-1835-10E by 30.9%, 13% and 4.9%, respectively while TGx-1937-1F did not respond to introduction of *B. japonicum* strain WB74. Uninoculated seed achieved a slightly higher crude protein than the inoculated TGx-1937-1F (Figure 3.4B). Tahir *et al.* (2009) reported 9% increase in soybean protein due to inoculation. Non-saturated carbohydrate content of seeds of Dundee and TGx-1740-2F varieties did not respond to inoculation while those of TGx-1937-1F and TGx-1835-10E were increased by 9.5% and 1.9%, respectively (Figure 3.4C).

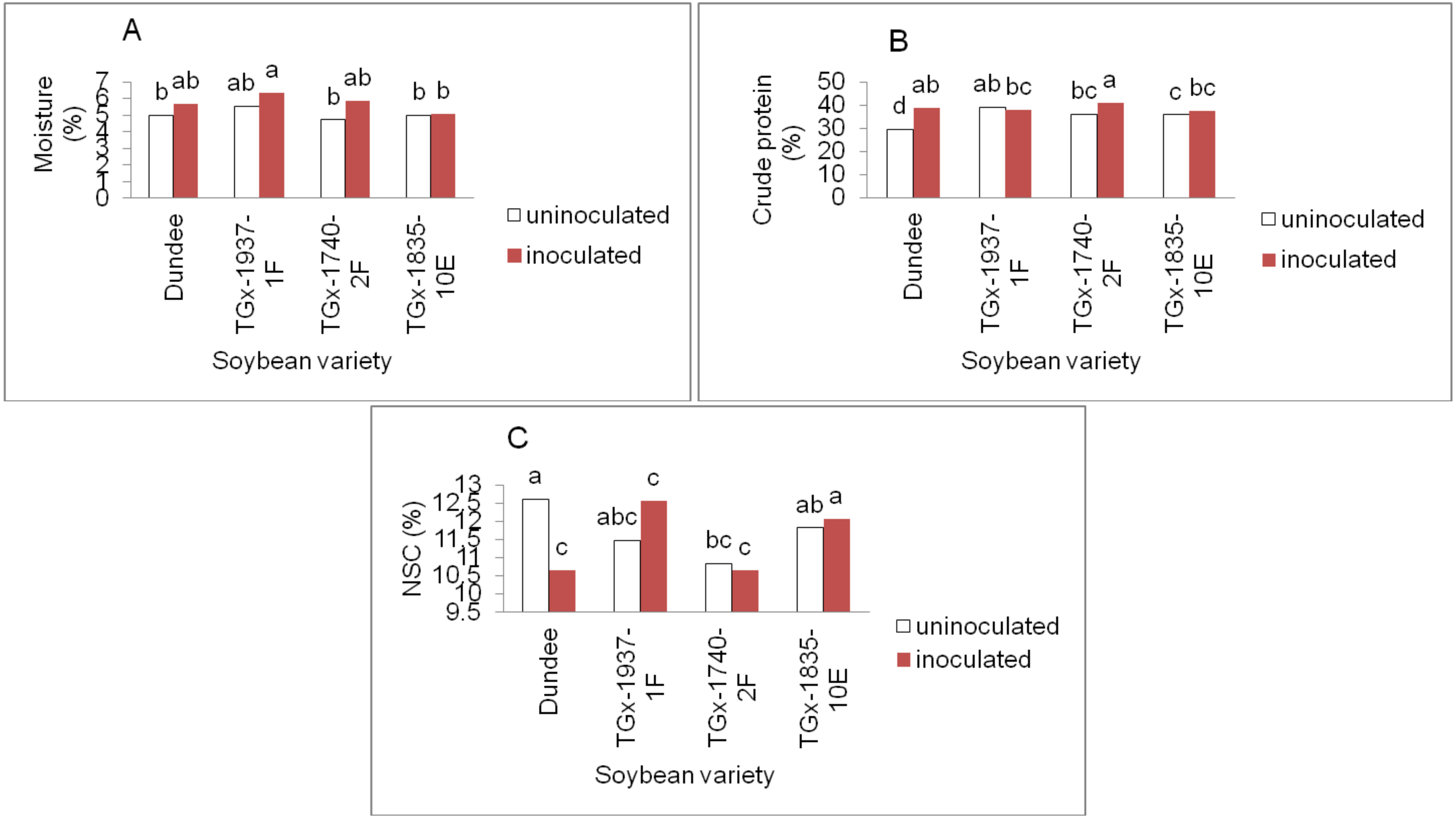

Figure 3.4: Interactive effects of inoculation with *B. japonicum* strain WB74 and soybean variety on seed moisture content (A) , crude protein (B) and Non saturated carbohydrates (NSC) (C). Bars followed by the same letter are not significantly different at P≤0.05

3.3.4.2 Seed N, P, K Ca, Mg and Na content

Inoculation had no significant ($P \leq 0.05$) effect on soybean seed P, K, Mg, and Na content but increased seed N by 10.3% (Table 3.6). The uninoculated plants achieved a slightly higher seed Ca compared to inoculated plants. Soybean varieties differed significantly ($P \leq 0.01$) in seed N, P, K, Mg and Na (Table 3.6) while seed Ca content was statistically similar among the four soybean varieties. All the promiscuous varieties had higher N and crude protein content than Dundee. TGx-1937-1F and TGx-1835-10E had significantly higher P than Dundee and TGx-1740-2F. Variety Dundee had higher Mg and Na content compared to all promiscuous varieties. The promiscuous varieties tested do not differ much from Dundee in terms of P, K, Ca, Mg and Na content. These results clearly show good nutrient content in promiscuous soybean variety seeds without inoculation.

Mahan and Escott-Stump (2008) revealed that calcium is needed to permit optimal gains in bone mass and density especially in adolescence years, and is required for nerve transmission and regulation of heart muscle function. Calcium ions play a critical role in smooth muscle contractility and the proper balance of calcium, sodium, potassium, and magnesium ions maintains skeletal muscle tone and control nerve irritability (Mahan and Escott-Stump, 2008). According to Ervine *et al.* (2004), phosphorus and magnesium act as components of enzymes in enzymatic reactions and plays essential role in energy metabolism, while calcium play an important additional role in blood clotting. Ervine *et al.* (2004) further indicated that sodium and potassium electrolytes along with calcium, phosphorus and magnesium play important roles in neural transmission, muscular activity, vascular constriction and dilation, maintaining normal acid-base balance, osmotic pressure and normal water balance.

Table 3.6: Effect of inoculation with *B. japonicum* strain WB74 and soybean variety on percentage of seed N, P, K, Ca, Mg and Na

Factor	N	P	K	Ca	Mg	Na
Inoculation						
Uninoculated	5.61b	0.84a	1.99a	0.23a	0.24a	0.01a
Inoculated	6.19a	0.77a	1.93a	0.20b	0.23a	0.01a
Significance	**	ns	ns	*	ns	ns
$LSD_{0.05}$	0.22	-	-	0.03	-	-
Variety						
Dundee	5.45c	0.75b	2.09a	0.21a	0.25a	0.02a
TGx-1937-1F	6.15a	0.86a	1.80b	0.21a	0.23c	0.00c
TGx-1740-2F	6.13ab	0.75b	1.85b	0.22a	0.23c	0.01b
TGx-1835-10E	5.86b	0.88a	2.08a	0.21a	0.24b	0.01b
Significance	**	**	**	ns	**	**
$LSD_{0.05}$	0.27	0.04	0.09	-	8.33	3.09

Means in the same column followed by the same letter are not significantly different at P≤0.05. LSD= Least significant difference, ns= non significant at P≤0.05, **= significant at P≤0.01,*=Significant at P≤0.05

Seed N, P, K and Ca were influenced by the interaction between inoculation and soybean varieties (Figure 3.5). An increase in seed N due to *B. japonicum* strain WB74 was achieved in Dundee, TGx-1740-2F and TGx-1835-10E while TGx-1937-1F did not respond to inoculation (Figure 3.5 A). The P content in the seeds of all tested varieties was not influenced by *B. japonicum* inoculation except for that of Dundee (Figure 3.5B). Tahir *et al.* (2009) reported 9% increase in soybean N due to rhizobium inoculation and no effect of rhizobium inoculant on P content in seed of soybean. The K content of TGx-1835-10E showed a slight (2.4%) increase due to inoculation while the seed K of other varieties was not influenced by *B. japonicum* inoculation (Figure 3.5C). The Ca in seeds of all tested varieties did not respond to inoculation but the uninoculated Dundee, TGx-1740-2F and TGx-1835-10E outperformed the inoculated ones (Figure 3.5D).

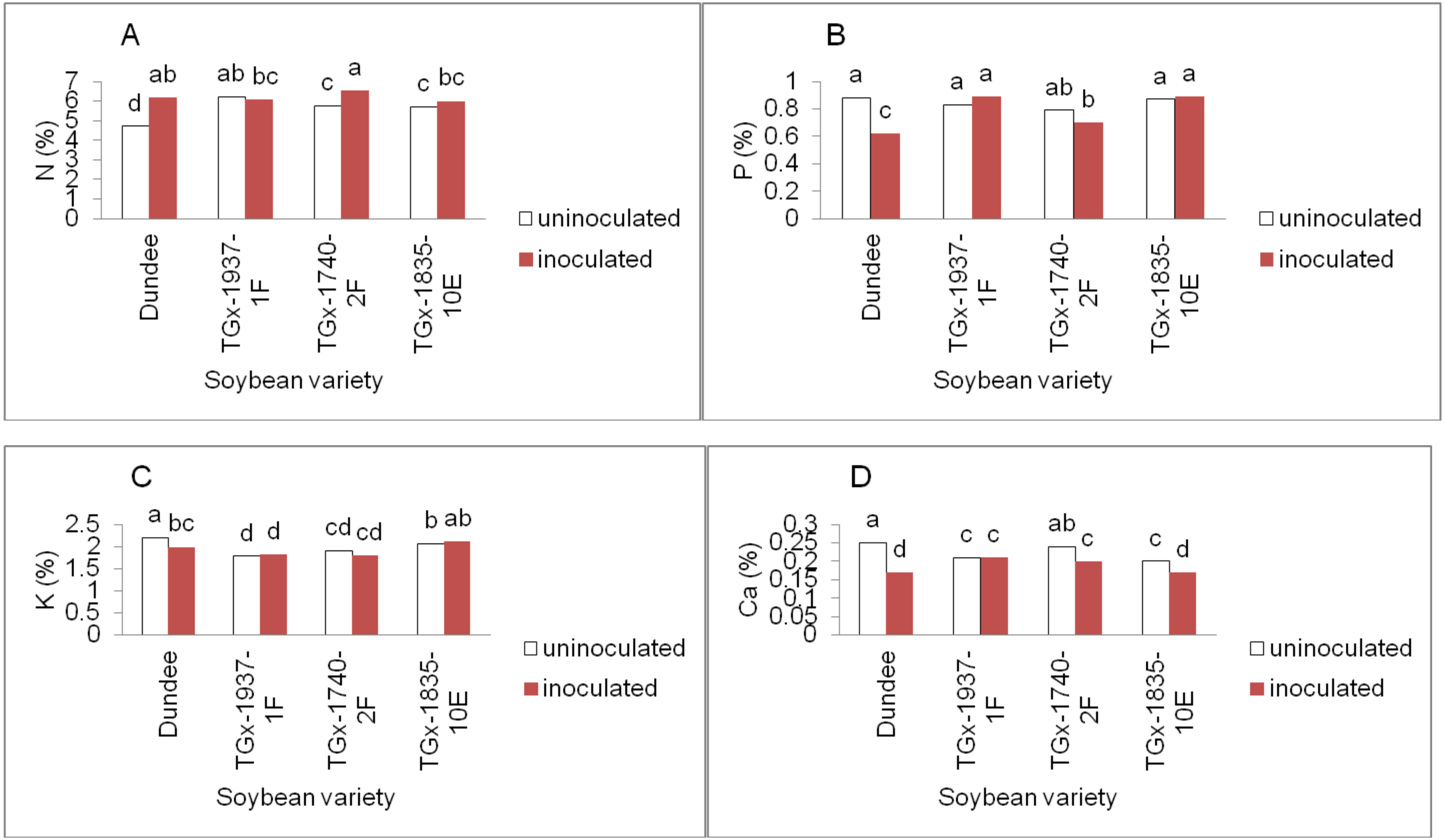

Figure 3.5: Interactive effects of inoculation with *B. japonicum* strain WB74 and soybean variety on seed Nitrogen (N) (A), Phosphorus (P)(B), Potassium (K) (C) and Calcium (Ca) (D). Bars followed by the same letter are not significantly different at P≤0.05

There was a significant interaction effect between inoculation and soybean variety on the Mg content of the seeds (Figure 3.6), wherein inoculation increased seed Mg of TGx-1835-10E marginally by 3.4% while the other evaluated varieties, did not respond to inoculation. In fact, there were a decrease in seed Mg content from inoculated plants for Dundee and TGx-17402F.

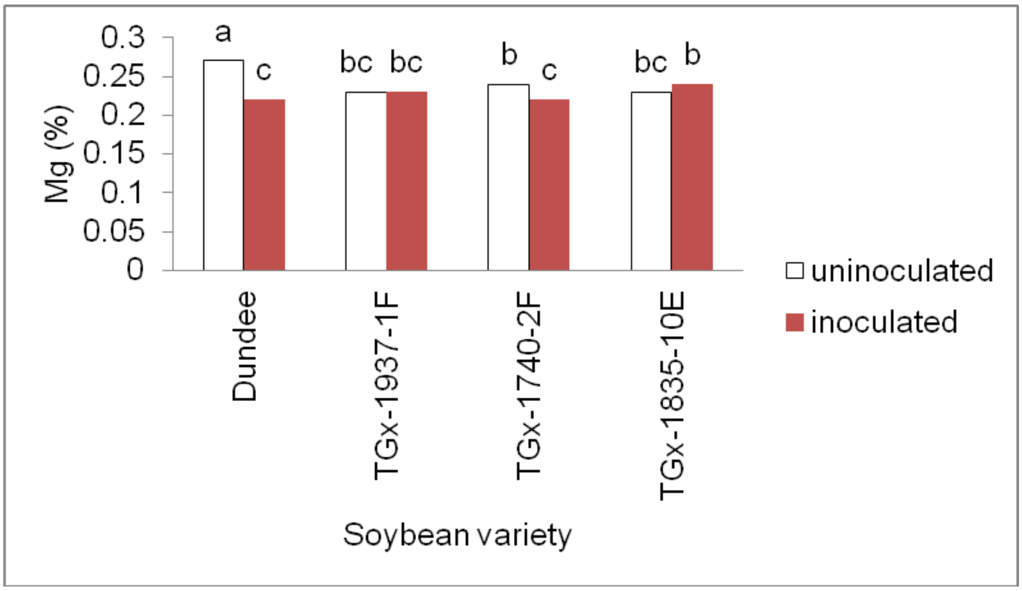

Figure 3.6: Interactive effects of inoculation with *B. japonicum* strain WB74 and soybean variety on seed Magnesium (Mg). Bars followed by the same letter are not significantly different at P≤0.05

3.3.4.3 Soybean seed Zn, Cu and Mn content

Inoculation with *B. japonicum* strain WB74 had no significant effect on the content of Zn, Cu and Mn content in soybean seed (Table 3.7). The tested soybean varieties showed highly significant (P≤0.01) differences in Zn, Cu and Mn concentration (Table 3.7), whereby TGx-1835-10E seeds accumulated high levels of Zn, Cu and Mn, being 50.63, 16.88 and 27 mg/kg, respectively (Table 3.7).

According to Mahan and Escott-Stump (2008), zinc is distributed abundantly throughout the human body and plays important structural role as a component of several proteins and functions; and also functions as intracellular signal in brain cells. Zinc is also involved in stabilisation of protein and nucleic acid structure and as well as in transport processes, immune function and expression of genetic information (Mahan and Escott-Stump, 2008). Copper is a component of many enzymes, and the symptoms of its deficiency are attributable to enzyme failures. Manganese is associated with the formation of connective and skeletal tissues, growth and reproduction, carbohydrate and lipid metabolism (Mahan and Escott-Stump, 2008).

Table 3.7: Effect of inoculation with *B. japonicum* strain WB74 and soybean variety on seed Zn, Cu and Mn (mg/kg)

Factor	Zn	Cu	Mn	Fe
Inoculation				
Uninoculated	46.3a	15.6a	28.1a	123.4a
Inoculated	41.4a	13.8a	26.8a	122.7a
Significance	ns	ns	ns	ns
$LSD_{0.05}$	-	-	-	-
Variety				
Dundee	42.9b	14.8b	26.8b	122.9ab
TGx-1937-1F	44.3b	14.9b	25.0c	146.8a
TGx-1740-2F	37.8c	12.4c	31.0a	95.4b
TGx-1835-10E	50.6a	16.8a	27.0b	127.1ab
Significance	**	**	**	ns
$LSD_{0.05}$	3.60	1.12	1.34	-

Means in the same column followed by the same letter are not significantly different at $p \leq 0.05$. LSD= Least significant difference, ns= non significant at $P \leq 0.05$, **= significant at $P \leq 0.01$,*=Significant at $P \leq 0.05$

Zinc concentration in Dundee and TGx-1740-2F seeds was higher when uninoculated than when inoculated; hence thus to say the introduced bacteria did not influence the concentration of zinc (Figure 3.7A). In TGx-1937-2F and TGx-1835-10E varieties, inoculation resulted in increase seed zinc concentration by 7% and 2.5%, respectively. Inoculated Dundee and TGx-1740-2F plants showed reduction in Cu while TGx-1937-1F was slightly increased by inoculation and TGx-1835-10E showed no response to inoculation (Figure 3.7B). Manganese concentration in seeds of Dundee, TGx-1937-1F and TGx-1740-2F was not increased by inoculation (Figure 3.7C).

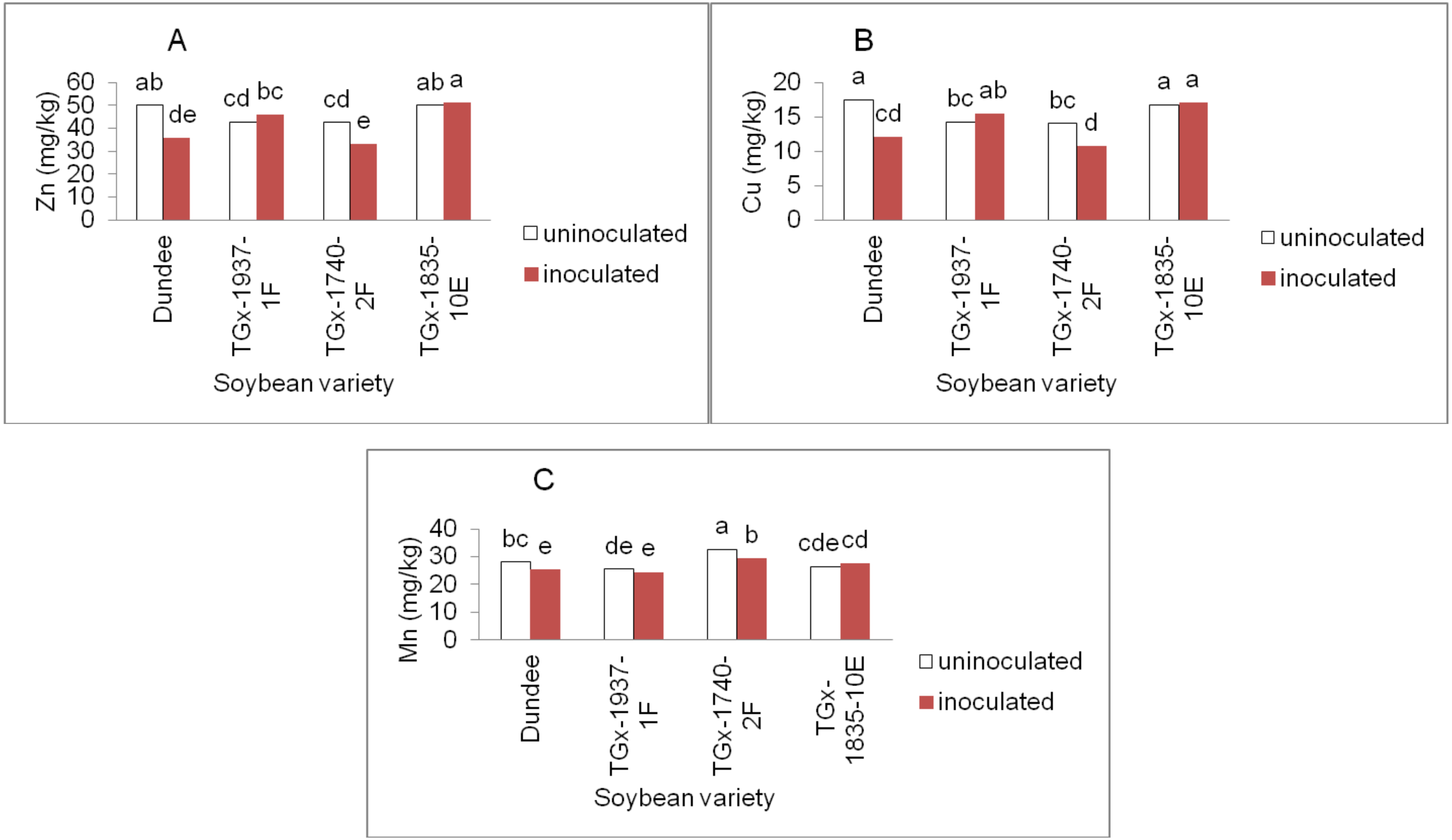

Figure 3.7: Interactive effects of inoculation with *B. japonicum* strain WB74 and soybean variety on seed Zinc (Zn) (A), Copper (Cu) (B) and Manganese (Mn) (C). Bars followed by the same letter are not significantly different at P≤0.05

Correlation analysis showed that nodulation parameters (number of nodules and active nodules) and seed nutrition (seed N and crude protein) were significantly related (Table 3.8). Highly positive and significant correlation ($P \leq 0.01$) was obtained between active nodules, seed N and crude protein. There was also positive significant correlation between seed N and crude protein. Tahir *et al.* (2009) similarly reported a significant correlation of nodules with seed N.

Table 3.8: Persons correlation for nodulation, plant height, seed N and crude protein of soybean

Parameters	ANN	SN%	CP%
NN	0.39*	0.46*	0.46*
ANN		0.66**	0.68**
SN			1.00***

NN= Number of Nodules, ANN=Active nodules number, SN= Seed N, CP= Crude protein

The number of nodules showed positive and significant correlation with chlorophyll content at flowering; dried plant biomass, plant height and grain yield (Table 3.9). Bekere *et al.* (2012) reported significant relationship between nodulation and growth parameters of the soybean crop. Chlorophyll content and dried plant biomass showed significant correlation with plant height, pods number and grain yield. There was also a positive and significant correlation between plant height and grain yield (R^2=0.55), number of pods and grain yield (R^2=0.50). In this study, chlorophyll content, plant height and number of pods per plant showed very strong positive influence on grain yield. Number of nodules and plant biomass had weak positive relationship with grain yield. Plant height also had very strong positive relationship with chlorophyll content. This suggests that chlorophyll content is a good indicator of soybean growth vigour.

Table 3.9: Persons correlation for nodulation, dried plant biomass, plant height, pods number and grain yield of soybean

Parameters	CC (CCI)	DPB (g/plant)	PH (cm)	PN/plant	GY (Kg/ha)
NN	0.59*	0.37*	0.55*	0.30	0.36*
CC		0.18	0.68**	0.39*	0.72**
DPB			0.48*	0.60**	0.46*
PH				0.434	0.74**
PN					0.71**

NN = Number of Nodules, CC= Chlorophyll Content, DPB = Dried plant biomass, PH = Plant height, PN = Pods number, GY=Grain yield

CHAPTER 4

GLASSHOUSE STUDY

4.1 Introduction

This chapter outlines the experimental area, design and data collected under materials and methods used to accomplish the stated objectives, as well as data analysis, results and discussion for the glasshouse study. This study focused on growth and nodulation performance of soybean in different soils of Limpopo, so as to evaluate if major soils of Limpopo Province contain compatible cowpea-type rhizobia capable of nodulating promiscuous soybean varieties. This would contribute to the determination of the potential for successful introduction of soybean to SH farmers.

4.2. Materials and methods

4.2.1 Study location

The pot experiment was conducted during summer growing season in January 2013 at the University of Limpopo glasshouse located Mankweng (23.88˚ S, 29.74˚ E).

4.2.2 Experimental design, procedures and treatments

The experiment was laid in a split-split plot design with three replications in polyethylene plastic pots of 30 cm diameter. The main plot factor was inoculation at two inoculation levels namely (uninoculated and inoculated), where *Bradyrhizobium japonicum* strain WB74 purchased at Soygro Pty Ltd, South Africa was used to inoculate the soybean seeds prior to planting. The subplot factor studied included three soybean varieties consisting of two naturally- nodulating soybean varieties (TGx-1937-1F and TGx-1740-2F) and one commercial (specific nodulating) soybean variety (Dundee). The sub-subplot factor included four soil types from different locations namely sandy clay loam from Syferkuil, loamy sand from Ga-Molepo, sandy loam from Gabaza-Mafarana (in Tzaneen) and loamy sand from University of Limpopo Horticultural unit. Four soybean seeds were planted in each pot and thinned to two at two weeks after emergence. The pots were irrigated three times a week and weeds were controlled by hand picking.

4.2.3 Soil sampling

The soils for the glasshouse experiment were obtained from two districts of Limpopo Province namely Capricorn and Mopani, and were collected at the beginning of the rainy season from land that was never grown to soybean. Four bulk samples of soil types with varying fertility status were collected at a depth of (0-20 cm).The sandy clay loam soil was obtained from University of Limpopo experimental farm, Syferkuil while the sandy loam was obtained from Gavaza-Mafarana dry planting station in Greater Tzaneen local municipality. The loamy sand soil was collected from Lebopo co-operative crop field at Ga-Molepo, while loamy sand was collected from the Horticultural unit of the University of Limpopo. The collected soils were analysed for selected soil chemical/physical properties such as soil pH, organic carbon, phosphorus, mineral nitrogen and soil particle size/texture (Table 4.1).

Table 4.1: Characteristics of soils evaluated with promiscuous soybean cultivars

	Location			
Chemical characteristics	Syferkuil	Tzaneen (Gavaza Mafarana)	Ga-Molepo	ULHorticultural unit
% sand	70	63	80	83
% Clay	23	13	10	3
% Silt	7	24	10	14
Soil texture	Sandy clay loam	Sandy loam	Loamy sand	Loamy sand
% Organic carbon	0.73	0.24	1.46	1.11
pH (H_2O; 1:2.5)	7.11	6.66	7.59	6.50
Phosphorus (mg kg^{-1})	19.99	3.43	10.63	31.00
Mineral N (mg kg^{-1})	64.58	107.06	53.87	35.84

4.2.4. Data collection

4.2.4.1 Growth parameters data

Days to flowering were recorded and prior to termination of the experiment, the number of primary branches were counted. Plant height and chlorophyll content were also measured.

4.2.4.2 Nodulation data

To carefully remove the soybean plants without losing the root nodules, a pair of scissors was used to cut the polythene plastic pots; after which; the bags were shaken to loosen the plant roots from the soil. The whole plant was then removed from the soil by hand. Thereafter, same procedures as described under nodulation data of the field experiment were also followed for the glasshouse study. The following data were also recorded: (a) number of nodules/plant, (b) number of effective nodules/plant, (c) nodule dry weight and (d) shoot and roots dry weight/plant.

4.2.5 Data analysis

Growth and root data were also subjected to Analysis of Variance (ANOVA) using Statistix 9.0, followed by Tukey Honestly Significant Difference (HSD) test at ($\alpha \leq 0.05$) for mean comparison.

4.3 Results and discussion

4.3.1 Soil analysis results

The soil for the glasshouse study had pH ranging between 6.50-7.59 (Table 4.1). These pH ranges are suitable for rhizobial growth that may be why the process of nodulation was not limited in the glasshouse study. According to DAFF (2010), soybeans are better adapted to low pH than other legumes; however the pH of less than 5.2 hampers nitrogen fixation. It is recommended that the acid saturation percentage be kept below 10% (FSSA, 2007). The studied soils show P deficiency except the soil from horticultural unit which shows adequate P-status for production of soybean. According to FSSA (2007), an optimal soil P ranges from 25 to 30 mg/kg (Bray 1) for soybean. Soil P status was 3.43, 10.63 and 19.99 mg/kg for soils from Tzaneen (Gavaza Mafarana), Ga-Molepo and Syferkuil, respectively (Table 4.1). Phosphorus is linked with cell division, root growth, flowering and ripening (FSSA, 2007). All studied soils show sufficient Nitrogen (N) for the production of soybean under inoculation. The mineral N ranged between 35.8 and 107 mg/kg (Table 4.1). According to FSSA (2007), inoculation with correct nitrogen-fixing bacterium (e.g *Rhizobium japonicum*) prior to planting will increase yield in these soils without the application of N fertilizers. However, an initial application of 10 – 20 kg/ha N would be advantageous in the soil from Horticultural unit where less than 10 % clay was observed.

Organic carbon was ranged between 0.24 and 1.46% (Table 4.1). Soils from Syferkuil and Tzaneen showed sandy clay loam and loamy sand textural classes, respectively. Soils from Ga-Molepo and UL showed similar textural class of loamy sand. Tzaneen, Ga-Molepo and UL Horticultural unit soils can be good for soybean production provided that other growth factors are favourable including nutrient status, as these soils are characterized by good aeration, good infiltration and good drainage. However, the low clay content (3%) with high sand in soil from UL Horticultural unit might suggests a potential high leaching of nutrients out of the soil profile and this soil might require intensive fertilization and irrigation for better yield. Although soil from Syferkuil farm is chemically active due to high clay content, soybean production may be limited due to poor infiltration capacity, low drainage rate, poor root distribution and poor crop emergence due to possible compaction that are associated with high clay soils.

4.3.2 Effect of inoculation with *B. japonicum* strain WB74, soybean variety, soil type and their interaction on:

4.3.2.1 Days to flowering

Inoculation exerted non-significant effect on days to flowering. Soybean variety significantly influenced the days to first flowering at ($P \le 0.01$) wherein Dundee flowered much earlier (53 days) than TGx-1937-1F which took about 68 days (Table 4.2). Soil type exerted a significant ($P \le 0.01$) effect on days to flowering. The sandy loam soil from Tzaneen and loamy sand soil from Ga-Molepo influenced the soybean plants to reach first flowering 58 days after planting, and were significantly earlier ($P \le 0.01$) from that of sandy clay loam from Syferkuil and loamy sand from UL Horticultural unit by 4 and 3 days, respectively (Table 4.2).

4.3.2.2 Chlorophyll content

Inoculation significantly influenced the chlorophyll content ($P \le 0.05$), thus inoculation affected the greenness of the leaves as Table 4.2 shows that inoculated plants had higher chlorophyll content than the uninoculated plants. Promiscuous varieties (TGx-1740-2F and TGx-1937-1F achieved higher chlorophyll content at 18.9 and 18.3 cci compared to the commercial variety Dundee at 16.7 cci (Table 4.2). Soil type significantly influenced chlorophyll content (P≤0.01), meaning the type of soil affect the healthiness of the leaf. Loamy sand soil from Ga-Molepo produced leaves with high (19.8 cci) chlorophyll content (Table 4.2). From the results in Table 4.1, it would be expected that soybean plants grown in the soil from Tzaneen would have higher chlorophyll content since it had higher mineral N. The low soil P in soil from Tzaneen may have influenced the low chlorophyll reading since P is a key component of energy generation in plants and plays an important role in the process of photosynthesis. According to Sultenfuss and Doyle (1999), when P is limiting it reduces the leaf expansion, leaf surface area as well as number of leaves; and ultimately affects the chlorophyll content which is an important component in photosynthesis.

4.3.2.3 Plant height

Statistical analysis indicated that there were no significant differences on plant height/plant (P≤0.05) due to inoculation; however the inoculated plants produced taller plants compared to the uninoculated plants (Table 4.2). Variety Dundee produced taller plants than the promiscuous varieties at 58.2 cm, however statistical analysis showed no significant differences between the height of TGx-1740-2F and Dundee varieties; but both Dundee and TGx-1740-2F varieties were significantly taller than TGx-1937-1F (Table 4.2). Plant height was significantly (P≤0.01) affected by the soil type. Loamy sand soil from Ga-Molepo produced tallest plants at (61.0 cm) and significantly differed from the other soil types (e.g. 50.4 cm on sandy clay loam from Syferkuil, 49.0 cm on sandy loam from Tzaneen, and 51.9 cm on loamy sand from UL Horticultural unit centre (Table 4.2). Loamy sand soil from Ga-Molepo influenced growth (plant height) better than the other soils.

4.3.2.4 Number of primary branches

Both inoculation and soybean variety had no significant effect on number of primary branches per plant (Table 4.2). Soil type significantly (P≤0.05) influenced number of primary branches per plant, whereby the loamy sand soil from Ga-Molepo produced high number of primary branches which was significantly higher than those of other soil types (Table 4.2).

Table 4.2: Effect of inoculation with *B. japonicum* strain WB74, soybean variety and soil type on phenological development growth of soybean

Treatment factor	Days to flowering	Chlorophyll content (CCI)	Plant height (cm)	No of primary branches/ plant
Inoculation				
Uninoculated	58.9 a	15.8b	45.8a	2.4a
Inoculated	60.1 a	20.1a	60.4a	2.3a
Significance	ns	*	ns	ns
$HSD_{0.05}$	-	2.02	-	-
Variety				
Dundee	53.2b	16.7b	58.2a	2.3a
TGx-1937-1F	67.8a	18.9a	44.8b	2.3a
TGx-1740-2F	57.5b	18.3ab	56.3a	2.6a
Significance	**	*	**	ns
$HSD_{0.05}$	7.42	2.09	9.28	-
Soil type (location)				
SCL (Syferkuil)	61.7a	18.6a	50.4b	2.2ab
SL (Gabaza-Mafarana	57.8b	17.9ab	49.0b	2.2ab
LS (Ga-Molepo)	57.6b	19.8a	61.0a	3.1a
LS (Greenhouse)	60.9ab	15.5b	51.9b	2.1 b
Significance	**	**	**	*
$HSD_{0.05}$	3.83	2.64	7.10	0.98

Means in the same column followed by the same letter are not significantly different HSD=Honestly Significant Difference, ns=non-significant, ** significant at $P \leq 0.01$,* significant at $P \leq 0.05$. SCL=Sandy clay loam, SL=Sandy loam, LS=Loamy sand

Inoculation x variety interaction exerted a significant effect on days to first flowering ($P \leq 0.05$). The application of inoculation to TGx-1740-2F variety shortened the number of days it took to flower from 61 to 54 days (Figure 4.1A), which suggests that seed inoculation is beneficial in speeding up the growth duration of TGx-1740-2F variety. TGx-1937-1F did not respond to inoculation, while in Dundee inoculation did not show a positive effect as it resulted in increased number of days to flowering from 48 to 58 days (Figure 4.1A).

Inoculation x soybean variety interaction showed significant effect on chlorophyll content ($P \leq 0.01$). Huge effect of inoculation was observed on variety Dundee with increase in chlorophyll content from 11.8 cci to 21.6 cci (+83%) while a slight effect of inoculation was observed on chlorophyll content of TGx-1740-2F as it increased from 16.1 to 20.5 (+27.3%). TGx-1937-1F however did not respond to inoculation (Figure 4.1B). Inoculation x variety interaction enhanced a significant ($P \leq 0.05$) effect on plant height. Inoculation resulted in increased plant height from 49.7 to 66.6 cm (+34.0%) in Dundee and from 44.8 to 67.9 cm (+51.6%) in TGx-1740 2F (Figure 4.1C). Statistical analysis indicated no differences among inoculated and uninoculated TGx-1937-1F variety, although inoculated plants were much taller (Figure 4.1C).

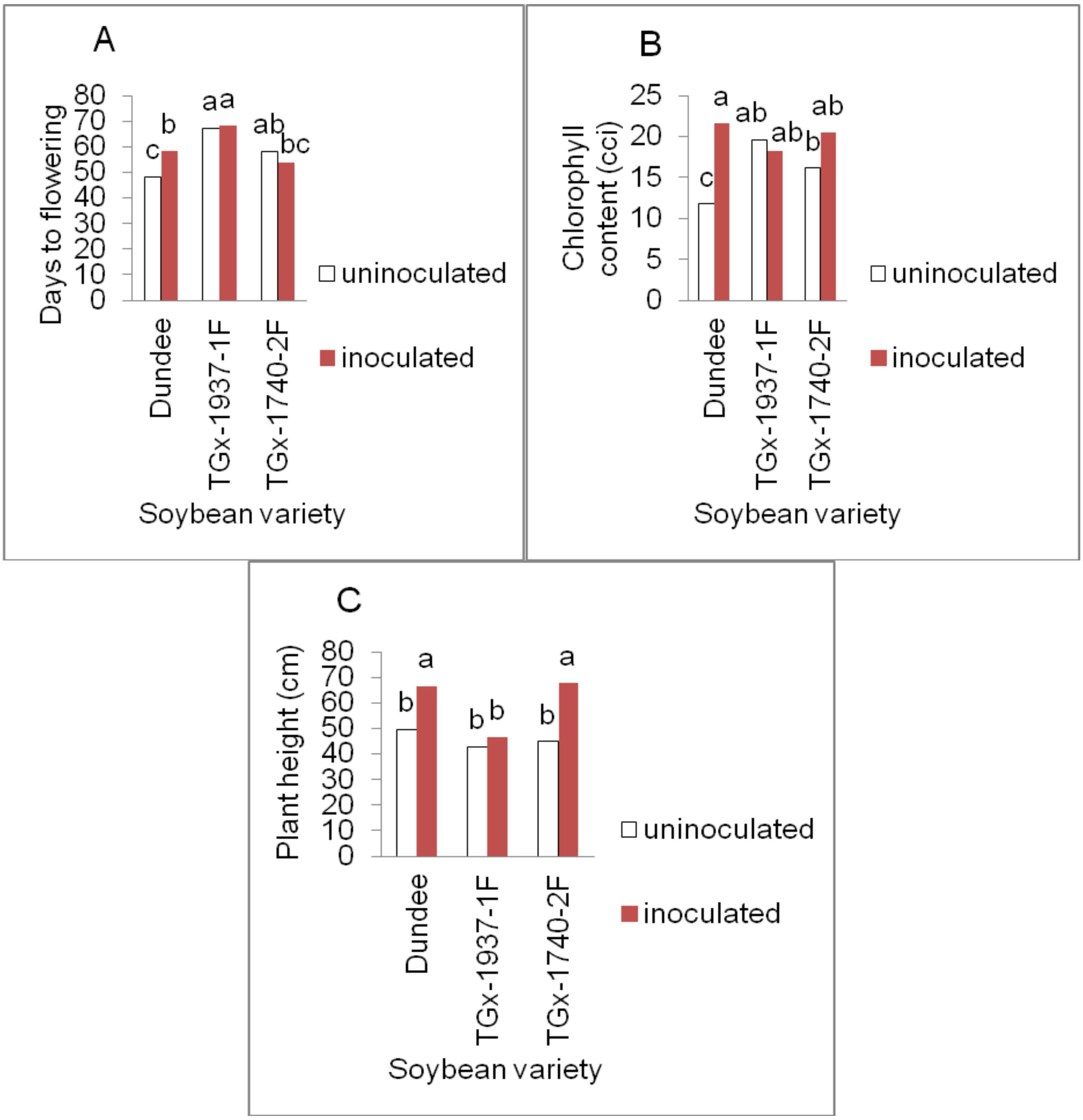

Figure 4.1: Interactive effects of inoculation with *B. japonicum* strain WB74 x soybean variety on days to flowering (A), chlorophyll content (B) and plant height (C). Bars followed by the same letter are not significantly different at P≤0.05

Inoculation x soil type showed a highly significant (P≤0.01) effect on plant height; with plants from the inoculated pots being generally taller compared to the uninoculated plants. Figure 4.2 shows that inoculation had influence on plants planted on all soil types, except on those planted on sandy clay loam from Syferkuil. This may imply that inoculation contribute to improvement of soil status, which therefore leads to improvement of growth. The results suggest that there may be differences in population of cowpea type rhizobia in the soils used in this study, with a possibility that soil from Syferkuil farm has more cowpea-type rhizobia that mostly out-competed the introduced *Bradyrhizobia*. In the other soils, the population of cowpea type rhizobia was likely low hence the noted influence of inoculation.

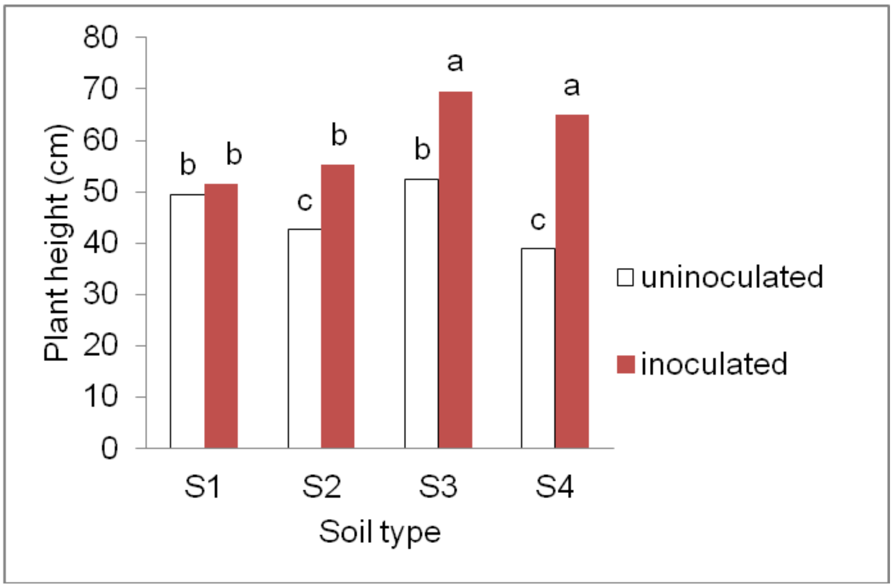

Figure 4.2: Interactive effects of inoculation with *B. japonicum* strain WB74 x soil type on plant height. S1= Sandy clay loam (from Syferkuil), S2= Sandy loam soil (from Tzaneen), S3= Loamy sand soil (from Ga-Molepo), S4= Loamy sand soil (from UL horticultural unit centre). Bars followed by the same letter are not significantly different at P≤0.05

Variety x soil type interaction had significant effect on chlorophyll content (P≤0.01). Dundee variety produced leaves with high chlorophyll content of about 20.6 cci when planted on sandy clay loam soil from Syferkuil and slightly differed significantly with the promiscuous varieties (Figure 4.3). All tested varieties responded similarly when planted in sandy loam from Tzaneen. Both promiscuous TGx-1937-1F and TGx-1740-2F varieties evaluated had high chlorophyll content when planted on loamy sand soil (from Ga-Molepo) (Figure 4.3). On loamy sand soil from UL Horticultural unit variety Dundee produced leaves with lowest chlorophyll content of about 10.9 cci as compared to promiscuous TGx-1937-1F and TGx-1740-2F varieties (Figure 4.3). These results show the potential of soybean varieties to perform differently in different soils.

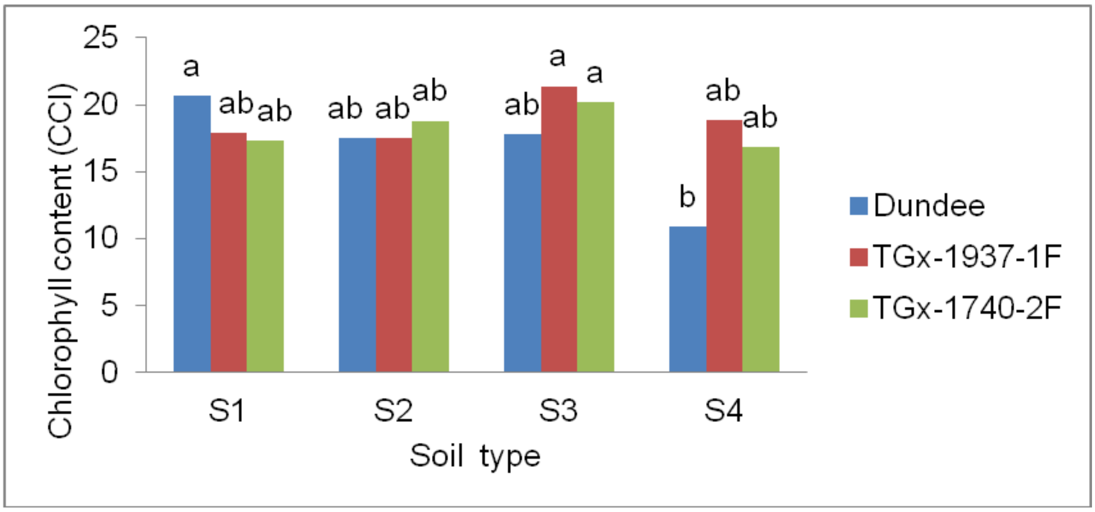

Figure 4.3: Interactive effects of soybean variety x soil type on leaf chlorophyll content. S1= Sandy clay loam (from Syferkuil), S2= Sandy loam soil (from Tzaneen), S3= Loamy sand soil (from Ga-Molepo), S4= Loamy sand soil (from UL horticultural unit centre). Bars followed by the same letter are not significantly different at P≤0.05

Inoculation x variety x soil type had significant effect on chlorophyll content (P≤0.05). The chlorophyll content of Dundee variety showed positive response to inoculation in all soil types used for this study e.g with increase of 33.3%, 99.2%, 100.8% and +169.6% in S1, S2, S3 and S4, respectively (Figure 4.5). TGx-1937-1F did not respond to inoculant application in all the soil types tested as the leaf chlorophyll content of the inoculated plants were mostly outperformed by those of the uninoculated treatment. Chlorophyll content of TGx-1740-2F was also influenced by application of *B. japonicum* strain WB74 inoculant, in all soils tested except on loamy sand soil from UL Horticultural unit centre as the leaf chlorophyll content was reduced from 17.7 to 15.9 cci due to inoculation (Figure 4.5). Figure 4.6 support the chlorophyll content results for response of Dundee variety in soil from Ga-Molepo, by showing the dark green leaves of inoculated plants compared to the uninoculated ones, while Figure 4.7 shows the plants of uninoculated and inoculated TGx-1937-1F plants, supporting the results of chlorophyll content wherein the leaves of both inoculated and uninoculated plants did not show difference in terms of the degree of greenness of the leaves.

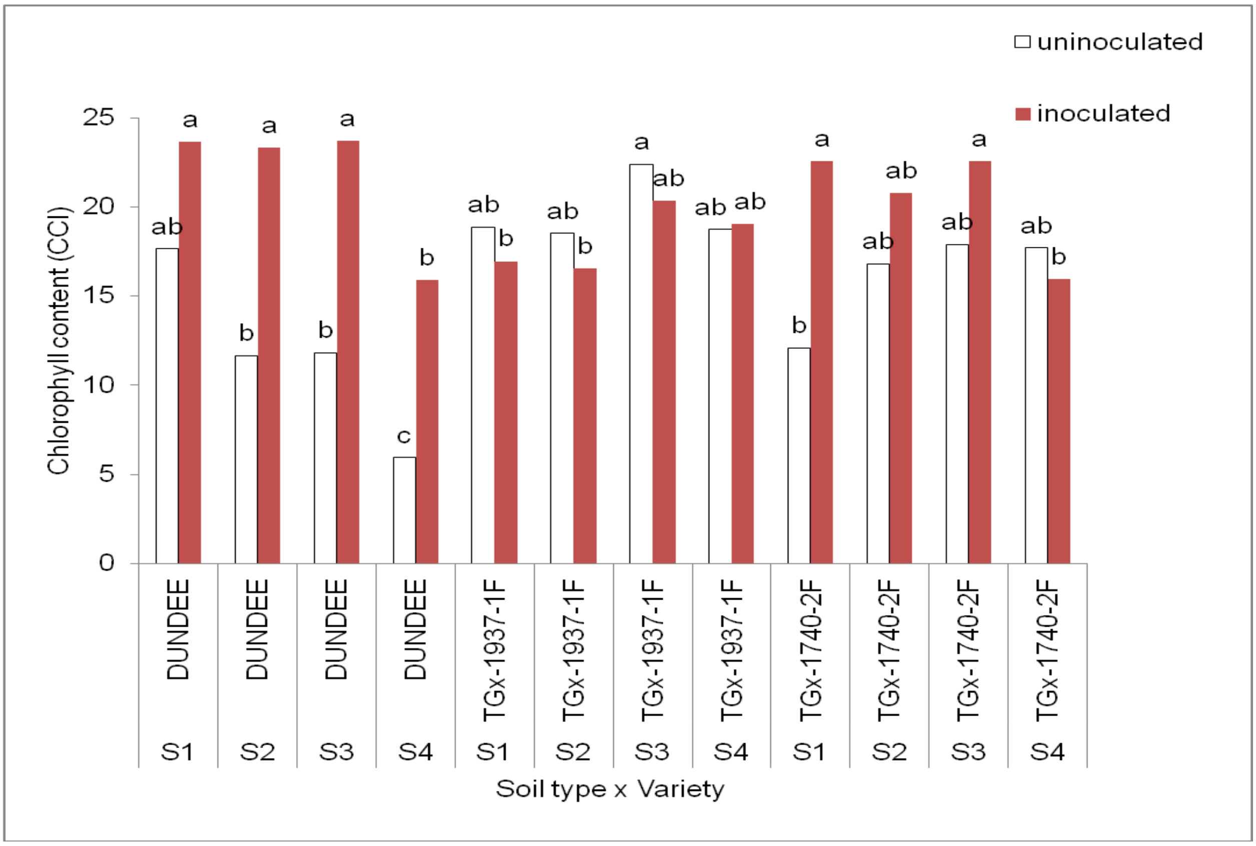

Figure 4.5: Interactive effects of inoculation x soybean variety x soil type on chlorophyll content. S1= Sandy clay loam (from Syferkuil), S2= Sandy loam soil (from Tzaneen), S3= Loamy sand soil (from Ga-Molepo), S4= Loamy sand soil (from UL horticultural unit centre). Bars followed by the same letter are not significantly different at P≤0.05.

Figure 4.6: Comparison of inoculated (left) and uninoculated (right) Dundee variety in soil from Ga-Molepo

Figure 4.7 : Inoculated and uninoculated TGx-1937-1F planted in soil from Syferkuil (A) , Tzaneen (Gabaza-Mafarana) (B), Ga-Molepo (C) and University of Limpopo Horticultural unit (D). Inoculated plants on the left for each soil

4.3.2.5 Number of nodules

Inoculation had no significant effect on number of nodules. Soybean variety significantly ($P \leq 0.01$) influenced number of nodules, whereby TGx-1937-1F achieved the highest number of nodules/plant at 27, which was significantly different from those of TGx-1740-2F and Dundee at; 17 and 18, respectively (Table 4.3). Soil type significantly ($P \leq 0.05$) differed in the number of nodules, wherein loamy sand soil from Ga-Molepo produced higher number of nodules/plant (29) relative to other soil types e.g. Sandy clay loam from Syferkuil and sandy loam soil from Tzaneen produced 19 nodules/plant, while loamy sand soil from UL horticultural unit centre produced 17 nodules/plant (Table 4.3). Given that the soils all had acceptable soil pH, the difference in nodules per plant could be explained by mineral N. Nodulation was expected to be least in the Tzaneen and Syferkuil soils given their high mineral N content.

4.3.2.6 Nodule dry weight

Inoculation, soybean variety and soil type had no significant effect on nodule dry weight (Table 4.3).

4.3.2.7 Dried plant biomass

Inoculation and soybean variety had no significant ($P \leq 0.05$) effect on dried plant biomass; however variety Dundee produced lower dried plant biomass (5.8 g) relative to promiscuous varieties (Table 4.3). Dried plant biomass was highest on plants planted in pots with loamy sand soil (from Ga-Molepo) at 9.6 g and differed significantly from those of other soil types. This is consistent with the degree of nodulation observed.

4.3.2.8 Root dry weight

Inoculation had no significant effect on roots dry weight (Table 4.3). TGx-1937-1F gave high roots dry weight at 1.5 g which was significantly different from that of variety Dundee and TGx-1740-2F (Table 4.3). Root weights differed between soil types with significantly ($P \leq 0.01$) lower root development in the Syferkuil soil. Root development in grain legumes is important in that it influences the organic matter and the legume leaves in the soil.

Table 4.3: Effect of inoculation with *B. japonicum* strain WB74, soybean variety and soil type on nodulation of soybean at flowering to early podding

Treatment factor	Number of nodules/plant	Nodule dry weight/plant (g)	Dried plant biomass (g)	Root dry weight/plant (g)
Inoculation				
Uninoculated	20.6a	0.3a	6.0a	1.1a
Inoculated	21.2a	0.4a	7.8a	1.2a
Significance	ns	ns	ns	ns
$HSD_{0.05}$	-	-	-	-
Variety				
Dundee	17.2b	0.5a	5.8a	1.0b
TGx-1937-1F	27.0a	0.4a	7.9a	1.5a
TGx-1740-2F	18.4b	0.3a	7.0a	1.0b
Significance	**	ns	ns	**
$HSD_{0.05}$	6.62	-	-	0.35
Soil type (location)				
SCL (Syferkuil)	18.8b	0.4a	7.1b	0.9b
SL (Gabaza-Mafarana)	18.6b	0.3a	5.5b	1.2a
LS (Ga-Molepo)	29.0a	0.4a	9.6a	1.3a
LS (Greenhouse)	17.0b	0.5a	5.5b	1.3a
Significance	*	ns	**	**
$HSD_{0.05}$	10.14	-	2.36	0.27

Means in the same column followed by the same letter are not significantly different HSD=Honestly Significant Difference, ns=non-significant, ** significant at $P \leq 0.01$,*= significant at $p \leq 0.05$. SCL=Sandy clay loam, SL=Sandy loam, LS=Loamy sand

Inoculation x soil type interaction was significant at (P≤0.05) on dried plant biomass. Effect of inoculation was observed in loamy sandy soil (from Ga-Molepo) wherein the dried plant biomass increased from 7.4 to 11.8 g (thus +59.5%), while plants planted on other soils tested in this study did not respond to inoculant application (Figure 4.8). Plant biomass decreased by 4.4 % due to inoculation in sandy clay loam soil from Syferkuil. The results clearly showed the variable response of soybean to inoculation in different soil types.

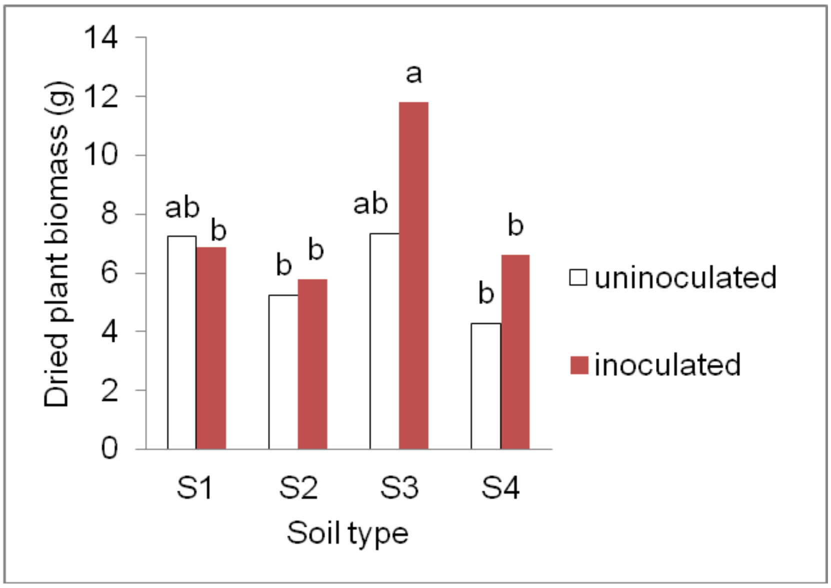

Figure 4.8: Interactive effects of inoculation with *B. japonicum* strain WB74 x soil type on dried plant biomass. S1= Sandy clay loam (from Syferkuil), S2= Sandy loam soil (from Tzaneen), S3= Loamy sand soil (from Ga-Molepo), S4= Loamy sand soil (from UL horticultural unit centre). Bars followed by the same letter are not significantly different at P≤0.05

4.3.2.9 Percent of active nodules

Both uninoculated and inoculated plants had high percentage of active nodules. However, the uninoculated plants showed an average value of 76.3% which was marginally higher than the 75.3% for inoculated plants. The two tested promiscuous varieties (TGx-1937-1F and TGx-1740-2F) had higher percentages of active nodules at 77.5 % and 77.4%, respectively when compared to commercial Dundee variety of 72.4% (Table 4.4). Sandy loam soil from Gabaza- Mafarana had highest number of active nodules at 77.3%, when compared to other soil type (Table 4.4). These high nodule numbers suggest that all the soils tested had high numbers of cowpea type rhizobia. High percentage of active nodules for uninoculated plants suggests that soils tested with promiscuous varieties in this study might have had compatible indigenous or cowpea-type rhizobia. There is however a need to conduct further research that focuses on characterization of rhizobia from nodules of different soybean varieties in soils from different locations of Limpopo Province before introducing the crop to the SH farming sector. Table 4.5 shows effect of inoculation, soybean variety and soil type interaction on percentage of active nodules Table 4.4 presents the percentage of active nodules.

Table 4.4: Inoculation, soybean variety and soil type means for percent of active nodules

Treatment	Percent of active nodules
Uninoculated	76.3
Inoculated	75.3
Dundee	72.4
TGx-1937-1F	77.5
TGx-1740-2F	77.4
SCL (Syferkuil)	75.7
SL (Gabaza-Mafarana)	77.3
LS (Ga-Molepo)	74.5
LS (Greenhouse)	75.5

SCL=Sandy clay loam, SL=Sandy loam, LS=Loamy sand

Table 4.5: Effect of inoculation, soybean variety and soil type interaction on percent of active nodules

Treatments	Percent of active nodules (%)
$I_0V_1S_1$	86
$I_0V_1S_2$	73
$I_0V_1S_3$	68
$I_0V_1S_4$	64
$I_0V_2S_1$	78
$I_0V_2S_2$	80
$I_0V_2S_3$	80
$I_0V_2S_4$	70
$I_0V_3S_1$	73
$I_0V_3S_2$	92
$I_0V_3S_3$	77
$I_0V_3S_4$	74
$I_1V_1S_1$	70
$I_1V_1S_2$	68
$I_1V_1S_3$	75
$I_1V_1S_4$	75
$I_1V_2S_1$	77
$I_1V_2S_2$	71
$I_1V_2S_3$	74
$I_1V_2S_4$	90
$I_1V_3S_1$	70
$I_1V_3S_2$	80
$I_1V_3S_3$	73
I1V_3S_4	80

S_1 = Soil from the University of Limpopo farm (Syferkuil), S_2 = Soil from Tzaneen, S_3= soil from Ga-molepo, S4 = Soil from University of Limpopo experimental farm. V_1 = Dundee, V_2 = TGx-1937-1F, V_3 =TGx-1740-2F

CHAPTER 5

GENERAL DISCUSSION

There was a clear effect of inoculation on chlorophyll content, number of nodules, percentage number of active nodules, nodule dry weight, hundred seed mass, number of pods and grain yield. Inoculation had effect on both promiscuous and the commercial varieties and this suggests that it is very important or beneficial to inoculate soybean seeds before planting, irrespective of type. The huge differences in grain yield achieved in this study as compared to the little cost of inoculants justify the use of inoculation in soybean production. Inoculation is also likely to be very important in the smallholder farming sector where soils are generally of low fertility. These findings agree with Pule-Meulenberg *et al.* (2011) who reported that inoculation had marked effect on the number of nodules and nodule mass. Even though inoculation showed effect on above mentioned parameters, it showed no effect on phenological development (e.g days to 50% flowering and PM), dried plant biomass, plant height, number of primary branches and shelling percentage. High nodule number of the inoculated soybean plants was also reported by Meghvansi *et al.* (2005) and Tahir *et al*. (2009).

Of the evaluated varieties TGx-1937-1F was a late maturing variety as it took about 168 days to reach its PM, Dundee and TGx-1740-2F were the two early maturing varieties from the four observed varieties while TGx-1835-10E can be regarded as medium maturing variety under Limpopo Province growing conditions. High nodulation, grain yield, plant height, number of pods, and biomass were produced by TGx-1937-1F making it the best variety among the evaluated varieties as it is the one that performed/did well in most important parameters evaluated. TGx-1740-2F was the second best performer after TGx-1937-1F in terms of nodule number and plant height, while Dundee was second place after TGx-1937-1F in terms of grain yield. The highest number of primary branches was observed in TGx-1835-10E. Variety effect was significant in almost all parameters evaluated.

The interaction effects of inoculation with *B.japonicum* strain WB74 with soybean variety resulted in increased chlorophyll content, nodule dry weight, 100 seed weight, shelling percentage and grain yield on variety Dundee. In TGx-1937-1F, inoculation caused a slight decrease in shelling percentage and grain yield implying that the introduced *B.japonicum* strain WB74 was probably outcompeted by the indigenous rhizobia that were present in the soil. These results agree with the findings reported by Okugun and Sanginga (2003) who reported that introduced strain had no effect on grain yield.

Of all varieties tested in this study TGx-1937-1F showed larger biomass which was significantly higher than that of other varieties tested. The large biomass in TGx-1937-1F indicates that it can play an important role in covering the soil layer thereby reducing the soil run-off and erosion, also suppresses weed growth by reducing the amount of light that can reach the ground. The inclusion of the TGx-1937-1F among crops grown by the SH farmers could possibly reduce the cost of labour for weeding. Its large leaf biomass will also contribute to soil fertility for example when they fall into the soil and decompose thereby adding organic matter, thus improving the nutrient conditions for the crop that will follow it. Large biomass of this variety can also provide benefits when it comes to fodder production for livestock. This agrees with Mpepereki *et al.* (2000) who reported that large leaf biomass in promiscuous varieties adds large benefits to the SH farmers by providing both fodder for livestock and an organic amendment for soil fertility improvement. Osunde *et al*. (2003b) reported significant differences in terms of biomass between two promiscuous varieties (TGx-1456-2E and TGx-1660-19F) at 103.5 and 80.1 g/plant. There was no significant effect due to inoculation; however the uninoculated plants gave larger biomass than the inoculated ones. The results in this study disagree with Osunde *et al*. (2003b) who reported lowest biomass in uninoculated plants. Local information about TGx varieties is limited and there is therefore need to conduct further research and make information available about promiscuous varieties performance in different locations of South Africa.

TGx-1937-1F showed higher yield compared to other three varieties evaluated while TGx-1740-2F and TGx-1835-10E varieties showed a comparable grain yield to Dundee variety (Table 3.4). The soybean varieties tested in the present study showed great potential in terms of yield, when compared to the yield of many TGx varieties reported in some studies. For example, Sanginga *et al.* (2002) reported grain yield range of between 1340 and 1494 kg/ha for three promiscuous varieties, where TGx-1519-1D (1340 kg/ha) was significantly lower in yield compared to TGx-1456-2E (1494 kg/ha) and TGx-1660-19F (1493 kg/ha) in Nigeria.

Correlation analysis showed strong positive influence of chlorophyll content, plant height and number of pods per plant on grain yield. Thus the higher the number of pods/plant the higher the grain yield. Kuruvadi and Valdez (1993) indicated that pod number is an important element of yield and that the pod number tends to increase with the environmental improvements. This suggests that TGx-1937-1F with the highest pod number per plant is well suited for Limpopo conditions though it is a late maturing variety. The strong influence of inoculation on grain yield of variety Dundee shows that it is much more dependent on inoculation. Osunde *et al*. (2003b) reported that growth and yield of soybeans was much more dependent on farmers’ field than inoculation or cultivars, while the inoculation effect was site dependent as tested varieties showed no significant response to inoculation.

TGx-1835-10E × inoculation interaction increased its chlorophyll content and grain yield. The overall assessment actually suggests that inoculation improves the nodulation, growth and yield of the soybean varieties Dundee, TGx-1937-1F, TGx-1740-2F and TGx-1835-10E varieties, even though TGx-1835-10E was the poorest performer in almost all parameters. Meghvansi *et al.* (2005) also reported significant effect of inoculation on overall nodulation and vegetative growth of soybean plants.

According to Lubungu *et al.* (2013), soybean offers a variety of potential benefits including diets and income for smallholder farmers and to production systems. Looking at commercial farmers, TGx-1937-1F could be a good variety for them especially when we consider the issue of biodiesel production, it would result in more yield, then more oil, and then more biodiesel. More oil would also mean the risks of stomach diseases and diabetes are reduced through the use of soybean oil as Singh (2005) indicated. More oil would also mean more products could then be produced in South Africa thereby boosting the South African economy.

High protein levels were observed in different soybean varieties and since protein from animal sources is unaffordable to many poor homesteads or farm families the soybean crop could be a good cheap protein source to those who are poor in Limpopo Province. According to CBU (2011) the soybean crop is emerging as an important feed, food and as well as raw materials for producing high quality protein products. Soybean could play an important role in giving the macronutrients that are most compromised in the diets of low income communities e.g carbohydrates and proteins, since the analysis of the promiscuous soybean evaluated showed that soybean is a healthy food source of protein and carbohydrates which could help reduce the problem of malnutrition.

In the glasshouse study, inoculation had effect on leaf chlorophyll content only, thus inoculation did not influence the other evaluated parameters such as days to flowering, plant height, number of primary branches, number of nodules, nodule dry weight and dried plant biomass.

Variety showed to have influenced days to flowering, chlorophyll content, plant height, number of nodules and roots dry weight, however it had no influence on primary branches, nodule dry weight, and dried plant biomass. TGx-1937-1F showed better performance in terms of achieving high nodule number and dry weight compared to Dundee and TGx-1740-2F varieties. The difference in nodule number between TGx-1937-F and TGx-1740-2F varieties indicates that promiscuous varieties have a variation in their ability to produce nodules. This result agrees with Arulnandhy (undated) who investigated 15 soybean varieties and indicated that there was a remarkable difference in nodule number and nodule dry weight among investigated varieties, indicating a wide variation in their ability to nodulate as a result of their interaction with the indigenous rhizobial strains available in soils. This therefore suggests the need for evaluating BNF for each variety under different environments.

Different soils used for the glasshouse study showed significant influence on days to flowering, leaf chlorophyll content, plant height, number of primary branches, dried plant biomass, roots dry weight and number of nodules; however it had no influence on nodule dry weight. Higher chlorophyll content, plant height, number of primary braches, dried plant biomass and number of nodules were achieved by loamy sand soil from Ga-Molepo, making it the best soil type among the other soils evaluated, thus to say the area Ga-Molepo could be a good target for producing the soybean crop. However, this study could not pinpoint the exact soil characteristics responsible for the observed differences. There is need to characterize rhizobia in the soybean nodules from the different varieties and in different soils.

Inoculation x variety interaction influenced days to flowering, leaf chlorophyll content and plant height only, while inoculation x soil type interaction showed to have influenced plant height and dried plant biomass. The interactive effect of variety x soil type had influence on chlorophyll content only. The interaction between inoculation x variety x soil type had influence on one parameter (chlorophyll content), making it the only parameter which was influenced by all individual treatment factors (inoculation, variety and soil type) and their interactions beside inoculation x soil type interaction.

Inoculation showed to have influenced chlorophyll content in both glasshouse and field condition and the results have shown that it will be unlikely to produce the commercial Dundee variety without the application of inoculant. Mpepereki *et al.* (1996), in Mpepereki *et al*. (2000), reported that SH farmers noticed that inoculated soybean plants developed a darker green colour than the uninoculated soybeans, which is similar to observations in the present study. This simply underlines that inoculation results in healthier crops. Field and glasshouse experiments revealed that the soybean crop behaves differently depending on the conditions that they are grown in. Soybean plants grew and spread well under field conditions than under the glasshouse conditions. The standability was reduced when grown in pots than when grown in the field, e.g. the stems of the plants in pots were thin compared to the plants in the field. The crop growth itself was reduced when plants are planted in the pots probably due to restriction of root growth. This suggests that field conditions are the best for soybean evaluation.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

In conclusion the overall evaluation showed that it is beneficial to inoculate the soybean varieties studied with *B.japonicum* strain WB74, especially the commercial variety Dundee, in order to enhance their growth nodulation and yield. This therefore suggests that it is of particular importance to inoculate the soybean seeds before planting in order to improve/enhance nodulation for the improvement of soil fertility through BNF as the results of the study clearly indicated that nodule numbers were very low when both TGx lines and the commercial variety were not inoculated. This however will only be possible to farmers with access to inoculants. In areas where SH farmers are poor and have little access to agrochemical inputs, TGx-1937-1F even though is late maturing, could be a good variety to be introduced in Limpopo Province for the SH farmers as it did form acceptable number of nodules and also gave high biomass and yield well without inoculation.

The most important factor of producing a soybean crop is to get good yield. The promiscuous variety, TGx-1937-1F can give SH farmers a chance to produce soybean with the minimal inputs that they have and still give them good yield thereby ensuring that they get the protein from the soybean plants, and therefore improve nutrition or diet of resource poor households. The variety TGx-1937-2F could however be risky for dryland production given that most SH farmers are located in areas of low rainfall and short rainy season. It is recommended that SH farmers in dryland areas can use TGx-1740-2F with inoculation, as it matures earlier. Loamy sand soil from Ga-Molepo has shown great potential. If the resource-poor SH farmers of Limpopo can have access to rhizobial inoculants, promiscuous soybean and also taught well or given good experience of growing the soybean crop the farmers of Limpopo Province will then produce adapted soybean cultivars and gets all the possible benefits from the crop.

The glasshouse study showed that there is need to test promiscuous soybeans in different soils, climatic locations and also to characterize rhizobia in root nodules of soybean in order to make sure that they can nodulate naturally (ensure availability of compatible cowpea type rhizobia) before introducing the crop for SH farmers.

REFERENCES

Abaidoo, R.C., Keyser, H.H., Singleton, P.W. and D. Borthakur. 2000. *Bradyrhizobium* spp. (TGx) isolates nodulating the new soybean cultivars in Africa are diverse and distinct from Bradyrhizobia that nodulate North American soybeans. *International Journal of Systematic and Evolutionary Microbiology*, 50: 225-234.

Abaidoo, R.C., Keyser, H.H., Singleton, P.W., Dashiell, K.E. and N. Sanginga. 2007. Population size, distribution, and symbiotic characteristics of indigenous *Bradyrhizobium spp*. that nodulate TGx soybean genotypes in Africa. *Applied Soil Ecology*, 35: 57-67.

Adjei, M.B., Quesenberry, K.H. and C.G. Chambliss. 2002. Nitrogen Fixation and Inoculation of Forage Legumes. Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, Gainesville, Fla. http://ufdc.ufl.edu/IR00001583/00001. Accessed 2013/04/01.

Akande, S.R., Owolade, O.F. and J.A. Ayanwole. 2007. Field evaluation of soybean varieties at Ilorin in the southern guinea savanna ecology of Nigeria. *African Journal of Agricultural Research*, 2: 356-359.

Arulnandhy. Undated. Promiscuous nodulation in soyabeans. Soybean Foods Research Centre, Gannoruwa, Peradeniya. http://www.goviya.lk/agrilearning/Pulses/Pulses_research/soyaaa/So5.pdf. Accessed 2012/07/04.

Bala, A., Abaidoo, R. and P. Woomer. 2010. Rhizobia Strain Isolation and Characterisation Protocol. http://www.n2africa.org. Accessed 2013/03/24.

Balasevic-Tubic, S., Dukic, V., Marinkovic, J., Dozet, G., Petrovic, K. and M. Tatic. 2011. Importance of microbiological fertilizer used in soybean production: Agronomical and biological aspects. *African Journal Microbiology Research*, 5: 4909-4916.

Bejandi, T.K., Sharifii, R.S., Sedghi, M. and A. Namvar. 2012. Effects of plant density, *Rhizobium* inoculation and microelements on nodulation, chlorophyll cotent and yield of chickpea (*Cicer arietinum* L.). *Annals of Biological Research*, 3: 951-958.

Bekere, W., Wolde-Meskel, E. and T. Kebede. 2012. Growth and nodulation response of soybean (*Glycine max* L) to *Bradyrhizobium* inoculation and phosphorus levels under controlled condition in South Western Ethiopia. *African Journal of Agricultural Research*, 7:4266-4270.

Bray, R.H. and L.T. Kurtz. 1945. Determinationof total Organic and available form of phosphorus in soils. *Soil Science*, 59: 39-45.

Crop Biotech Update, CBU. 2011. IITA releases better soybean varieties for African farmers. Newsletter. http://www.iita.org/soybean. Accessed 2014/03/01.

Department of Agriculture Forestry and Fisheries. DAFF. 2011. Soyabean Market Value Chain Profile 2010-2011. http://www.daff.gov.za/docs/AMCP/SoyabeanMVCP2010-2011.pdf. Accessed 2012/06 /18.

Department of Agriculture Forestry and Fisheries, DAFF. 2010. Soya beans production guidelines. Government printers: Pretoria.

Ervin, R.B., Wang, C.Y. and J.D. Wright. 2004. Dietary intake of selected minerals for the United States population: 1999–2000. *Energy*, 1:6.

Fageria, N.K., Baligar, V.C. and B.A. Bailey. 2005. Role of cover crops in improving soil and row crop productivity. *Communications in Soil Science and Plant Analysis*, 36: 2733-2757.

Fertilizer Society of South Africa, FSSA. 2007. Fertilizer Handbook, 6th revised edition. The Fertilizer Society of South Africa. Lynnwood Ridge, South Africa, pp88.

Gwata, E.T., Wofford, D.S., Pfahler, P.L. and K.J. Boote. 2004. Genetics of promiscuous nodulation in soybean: Nodule dry weight and leaf color score. *Journal of Heredity,* 95: 154-157.

Havlin, J.L., Beaton, J.D., Tisdale, S.L. and W.L Nelson. 2005. Soil Fertility and Fertilizers: An Introduction to Nutrient Management. 7th edition, Prentice Hall publishers, pp 102 -107.

Hungria, M. and M.A.T. Vargas. 2000. Environmental factors affecting N2 fixation in grain legumes in the tropics, with an emphasis on Brazil. *Field Crops Research.* 65: 151-164.

Ikeogu, U.N., and G.E. Nwofia. 2013. Yield parameters and stability of soybean [*Glycine max*.(L.) Merril] as influenced by phosphorus fertilizer rates in two ultisols. *Journal of Plant Breeding and Crop Science*, *5*: 54-63.

Javaid, A. and N. Mahmood. 2010. Growth, nodulation and yield response of soybean to biofertilizers and organic manures. *Pakistan Journal of Botany,* 42: 863-871.

Kuruvadi, S. and I. Valdez.1993. Range of yield components and phenotyp5ic correlations in tepary beans (Phaseolus acutifolius) under dryland conditions. *New Crops. Wiley, New York*, 594-596.

Lindemann, W.C. and C.R. Glover. 2003. Nitrogen fixation by legumes. New Mexico State University. Electronic Distribution.

Lubungu, M., Burke, W. and N.J. Sitko. 2013. Challenges of Smallholder Soybean Production and Commercialization in Eastern Province of Zambia (No. 161375). Michigan State University, Department of Agricultural, Food, and Resource Economics.

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

Mahan L.K. and S. Escott-Stump. 2008. Krause's Food and Nutrition Therapy.12th edition. Philadelphia: W.B Saunders, pp 39-137.

Maingi, J.M., Gitonga, N.M., Shisanya, C.A., Hornetz, B. and G.M. Muluvi. 2006. Population levels of indigenous *Bradyrhizobia* nodulating promiscuous soybean in two Kenyan soils of the semi-arid and semi-humid agroecological zones. *Journal of Agriculture and Rural Development in the Tropics and Subtropics,* 107:149-159.

Maluleke, N. 2008. Sekhukhune farmers receive seeds and fertilizers. Official Newsletter of Mapfura-Makhura Incubator.Volume 1.

Meghvansi, M.K., Prasad, K. and S.K Mahna. 2005. Identification of pH tolerant Bradyrhizobium japonicum strains and their symbiotic effectiveness in soybean [*Glycine max* (L.) Merr.] in low nutrient soil. *African Journal of Biotechnology*, 4: 663-666.

Mohammadi, K., Sohrabi, Y., Heidari, G., Khalesro, S. and M. Majidi. 2012. Effective factors on biological nitrogen fixation. *African Journal of Biotechnology*, 7: 1782-1788.

Moshia, M.E. 2005. Statistical correlations between extractable Ca, Mg, K and P from fresh and laboratory prepared soil samples. Masters dissertation. University of Limpopo, South Africa. pp 18-69.

Mpangane, P.N.Z, Ayisi, K.K., Mishiyi, M.G. and A. Whitbread. 2004. Grain yield of maize grown in sole and binary cultures with cowpea and lablab in the Limpopo Province of South Africa. Tropical Legumes for Sustainable Farming Systems in Southern Africa and Australia. In: Whitbread, A.M., and B.C. Pengelly (eds.), ACIAR Proceedings No.115: 106-114.

Mpepereki, S., Javaheri F., Davis, P. and K.E. Giller. 2000. Soyabeans and sustainable agriculture: Promiscuous soyabeans in southern Africa. *Field Crops Research,* 65:137-149.

Odhiambo, J.J.O. 2003. Physical features, soils and land utilization*,* in *Agriculture as the cornerstone of the economy of Limpopo province,* eds A.E Nesanvumi, S.A Oni, J.J Odhiambo and N.D Nthakheni, Livhu Printing, Johannesburg, South Africa.

Okalebo, J.R., Gathua, K.W. and P.L. Woomer. 2002. Laboratory methods of soil and water analysis: A working manual, second edition, pp 128.

Okogun, J.N. and N. Sanginga. 2003. Can introduced and indigenous rhizobial strains compete for nodule formation by promiscuous soybean in the moist savanna agroecological zone of Nigeria? *Biology and Fertility of Soils,* 38: 26-31.

Olatunji, S.O., Agumagu, A.C. and O.M Adesope. 2011. Utilization of Soybean Products by Farm Families in Abia State Nigeria. *Journal of Agricultural Extension and Rural Development*, 3: 186-192.

Osunde, A.O., Bala, A., Gwam, M.S., Tsado, P.A., Sanginga, N. and J.A Okogun. 2003a. Residual benefits of promiscuous soybean to maize (*Zea mays* L.) grown on farmers' fields around Minna in the southern Guinea savanna zone of Nigeria. *Agriculture, Ecosysystems and Environment,* 100: 209-220.

Osunde, A.O., Gwam, S., Bala, A., Sanginga, N. and J.A Okogun. 2003b. Responses to rhizobial inoculation by two promiscuous soybean cultivars in soils of the Southern Guinea savanna zone of Nigeria. *Biology and Fertility of Soils*, 37: 274-279.

Pule-Meulenberg, F., Gyogluu, C., Naab, J. and F.D. Dakora. 2011. Symbiotic N nutrition, bradyrhizobial biodiversity and photosynthetic functioning of six inoculated promiscuous-nodulating soybean genotypes. *Journal of Plant Physiology*, 168: 540-548.

Riekert, S. and S. Bainbridge.1998. Analytical methods of the Cedara plant and soil laboratory. KwaZulu-Natal Department of Agriculture and Environmental Affairs, South Africa, Pietermaritzburg.

Sanginga, N., Abaidoo, R., Dashiell, K., Carsky, R.J. and A. Okogun. 1996. Persistence and effectiveness of rhizobia nodulating promiscuous soybeans in moist savanna zones of Nigeria. *Applied Soil Ecology,* 3: 215-224.

Sanginga, N., Okogun, J., Vanlauwe, B. and K. Dashiell. 2002. The contribution of nitrogen by promiscuous soybeans to maize based cropping the moist savanna of Nigeria. *Plant and Soil*, 241: 223-231.

Schulz, T.J., Thelen, K.D. and D. Wang. 2005. The Effect of *Bradyrhizobium japonicum* inoculant on soybean growth and yield. Department of Crop and Soil Sciences, Michigan State University, East Lansing.

Sessitsch, A., Howieson, J.G., Perret, X., Antoun, H. and E. Martinez- Romero. 2002. Advances in Rhizobium research. *Critical Reviews in Plant Sciences*, 21: 323-378.

Shiferaw, B., Bantilan, M.C.S. and R Serraj. 2004. Harnessing the potential of BNF for poor farmers: technological policy and institutional constraints and research need.

Symbiotic nitrogen fixation; prospects for enhanced application in tropical agriculture. Oxford & IBH, New Delhi, *3*.

Silva, J.A. and R. Uchida. 2000. Biological nitrogen fixation. Nature’s partnership for sustainable Agricultural Production. Niftal center for BNF Technologies. Available at: http://www.ctahr.hawaii.edu/oc/freepubs/pdf/pnm. Accessed 2013/08/29.

Singh, M.S. 2005. Effect of *Bradyrhizobium* Inoculation on Growth, Nodulation and Yield Attributes of Soybean. *Agricultural Reviews,* 26: 305-308.

Singh, S.P. and D. Singh. 2010. Biodiesel production through the use of different sources and characterization of oils and their esters as the substitute of diesel: a review. *Renewable and Sustainable Energy Reviews*, 14: 200-216.

Sultenfuss, J. H and W.J. Doyle.1999. Phosphorus for agriculture. *Better Crops with Plant Food*, *83*: 1-40. http://greenpi.info/. Accessed 2014/06/8.

Tahir, M.M., Abbasi, M.K., Rahim, N., Khaliq, A. and M.H. Kazmi. 2009. Effect of Rhizobium inoculation and NP fertilization on growth, yield and nodulation of soybean (Glycine max L.) in the sub-humid hilly region of Rawalakot Azad Jammu and Kashmir, Pakistan. *African Journal of Biotechnology*, 8: 6191-6200.

Tefera, H. 2011. Breeding for Promiscuous Soybeans at IITA. *Soybean-Molecular Aspects of Breeding (Aleksandra Sudaric ed.). ISBN*, 978-953.

Tran, Y.T. 2004. Response to and benefits of rhizobial inoculation of soybean in the south of Vietnam. In *The 4th International Crop Science Congress.* 26 September - 1 October 2004. Brisbane: Australia.

Uaboi-Egbenni, P.O., Okolie, P.N., Okafor, C.N., Akinyemi, O., Bisi-Johnson, M.A. and O.D. Teniola. 2010. Effect of soil types and mixtures on nodulation of some beans and groundnut varieties. *African Journal of Food, Agriculture, Nutrition and Development*, 10: 2272-2290.

United Soybean Board, USB. 2008. Food and fuel. Meeting the challenges of feeding the world and creating renewable fuels.
http://www.soyconnection.com/soybean_oil/pdf/foodvsfuel_soy_biofuels.pdf.
Accessed 2012/07/04.

Walkley, A. and I.A. Black.1934. An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Science, 37:29-37.

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

Zhang, H., Daoust, F., Charles, T.C., Driscoll, B.T., Prithiviraj, B., and D.L Smith. 2002. *Bradyrhizobium japonicum* mutants allowing improved nodulation and nitrogen fixation of field-grown soybean in a short season area. *The Journal of Agricultural Science*, 138: 293-300.

LIST OF APPENDICES

Analysis of Variance (ANOVA) tables for field study

Appendix 1: Days to 50% flowering

Source of variance	DF	SS	MS	F	P
Replication (A)	3	1.4	0.46		
Inoculation (B)	1	1.4	0.13	1.00	0.3910
Error A*B	3	0.4	0.13		
Variety (C)	3	10398.4	3466.12	11883.9	0.0000
B*C	3	0.4	0.12	0.43	0.7350
Error A*B*C	18	5.3	0.29		
Total	31	10405.9			

Appendix 2: Days to physiological maturity

Source of variance	DF	SS	MS	F	P
Replication (A)	3	1.4	0.5		
Inoculation (B)	1	0.1	0.1	1.00	0.3910
Error A*B	3	0.4	0.1		
Variety (C)	3	11102.4	3700.79	12688.4	0.0000
B*C	3	0.4	0.1	0.43	0.7350
Error A*B*C	18	5.3	0.3		
Total	31	11109.9			

Appendix 3: Chlorophyll content

Source of variance	DF	SS	MS	F	P
Replication (A)	3	156.35	52.118		
Inoculation (B)	1	550.46	550.456	95.20	0.0023
Error A*B	3	17.35	5.782		
Variety (C)	3	182.14	60.714	13.62	0.0001
B*C	3	144.03	48.009	10.77	0.0003
Error A*B*C	18	80.25	4.459		
Total	31	1130.58			

Appendix 4: Dried plant biomass

Source of variance	DF	SS	MS	F	P
Replication (A)	3	3209.2	1069.7		
Inoculation (B)	1	778.0	778.0	1.21	1.21
Error A*B	3	1926.5	642.2		
Variety (C)	3	67045.1	22348.4	33.86	0.0000
B*C	3	4010.3	1336.8	2.03	0.1464
Error A*B*C	18	11882.0	660.1		
Total	31	88851.0			

Appendix 5: Number of nodules

Source of variance	DF	SS	MS	F	P
Replication (A)	3	105.6	35.21		
Inoculation (B)	1	2520.5	2520.50	272.49	0.0005
Error A*B	3	27.8	9.25		
Variety (C)	3	2604.4	868.13	2.47	0.0951
B*C	3	1182.5	394.17	1.12	0.3668
Error A*B*C	18	6330.1	351.67		
Total	31	12770.9			

Appendix 6: Nodule dry weight

Source of variance	DF	SS	MS	F	P
Replication (A)	3	0.5799	0.1933		
Inoculation (B)	1	12.2265	12.2265	44.02	0.0070
Error A*B	3	0.8333	0.2778		
Variety (C)	3	7.9340	2.6447	5.26	0.0088
B*C	3	10.1377	3.3792	6.72	0.0031
Error A*B*C	18	9.0539	0.5030		
Total	31	40.7654			

Appendix 7: percentage number of active nodules

Source of variance	DF	SS	MS	F	P
Replication (A)	3	1705.2	568.4		
Inoculation (B)	1	13530.1	13530.1	12.34	0.0391
Error A*B	3	3288.1	1096.0		
Variety (C)	3	3530.8	1176.9	1.82	0.1804
B*C	3	3634.6	1211.5	1.87	0.1710
Error A*B*C	18	11668.6	648.3		
Total	31	37357.5			

Appendix 8: Plant height

Source of variance	DF	SS	MS	F	P
Replication (A)	3	1835.31	611.770		
Inoculation (B)	1	260.49	260.490	4.08	0.1366
Error A*B	3	191.37	63.789		
Variety (C)	3	2638.35	879.449	10.91	0.0003
B*C	3	688.80	229.601	2.85	0.0666
Error A*B*C	18	1451.49	80.638		
Total	31	7065.80			

Appendix 9: Number of primary branches

Source of variance	DF	SS	MS	F	P
Replication (A)	3	15.344	5.1146		
Inoculation (B)	1	0.781	0.7813	1.47	0.3120
Error A*B	3	1.594	0.5312		
Variety (C)	3	66.594	22.1979	26.97	0.0000
B*C	3	2.344	0.7812	0.95	0.4377
Error A*B*C	18	14.812	0.8229		
Total	31	101.469			

Appendix 10: Number of pods

Source of variance	DF	SS	MS	F	P
Replication (A)	3	27844	9281.4		
Inoculation (B)	1	19751	19750.8	44.16	0.0069
Error A*B	3	1342	447.3		
Variety (C)	3	75504	25168.1	16.73	0.0000
B*C	3	1139	379.8	0.25	0.8585
Error A*B*C	18	27077	1504.3		
Total	31	152658			

Appendix 11: Hundred seed weight

Source of variance	DF	SS	MS	F	P
Replication (A)	3	16.519	5.5062		
Inoculation (B)	1	21.076	21.0763	20.61	0.0200
Error A*B	3	3.068	1.0226		
Variety (C)	3	268.902	89.6340	72.07	0.0000
B*C	3	25.939	8.6462	6.95	0.0026
Error A*B*C	18	22.386	1.2437		
Total	31	357.889			

Appendix 12: Shelling percentage

Source of variance	DF	SS	MS	F	P
Replication (A)	3	33.68	11.226		
Inoculation (B)	1	195.43	195.426	7.83	0.0679
Error A*B	3	74.87	24.957		
Variety (C)	3	239.10	79.702	5.12	0.0098
B*C	3	366.28	122.094	7.84	0.0015
Error A*B*C	18	280.46	15.581		
Total	31	1189.83			

Appendix 13: Grain yield

Source of variance	DF	SS	MS	F	P
Replication (A)	3	3.257E+07	1.085E+07		
Inoculation (B)	1	2.228E+07	2.228E+07	44.71	0.0068
Error A*B	3	1495120	498373		
Variety (C)	3	2.319E+07	7731588	8.03	0.0013
B*C	3	2.150E+07	7165161	7.44	0.0019
Error A*B*C	18	1.734E+07	963205		
Total	31	1.183E+08			

Appendix 14: Moisture content

Source of variance	DF	SS	MS	F	P
Replication (A)	3	4.3731	1.45771		
Inoculation (B)	1	3.6046	3.60461	4.38	0.1275
Error A*B	3	2.4705	0.82351		
Variety (C)	3	3.3832	1.12775	2.62	0.0825
B*C	3	1.1617	0.38725	0.90	0.4608
Error A*B*C	18	7.7522	0.43068		
Total	31	22.7455			

Appendix 15: Fat content

Source of variance	DF	SS	MS	F	P
Replication (A)	3	13.315	4.4385		
Inoculation (B)	1	20.672	20.6724	34.45	0.0099
Error A*B	3	1.800	0.6001		
Variety (C)	3	146.745	48.9151	43.00	0.0000
B*C	3	10.151	3.3837	2.97	0.0592
Error A*B*C	18	20.476	1.1376		
Total	31	213.161			

Appendix 16: Crude protein

Source of variance	DF	SS	MS	F	P
Replication (A)	3	30.842	10.281		
Inoculation (B)	1	107.128	107.128	71.88	0.0034
Error A*B	3	4.471	1.490		
Variety (C)	3	99.338	33.113	12.75	0.0001
B*C	3	113.379	37.793	14.56	0.0000
Error A*B*C	18	46.738	2.597		
Total	31	401.895			

Appendix 17: Non saturated carbohydrates

Source of variance	DF	SS	MS	F	P
Replication (A)	3	14.7839	4.92796		
Inoculation (B)	1	0.3445	0.34445	2.94	0.1847
Error A*B	3	0.3510	0.11701		
Variety (C)	3	8.0641	2.68803	3.79	0.0288
B*C	3	9.9590	3.31968	4.68	0.0138
Error A*B*C	18	12.7754	0.70974		
Total	31	46.2778			

Appendix 18: Nitrogen (N)

Source of variance	DF	SS	MS	F	P
Replication (A)	3	0.8037	0.26789		
Inoculation (B)	1	2.7378	2.73780	72.70	0.0034
Error A*B	3	0.1130	0.03766		
Variety (C)	3	2.5440	0.84799	12.82	0.0001
B*C	3	2.9051	0.96836	14.63	0.0000
Error A*B*C	18	1.1911	0.06617		
Total	31	10.2946			

Appendix 19: Phosphorus (P)

Source of variance	DF	SS	MS	F	P
Replication (A)	3	0.00378	0.00126		
Inoculation (B)	1	0.03850	0.03850	5.94	0.0927
Error A*B	3	0.01943	0.00648		
Variety (C)	3	0.12138	0.04046	28.55	0.0000
B*C	3	0.12273	0.04091	28.87	0.0000
Error A*B*C	18	0.33135	0.00142		
Total	31	0.02551			

Appendix 20: Potassium (K)

Source of variance	DF	SS	MS	F	P
Replication (A)	3	0.01091	0.00364		
Inoculation (B)	1	0.02940	0.02940		
Error A*B	3	0.02936	0.00979	26.80	0.0000
Variety (C)	3	0.56928	0.18976	4.47	0.0163
B*C	3	0.09498	0.03166		
Error A*B*C	18	0.12746	0.00708		
Total	31				

Appendix 21: Calcium (Ca)

Source of variance	DF	SS	MS	F	P
Replication (A)	3	0.00130	0.00043		
Inoculation (B)	1	0.00720	0.00720	14.90	0.0307
Error A*B	3	0.00145	0.00048		
Variety (C)	3	0.00083	0.00028	0.99	0.4198
B*C	3	0.00917	0.00306	11.01	0.0002
Error A*B*C	18	0.00500	0.00028		
Total	31	0.02495			

Appendix 22: Magnesium (Mg)

Source of variance	DF	SS	MS	F	P
Replication (A)	3	0.00076	0.00025		
Inoculation (B)	1	0.00138	0.00138	3.22	0.1707
Error A*B	3	0.00128	0.00043		
Variety (C)	3	0.00146	0.00049		
B*C	3	0.00925	0.00108	7.74	0.0016
Error A*B*C	18	0.00113	0.00006	17.15	0.0000
Total	31	0.00925			

Appendix 23: Sodium (Na)

Source of variance	DF	SS	MS	F	P
Replication (A)	3	0.00013	4.479E		
Inoculation (B)	1	0.00003	2.813E	2.45	0.2152
Error A*B	3	0.00003	1.145E		
Variety (C)	3	0.00161	5.365E	61.80	0.0000
B*C	3	0.00006	8.681E	2.28	0.1140
Error A*B*C	18	0.00016	1.979E		
Total	31	0.00202			

Appendix 24: Zinc (Zn)

Source of variance	DF	SS	MS	F	P
Replication (A)	3	80.00	26.667		
Inoculation (B)	1	190.12	190.125	6.04	0.0910
Error A*B	3	94.38	31.458		
Variety (C)	3	673.75	224.583	19.10	0.0000
B*C	3	417.62	139.208	11.84	0.0002
Error A*B*C	18	211.62	11.757		
Total	31	1667.50			

Appendix 25: Copper (Cu)

Source of variance	DF	SS	MS	F	P
Replication (A)	3	4.344	1.4479		
Inoculation (B)	1	26.281	26.2812	6.80	0.0798
Error A*B	3	11.594	3.8646		
Variety (C)	3	81.344	27.1146	24.03	0.0000
B*C	3	58.594	19.5312	17.31	0.0000
Error A*B*C	18	20.313	1.1285		
Total	31	202.469			

Appendix 26: Manganese (Mn)

Source of variance	DF	SS	MS	F	P
Replication (A)	3	0.375	0.1250		
Inoculation (B)	1	15.125	15.1250	6.15	0.0892
Error A*B	3	7.375	2.4583		
Variety (C)	3	154.375	51.4583	31.67	0.0000
B*C	3	19.375	6.4583	3.97	0.0246
Error A*B*C	18	29.250	1.6250		
Total	31	225.875			

Analysis of Variance (ANOVA) tables for glasshouse study

Appendix 27: Days to first flowering

Source of variance	DF	SS	MS	F	P
Replication (A)	2	205.75	102.88		
Inoculation (B)	1	22.22	22.22	0.07	0.8145
Error A*B	2	623.69	311.85		
Variety (C)	2	2696.08	1348.04	16.61	0.0014
B*C	2	910.36	455.18	5.61	0.0300
Error A*B*C	8	649.39	81.17		
Soil type (D)	3	239.67	79.89	4.39	0.0099
B*D	3	34.78	11.59	0.64	0.5961
C*D	6	233.58	38.93	2.14	0.0725
B*C*D	6	85.31	14.22	0.78	0.5902
Error A*B*C*D	36	655.17	18.20		
Total	71	6356.00			

Appendix 28: Chlorophyll content

Source of variance	DF	SS	MS	F	P
Replication (A)	2	39.61	19.807		
Inoculation (B)	1	328.32	328.320	82.97	0.0118
Error A*B	2	7.91	3.957		
Variety (C)	2	63.05	31.525	4.89	0.0409
B*C	2	382.21	191.104	29.66	0.0002
Error A*B*C	8	51.55	6.444		
Soil type (D)	3	173.95	57.984	6.70	0.0010
B*D	3	12.64	4.214	0.49	0.6934
C*D	6	228.35	38.059	4.40	0.0020
B*C*D	6	139.46	23.244	2.69	0.0294
Error A*B*C*D	36	311.44	8.651		
Total	71	1738.51			

Appendix 29: Plant height

Source of variance	DF	SS	MS	F	P
Replication (A)	2	642.2	321.10		
Inoculation (B)	1	3800.0	3800.03	13.33	0.0675
Error A*B	2	570.3	285.13		
Variety (C)	2	2543.2	1271.58	10.00	0.0067
B*C	2	1184.9	592.45	4.66	0.0455
Error A*B*C	8	1016.8	127.11		
Soil type (D)	3	1583.8	527.94	8.45	0.0002
B*D	3	1356.4	452.12	7.24	0.0006
C*D	6	531.1	88.52	1.42	0.2351
B*C*D	6	159.7	26.62	0.43	0.8567
Error A*B*C*D	36	2248.8	62.47		
Total	71	15637.2			

Appendix 30: Number of primary branches

Source of variance	DF	SS	MS	F	P
Replication (A)	2	14.194	7.09722		
Inoculation (B)	1	0.222	0.22222	0.09	0.7909
Error A*B	2	4.861	2.43056		
Variety (C)	2	2.028	1.01389	1.17	0.3589
B*C	2	4.861	2.43056	2.80	0.1197
Error A*B*C	8	6.944	0.86806		
Soil type (D)	3	11.000	3.66667	3.09	0.0390
B*D	3	3.444	1.14815	0.97	0.4181
C*D	6	7.417	1.23611	1.04	0.4142
B*C*D	6	5.472	0.91204	0.77	0.5988
Error A*B*C*D	36	42.667	1.18519		
Total	71	103.111			

Appendix 31: Number of nodules

Source of variance	DF	SS	MS	F	P
Replication (A)	2	33.8	16.889		
Inoculation (B)	1	6.7	6.722	0.04	0.8649
Error A*B	2	361.4	180.722		
Variety (C)	2	1392.7	696.347	10.78	0.0054
B*C	2	367.4	183.681	2.84	0.1167
Error A*B*C	8	516.6	64.576		
Soil type (D)	3	1625.8	541.944	4.25	0.0114
B*D	3	189.6	63.204	0.50	0.6874
C*D	6	516.1	86.014	0.67	0.6705
B*C*D	6	787.0	131.162	1.03	0.4224
Error A*B*C*D	36	4587.5	127.431		
Total	71	10384.6			

Appendix 32: Nodule dry weight

Source of variance	DF	SS	MS	F	P
Replication (A)	2	0.5888	0.29442		
Inoculation (B)	1	0.2113	0.21125	0.59	0.5238
Error A*B	2	0.7204	0.36022		
Variety (C)	2	0.4846	0.24229	0.61	0.5676
B*C	2	0.7227	0.36135	0.91	0.4414
Error A*B*C	8	3.1858	0.39822		
Soil type (D)	3	0.2907	0.09691	0.28	0.8428
B*D	3	1.3764	0.45881	1.30	0.2882
C*D	6	2.4723	0.41206	1.17	0.3435
B*C*D	6	1.9587	0.32645	0.93	0.4871
Error A*B*C*D	36	12.6714	0.35198		
Total	71	24.6832			

Appendix 33: Dried plant biomass

Source of variance	DF	SS	MS	F	P
Replication (A)	2	1.246	0.6230		
Inoculation (B)	1	54.201	54.2014	17.62	0.0523
Error A*B	2	6.152	3.0761		
Variety (C)	2	54.085	27.0423	3.62	0.0760
B*C	2	17.901	8.9507	1.20	0.3508
Error A*B*C	8	59.803	7.4753		
Soil type (D)	3	202.326	67.4421	9.75	0.0001
B*D	3	61.177	20.3924	2.95	0.0457
C*D	6	54.220	9.0366	1.31	0.2793
B*C*D	6	10.125	1.6875	0.24	0.9586
Error A*B*C*D	36	248.999	6.9166		
Total	71	770.235			

Appendix 34: Roots dry weight

Source of variance	DF	SS	MS	F	P
Replication (A)	2	0.3042	0.15212		
Inoculation (B)	1	0.0896	0.08961	2.10	0.2843
Error A*B	2	0.0853	0.04267		
Variety (C)	2	4.0382	2.01910	11.01	0.0050
B*C	2	0.1300	0.06502	0.35	0.7119
Error A*B*C	8	1.4665	0.18332		
Soil type (D)	3	2.3817	0.79391	8.52	0.0002
B*D	3	0.3689	0.12297	1.32	0.2829
C*D	6	0.7748	0.12913	1.39	0.2467
B*C*D	6	1.1949	0.19915	2.14	0.0727
Error A*B*C*D	36	3.3534	0.09315		
Total	71	14.1876			