PLANT GROWTH AND SYMBIOTIC FUNCTIONING OF PROMISCUOUS-NODULATING SOYBEAN GENOTYPES INOCULATED WITH \textit{BRADYRHIZOBIUM JAPONICUM} STRAIN WB74

by

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January 2011
DECLARATION

“I hereby declare that the dissertation submitted for the degree MTech: Agriculture at the Tshwane University of Technology is my own original work and has not previously been submitted to any other institution of higher education. I further declare that all sources cited or quoted are indicated and acknowledged by means of a comprehensive list of references”.

Cynthia Gyogluu

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DEDICATION

This work is dedicated to the blessed memory of my dear brother, Anthony Kanyiri Gyogluu (you will always be loved), to my dear parents Sylvester Gyoglueu and Joyce Yeri, who have always been my source of inspiration and motivation, and to my sisters for their unending love.
I thank the Almighty God for his many graces and guidance throughout my studies. My sincere gratitude and appreciation also go to my major supervisor, Prof FD Dakora for giving me the opportunity to carry out this work under his supervision, and for his encouragement, motivation and exceptional guidance during the course of this study. My profound gratitude also goes to my co-supervisor, Dr Stephen K Boahen, for all his support and motivation during the course of my study.

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ABSTRACT

This study evaluated plant growth and symbiotic performance of four promiscuous-nodulating soybean genotypes and three commercial varieties supplied with a peat-based inoculant of *Bradyrhizobium japonicum* strain WB74 at three field sites in Mozambique and a pot experiment in South Africa. The sole aim was to assess whether these promiscuous-nodulating soybean genotypes can benefit from inoculation. The four promiscuous genotypes (namely, TGx1910-14F, TGx1937-1F, TGx1908-8F, TGx1844-4E) and the three commercial varieties (namely, 427/5/7, Ocepera and Solitaire) were planted in the field at Nampula, Ruace and Mutequelesse in Mozambique. An assessment of plant growth and N\textsubscript{2} fixation at flowering and grain yield at harvest revealed marked differences between inoculation and among the soybean genotypes in their symbiotic performance. The inoculated soybean plants showed better plant growth (increased biomass) and symbiotic performance at all sites. The $\delta^{15}$N values of inoculated plants were lower compared to uninoculated (0.5 to 2.5‰ vs. 1.2 to 3.6‰ in the field) thus resulting in higher %Ndfa values. Actual amounts of N-fixed were higher with inoculation than without inoculation (14.7 to 212.0 kg N/ha Vs 3.7 to 112.2 kg N/ha). Due to the genotypic differences realized at the different locations, wide spread testing of these genotypes is advisable to ascertain which genotypes are suitable for which location.
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<tr>
<td>A</td>
<td>Photosynthesis</td>
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<td>$A_{\text{max}}$</td>
<td>Light saturated photosynthesis</td>
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<td>ANOVA</td>
<td>Analysis of variance</td>
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<tr>
<td>BNF</td>
<td>Biological nitrogen fixation</td>
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<tr>
<td>Ci</td>
<td>Intercellular carbon dioxide</td>
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<tr>
<td>DM</td>
<td>Dry matter</td>
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<tr>
<td>DMRT</td>
<td>Duncan multiple range test</td>
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<tr>
<td>E</td>
<td>Transpiration rate</td>
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<tr>
<td>FAO</td>
<td>Food and Agriculture Organization</td>
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<tr>
<td>gs</td>
<td>Stomata conductance</td>
</tr>
<tr>
<td>IITA</td>
<td>International institute for tropical agriculture</td>
</tr>
<tr>
<td>TGx</td>
<td>Tropical glycine cross</td>
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<td>WUE</td>
<td>Water use efficiency</td>
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CHAPTER 1

GENERAL INTRODUCTION

1.1 Soybean production and utilization

In the late 20th century up to the present, soybean has played an important part in alleviating world hunger (SPP, 2010). Soybean is produced in many parts of the world for its seed. The soybean seed contains about 40 - 45 % protein, 18-20 % edible oil and 24 - 26 % carbohydrate (Kaul & Das, 1986). Major fatty acids that can be obtained from soybean include linoleic acid (about 54 %), linolenic acid (7 %), stearic acid (5 %), palmitic acid (10 %) and oleic acid (24 %). Approximately, one-third of the world’s edible oil and two-third of its protein meal are derived from soybean (Golbitz, 2004). In Nigeria, malnutrition in kids who grow up in soybean-producing communities is reported to be less than the national average (CGIAR, 2004-2005). It is also reported that the cost of protein when purchased as soybean in Nigeria is only about 10 – 20 % of the cost of protein from fish, meat, eggs or milk (IDRC, 1998). Through soybean production and processing, about 40 % of Nigerian women earn a decent income and thus provide for their families. In many other countries, especially in Asia, soybean products serve as source of protein and provide nearly 5 % of protein consumption in China, India and Thailand, and 4 - 5 % in Indonesia (CGIAR, 2004-2005). A wide range of industrial, pharmaceutical and environmentally-friendly products such as margarine, cooking oil, salad dressing, industrial paints, varnishes and printing ink are made from soybean (SPP, 2010). In South Africa, soybean has
become an important source of protein with utilization exceeding that of production by 300 % (Booysen & Smith, 1998). In the United States, soybean was initially grown as forage and hay crop for the animal industry, but by 1941, the cultivation of the crop for its grain far exceeded that for forage (Smith & Huyser, 1987). In many other countries, soybean cake, a by-product from the oil production, is used as a high protein feed for animals. Soybean also plays an important role in the transport industry as the oil can be converted into biodiesel, thus serving as an alternative to fossil fuels.

FAO (2005) data indicated that the total land area under soybean cultivation in the world was 95.2 million ha, and total production was 212.6 million tonnes. In Africa, soybean was grown on an average of 1.16 million ha with an average production of 1.26 million tonnes. The countries with the largest area of production were Nigeria (601 000 ha), South Africa (150 000 ha), Uganda (144 000 ha), Malawi (68 000 ha), and Zimbabwe (61 000 ha). Africa as a whole produces minimal soybean when compared to the rest of the world. According to the same report, USA produced 83 million tonnes (29 million ha), followed by Brazil with a production of 51 million tonnes (23 million ha) and Argentina produced 38 million tonnes on a total land area of 14 million ha. From production levels of 29 million tonnes in 1964 to 244 million tonnes in 2009 (USDA, 2009), world production of soybean is increasing annually with United States, Brazil and Argentina as the leading producers.
The International Institute for Tropical Agriculture (IITA) bred promiscuous soybean genotypes without inoculation requirement. This was to promote widespread adoption and cultivation of soybean by small holder African farmers. These soybean genotypes are therefore supposed to nodulate freely with indigenous soil rhizobia. However, inconsistent and poor nodulation have been reported for these genotypes at various locations (Abaidoo et al., 2000; Okereke & Eaglesham, 1992).

Soybean production in Africa is lower compared to the rest of the world, though this legume has the ability to increase yield in low N soils (Keyser & Li, 1992). Soybean is a strict nodulator and requires nodulation with specific Bradyrhizobium species (such as Bradyrhizobium japonicum and Bradyrhizobium elkanii), but populations of these compatible rhizobial species are not endemic in African soils (Hadley & Hymowitz, 1973). To obtain maximum biological nitrogen fixation (BNF) benefits and high grain yield, soybean-rhizobial inoculants are often applied. However, inoculants are not readily available and accessible to most small-holder resource-poor African farmers. Promiscuous soybean varieties developed by IITA to freely nodulate with indigenous bradyrhizobia in African soils was seen as a breakthrough for increased soybean production in Africa. However, many promiscuous soybean genotypes have been reported to exhibit poor and/or ineffective nodulation in various locations (Abaidoo, Keyser, Singleton & Borthakur, 2000; Okereke & Eaglesham, 1992), indicating the need for wide testing of these genotypes with commercial Bradyrhizobium japonicum bacteria. This study evaluated the response of four promiscuous-nodulating soybean genotypes to inoculation in Mozambique and South Africa. Plant growth and N₂
fixation were measured over a two-year period. It was hypothesized that high N₂ fixation and enhanced plant growth in these genotypes can complement the strict nodulating genotypes currently planted by commercial farmers and stimulate the adoption and promotion of soybean cultivation by emerging farmers in Southern Africa.

1.2 Research Objectives

The main aim of this study was to evaluate promiscuous soybean cultivars for enhanced plant growth and symbiotic performance for widespread adoption and cultivation in Southern Africa. Specific objectives include;

i) To evaluate symbiotic performance of promiscuous soybean genotypes in the field (Mozambique) and in pot experiments.

ii) To measure carbon assimilation and water use efficiency (WUE) in promiscuous-nodulating soybean genotypes in the field (Mozambique) and in pot experiments.

iii) To determine the isotopic composition of xylem sap collected from soybean genotypes planted in Mozambique.
CHAPTER 2

LITERATURE REVIEW

2.1 Soybean origin and early history

Soybean (*Glycine max* (L) Merr.) is a grain legume indigenous to the Manchurian region of China. It is a member of the Fabaceae family, belongs to the sub-family Papilionoideae, tribe Phaseoleae and genus *Glycine*. It is believed that domestication of soybean began in the 11th century B.C. in Northern China (Hymowitz, 1970), and spread to other parts of Asia where the seeds have been used to prepare a variety of foods (Probst & Judd, 1973). Soybean was introduced into Africa from North America in the late 1800’s through missionaries, though uncertainty surrounds the first African countries to grow it. Cultivation of this grain legume was first reported in South Africa in 1903, the first African country to grow soybean in a systematic way, where trials were carried out on an experimental farm at Cedera, Natal, and in the Transvaal (Shurtleff & Aoyagi, 2007). By 1907, soybean was introduced into Mauritius and a year later, was being cultivated on small scale in Nigeria and the Belgian Congo, and Ghana in 1909. In the 1920’s, soybean cultivation spread to other African countries including Egypt, Zimbabwe and Rwanda. The soybean crop is usually grown in the tropical, subtropical and temperate climates on a range of well drained soil types. Soybean grows well in medium textured well drained loamy soils with pH range of 6.0 – 6.5. Suitable cropping time for soybean is largely dependent on temperature and
day length since different Soybean varieties differ in their photoperiod response. However, optimum temperature range of 20 – 30 °C with short day length (14 hours or less) is suitable for soybean production. In Southern Africa, soybean is usually cultivated in November. The soybean plant has an advantage over other grain legumes such as groundnut (*Arachis hypogaea* L.) and common bean (*Phaseolus vulgaris* L.), in that, it contributes residual N benefit to subsequent crops due to its large leaf biomass (Mpepereki, Makonese & Giller, 1996).

### 2.2 Soybean as a bio-fertilizer

Low soil fertility, especially N and P, are major constraints limiting increased crop yield in the tropics (Dakora & Keya, 1997; DeWit, 1981; Graham, 1981; Nye & Greenland, 1960; Sanchez, Buresh & Leakey, 1997). It is estimated that about 660 kg N/ha, 75 kg P/ha, and 450 kg K/ha have been lost over the past 30 years from about 200 million ha of cultivated land in 37 African countries (Smaling, 1993; Smaling, Nandwa & Janssen, 1997; Stoorvogel & Smaling, 1990). This has arisen from intense cultivation on the same land to feed the ever-increasing human population (Sanginga, 2003; Shepherd & Soule, 1998; Tittonel, 2002), as well as from leaching, soil erosion, and inadequate return of crop residues at harvest (Gachengo, Palm, Jama & Othieno, 1999; Shepherd & Soule, 1998). Although increased use of chemical fertilizers in the developed countries has resulted in increased food production (FAO, 1990), Africa exhibits the lowest use of chemical fertilizers in the world due to high cost, lack of
access and poor marketing infrastructure (Kinzig & Sokolow, 1994; Vitousek, Moooney, Luchenco & Melillo, 1997). To replenish the already fragile African soils for increased and sustainable crop production requires effective management of N. BNF offers an economically viable and environmentally-friendly alternative to chemical fertilizers in improving soil fertility in Africa. Species of root-nodule bacteria, which include *Rhizobium, Bradyrhizobium, Sinorhizobium, Azorhizobium, Mesorhizobium and Allorhizobium*, form symbiosis with legumes and contribute over 80 % of biologically-fixed N to agricultural systems (Vance, 1998), release chemicals that protect plants against pathogens (Dakora & Phillips, 1996), reduce the effects of water stress in legumes (Figueiredo, Vilar, Burity & de Franca, 1999) and promote seed germination and seedling development (Smith, Prithiviraj & Zhang, 2002). It is estimated that BNF contributes about 175 million tonnes of fixed-N per year compared with about 80 million tonnes of ammonium produced annually through the Haber-Bosch process (Elkan, 1992). Though N₂ gas forms about 80 % of air, it is not readily available for plant use due to its inert nature (Elkan, 1992). Biologically fixed-N is however less susceptible to leaching, denitrification and volatilization as it can be used directly by plants.

The soybean-*Bradyrhizobium* symbiosis can fix about 300 kg N/ha (Keyser & Li, 1992), thus reducing the need for chemical fertilizers. Osunde *et al.* (2003) reported maize yield of 3 t/ha following a 2-year successive cropping of soybean without fertilizer application. Kaye *et al.* (2007) found that BNF contributed about 35 to 41 %N to grain yield in a soybean-sorghum rotation. Sanginga *et al.* (2002) also recorded a
significantly higher maize yield (1.2-to 2.3-fold increase) in a rotation with soybean for two consecutive years compared to sole maize control. Additionally, 10 to 22 kg N/ha was accumulated by the first maize crop from soybean residue (Sanginga et al., 2002).

2.3 Measurement of Biological Nitrogen Fixation

Several methods are used to quantify biological nitrogen fixation. The choice of a particular method depends largely on the type of experiment, legume species and the experimental site. Wide variations in the amounts of N-fixed have been reported due to factors such as soil types, nutrient status of soils, type of legume species (or varieties), water availability, temperature and effectiveness of the N₂-fixing bacteria (Ledgard & Steele, 1992). Also, the type of non-fixing reference plant used (Nyemba & Dakora, 2005) and the crop management practices can affect the amount of N₂-fixed by legumes. Some of the methods used for measuring N₂ fixation in legumes include dry matter determination, the N difference method, acetylene reduction assay, and the ¹⁵N natural abundance method.
2.3.1 Determination of dry matter yield

Biomass accumulation of a crop is dependent on its N nutrition. Since legumes can meet up to 90% of their N requirement from N$_2$ fixation, dry matter accumulation by plants can be used to measure N$_2$ fixation in different legume species. This method however has limitations because legume species differ in their ability to exploit available soil N, as well as nodulate with compatible rhizobia. These differences can have a significant effect on N$_2$ fixation and dry matter accumulation by the different plant types.

2.3.2 The Nitrogen difference method

The N difference method is commonly used for estimating N$_2$ fixation in trees and field crops. The method compares the total N uptake by the legume and that of a non-fixing reference plant. This method is based on the assumption that the rooting pattern, root depth and rate of N uptake by the reference plant are similar to that of the test legume. The method is not suitable for legume / cereal intercrops since competition for nutrients may affect soil N uptake by both the legume and non-legume. Also, the choice of a suitable reference plant that reflects soil N uptake by the legume can restrict the use of the N difference method (Shearer & Kohl, 1986). In situations where the reference plant utilizes less soil N than the legume, or when both legume and reference plant show equal N yields, estimation of N$_2$ fixation by the N difference method becomes difficult. The method is also not suitable for deep rooted
perennials because it becomes difficult to distinguish between N that was re-absorbed from decomposition of fallen leaves, senesced roots and nodules, and soil N (Danso, Bowen & Sanginga, 1992).

2.3.3 The acetylene reduction assay and hydrogen evolution methods

The acetylene reduction assay (ARA) is a technique that was developed in the 1960s based on the observations that the N\textsubscript{2}-fixing enzyme, nitrogenase catalyzes the reduction of acetylene (C\textsubscript{2}H\textsubscript{2}) to ethylene (C\textsubscript{2}H\textsubscript{4}) making it possible to measure N\textsubscript{2}-fixing activity at any given time. The ARA method is based on the assumption that all nodules are recovered and a conversion factor of 1 used when calculating N\textsubscript{2} fixation. The rate of nitrogenase activity during the measurement is equal to the pre-assay rate, and the electron coefficient to H\textsubscript{2} is 0.25 leading to a conversion factor of 4. The ARA has since played an important role in N\textsubscript{2} fixation research because of its simplicity, inexpensiveness, sensitivity, and rapid measurements. It provides an instantaneous measure of nitrogenase activity at any given point in time. The ARA has lost its widespread application in recent times due to certain limitations in the assumptions. The conversion ratio is under constant debate as in some cases 3 is used and this does not apply to all species and large errors are likely to occur. Furthermore, it is difficult to recover all nodules under field conditions for an accurate assessment of BNF, and the measure is instantaneous which does not reflect an integrated value of N\textsubscript{2} fixation over a long period of time.
Another method for measuring nitrogenase activity is the hydrogen (H\textsubscript{2}) evolution method. H\textsubscript{2} evolution by nodules was first observed on soybean in 1957 through the nitrogenase reaction (Hoch, Little & Burris, 1957). This method has been reported as a much easier, safer and more accurate method of assaying nitrogenase activity in nodulated roots of legumes (Hunt & Layzell, 1993). The nitrogenase enzyme which catalyzes N\textsubscript{2} fixation reaction, simultaneously reduces protons to H\textsubscript{2} (Burns & Bulen, 1965) and the rate of hydrogen released into the soil directly relates to nitrogenase activity (Hunt, 1996). However, the amount of electrons used for proton reduction varies with the turnover rate of the MoFe component of the enzyme (Burris, Arp, Benson, Emerich, Hageman, Jones, Luden & Sweet, 1980). H\textsubscript{2} evolution by legumes has however been considered as a waste of energy because the gas has had no known benefit. A major limitation of this method is that some N\textsubscript{2} fixation associations do not evolve H\textsubscript{2} (Schubert, Engelke, Russell & Evans, 1977).

2.3.4 The $^{15}$N abundance method

This technique is based on the differences in the natural abundance of stable isotopes of N, (i.e. $^{14}$N and $^{15}$N) between atmospheric N\textsubscript{2} and other sources of N (Broadbent, Nakashima & Chang, 1982; Herridge, Marcellos, Felton, Turner & Peoples, 1995; Rennie & Rennie, 1983; Ruschel, Vose, Victoria & Salati, 1979). The heavier $^{15}$N isotope occurs less in the atmosphere (0.3663 ‰) as compared to 99.6336 ‰ for $^{14}$N isotope (Mariotti, 1983). Most soils are enriched in $^{15}$N due to discrimination against this isotope during volatilization and denitrification. As a result,
the heavier isotope can accumulate in soil with time, and thus become enriched and gains a positive $\delta^{15}\text{N}$ value (Shearer & Kohl, 1986; Unkovich, Pate, Sanford & Amstrong, 1994). The $^{15}\text{N}$ value of soil N is often significantly higher than in the atmosphere (0 ‰) allowing for the estimation of the proportion of a legume $^{15}\text{N}$ derived from soil. The $^{15}\text{N}$ natural abundance of soil N taken up by the legume is usually estimated by measuring the $\delta^{15}\text{N}$ value of a suitable non-fixing reference plant. Like the N difference technique, the $^{15}\text{N}$ natural abundance method assumes that the rooting pattern and depth, and the rate of N uptake by the non-fixing reference plant are the same as the test legume (Peoples, Turner, Shah, Shah, Aslam, Ali, Markey, Afandi, Schwenke & Herridge, 1997). Because the non-fixing reference plants depend on the soil for their N nutrition, their $\delta^{15}\text{N}$ values therefore represent that of the soil N available to the test legume. Though this method is considered the most reliable and widely used method for estimating $\text{N}_2$ fixation, it has certain limitations. It is an expensive method because i) mass spectrometers capable of measuring accurate differences of 0.1 ‰ (about 0.00004 atom $^{15}\text{N}$) are needed, ii) the samples can be contaminated with $^{15}\text{N}$ enriched material if great care is not taken during sample preparation, and iii) the choice of a suitable reference plant that accurately measures the $^{15}\text{N}$ natural abundance of soil N taken up by the test legume can be difficult.
2.4 Factors affecting biological N$_2$ fixation

2.4.1 Edaphic factors

Several factors, including drought, excessive soil moisture, soil acidity, P deficiency and excess N, as well as Ca, Mo, Co and B deficiency (Mulongoy, 1995) can affect N$_2$ fixation in legumes. Effective nodulation of host plants by rhizobia can be affected by water logging or drought. Water logging prevents the development of root hairs, and can interfere with oxygen acquisition by the root system of the host plant. Drought can lower rhizobial populations, affect nodule formation and their longevity, inhibit the synthesis of leghaemoglobin, affect nodule function as well as inhibit plant growth and N$_2$ fixation (Hungria & Vargas, 2000; Mulongoy, 1995). Williams and De Mallorca (1984) reported low nodule formation on soybean under conditions of mild water stress, and reduced both nodule number and size in moderate and severe water stress conditions. Soil acidity, phosphorus deficiency, high soil mineral N, and micronutrient deficiency such as molybdenum (a component of nitrogenase), all reduce nodulation and N$_2$ fixation. However, certain rhizobial species can tolerate acidity better than others. The suitable pH range for rhizobial growth is considered to be between 6.0 and 7.0, with few rhizobia surviving at pH less than 5.0 (Hungria & Vargas, 2000).
2.4.2 Temperature

Extreme temperatures affect the survival and eventually the population of soil rhizobia, and can thus adversely affect N$_2$ fixation. However, different *Bradyrhizobium* strains have been reported to have different levels of tolerance to soil temperature. Montanez *et al.* (1995) showed that low temperature induced poor nodulation in soybean inoculated with different strains of *Bradyrhizobium*. Critical temperatures of 30 °C have been reported for clover and peas, and a range of 35 °C to 40 °C for soybean and peanuts (Zahran, 1999).

2.4.3 Rhizobial factors

Effective nodulation and N$_2$ fixation depends on the presence of host plant and compatible nodulating bacteria in sufficient numbers. In the absence of effective compatible indigenous soil bacteria, rhizobial strains are introduced through inoculation. However, the introduced strain must be highly competitive to overcome the indigenous strains for root hair colonization, nodulation, and subsequently N$_2$ fixation.

2.5 Soybean and water relations

Scarcity of water is one of the major factors affecting crop production worldwide. Inadequate (or excess) supply of water to plants can slow germination and retard
plant growth and subsequently affect yield. Irrigation ensures sustainable cropping, despite cost associated with installation and maintenance. Sufficient supply of water to plants through irrigation is a better alternative to improve crop yields. Efficient water use in plants improves the plant’s tolerance to drought, and sufficiently maintains soil moisture (Martin & Thorstenson, 1988) with improved plant growth and function.

Water-use efficiency (WUE) can be observed at both leaf and whole-plant level (Liu, Andersen, Jacobsen & Jensen, 2005). WUE$_{\text{leaf}}$ is defined as the ratio of the rate of light saturated photosynthesis ($A_{\text{max}}$) and the rate of stomatal conductance ($g_s$) for water vapour. In the situation where water is limiting, WUE$_{\text{leaf}}$ may be improved by partial closure of stomata so that intercellular CO$_2$ concentration ($C_i$) is just sufficient for saturation of $A_{\text{max}}$, thus reducing the rate of water loss. WUE$_{\text{plant}}$ is determined as the ratio of dry mass (DM) accumulation and plant water use (transpiration). Chen et al. (1993) reported that depending on the severity of stress, WUE$_{\text{plant}}$ is always higher in drought stressed soybean plants.

Several methods such as micrometeorological approaches and the use of portable photometer can measure photosynthesis, transpiration and WUE of individual leaves (Martin & Thorstenson, 1988), though limitations to their use have been reported. More recently, the use of the relative ratio of $^{13}$C/$^{12}$C (expressed relative to the PeeDee Belemnite (PDB) standard as $\delta^{13}$C) in plant tissues has been shown to reflect WUE in several crop species. The natural abundance of $^{13}$C relative to $^{12}$C in plant tissue is usually less compared to the C in atmospheric CO$_2$ (Farquhar, Hubick,
Condon & Richards, 1989). This gives an indication that $^{13}$C isotope discrimination occurs during the incorporation of CO$_2$ into plant biomass. In the event of water stress, this situation is reversed as less $^{13}$C discrimination occurs due to partial stomatal closure. The resultant $\delta^{13}$C enriched values in plant tissues usually indicate higher WUE in plants. In contrast, plants with less enriched $\delta^{13}$C are less water-use efficient (Farquhar & Richards, 1984).
CHAPTER 3
MATERIALS AND METHODS

3.1 Site description

The study was conducted at Nampula, in the Nampula Province, in Mozambique during the 2007/2008 cropping season. The Nampula IITA experimental site is located in the semi-arid savanna on latitude 15° 09’12.9”S and longitude 039°18’20.0”E, on an altitude of 398 m, with mean annual temperature of 20 - 26 °C. The annual rainfall is 800 to 1200 mm and occurs mostly in December to February each year. In the 2008/2009 cropping season, the experiment was replicated at Ruace and Mutequelesse experimental sites both in the Gurue district of Zambezi province in Mozambique. The Ruace site is located in the humid savanna agro-ecological zone on latitude 15°14’17.5”S and longitude 036° 43’44.8”E, at an altitude of 707 mm, with a mean temperature varying from 15 to 23 °C, and rainfall from 2000 to 2500 mm, most of which occurs in late November to April. The Mutequelesse experimental site is also located in the tropical humid savanna agro-ecological zone on latitude 15° 19’09.5”S and longitude 036° 42’43.9”E at an altitude of 678 mm, with mean temperatures varying from 15 to 23 °C, and annual rainfall varying from 1800 to 2000 mm, mostly occurring in November to early March. A pot experiment was carried out at the Tshwane University of Technology skills centre, Pretoria, which is located on latitude 25°43’53.55”S and longitude 28°09’40.38”E. The soil in Nampula is sand-clayey-loam whilst soils in both Ruace and Mutequelesse are clayey-loam.
The Nampula and Ruace sites have previous cropping history of soybean whilst Mutequelesse site was fallowed.

3.2 Field design, cropping and pest management

Four TGx inbred lines (TGx1910-14F, TGx1937-1F, TGx1844-4E and TGx1908-8F) and three commercial varieties (427/5/7, Ocepera and Solitaire) were planted in a randomised complete block design (RCBD) and replicated four times with two levels of *Bradyrhizobium* treatment (inoculated and uninoculated). Solitaire was obtained from the Seed Company in Zimbabwe whiles Ocepera and 427/5/7 are two local Malawian varieties. A peat-based *Bradyrhizobium japonicum* inoculant (strain WB74) was used for inoculating the soybean genotypes. The *Bradyrhizobium japonicum* inoculant was purchased from Soygro Pty Ltd, South Africa. At cropping, the seed was drilled into the soil and the inoculant measured with a container and poured onto the seed material to attain cell number of about $10^8$ cells per seed (Vincent 1970) before covering with soil. Uninoculated plots were planted first, followed by inoculated treatments to reduce cross-contamination. Each plot measured 9 m x 3 m (27 m$^2$), inter-row spacing of 0.5 m and intra-row spacing of 0.2 m. Weed control was always carried out in the uninoculated plots first by hand hoeing, to avoid contamination. The soybean field experiments for both years were planted in early December whiles the pot experiment was carried out in early March.
3.3 Pot experiment

A complete randomised design with four replications for each genotype was also used for the pot experiment. Plastic bags of size 10 kg were filled with a 1:1 mixture of sandy clay and loam soil obtained from the Tshwane University of Technology agricultural farm. A peat-based *Bradyrhizobium japonicum* inoculant (strain WB74) was applied to the inoculated seed material at cropping. Five seeds were planted per pot and later thinned out to three after germination. Weeds were controlled by hand picking and the plants watered three times a week.

3.4 Plant sampling and processing

About 10 plants were sampled per plot at flowering to early podding and separated into shoots and roots. At Nampula, the soybean shoots and roots were oven-dried (60 °C) for 48 hours, weighed and ground (0.85 mm sieve size). At physiological maturity, grain was processed and together with shoots, analysed for %N, $^{15}$N and $^{13}$C in the case of Ruace and Mutequelesse. The non-legume reference plants were similarly harvested and analyzed to ascertain the amount of soil-N taken up by the soybean genotypes. Xylem sap collected from root stocks of decapitated plants was stored in vials for $^2$H, $^{18}$O and $^{13}$C isotopic analysis.
3.5 Measurement of nitrogen fixation

3.5.1 \(^{15}\text{N}/^{14}\text{N}\) and \(^{13}\text{C}/^{12}\text{C}\) isotopic ratio analysis

The ratio of \(^{15}\text{N}/^{14}\text{N}\), the N concentration (\%N) and \(^{13}\text{C}/^{12}\text{C}\) in plant samples were determined with a Carlo Erba NA1500 elemental analyzer coupled to a Finnigan MAT 252 mass spectrometer (Finnigan MAT GmbH, Bremen, Germany) via Conflo II open-split device. A mass (2.00 mg) of ground plant sample was weighed into aluminium capsules, loaded onto the Carlo-Erba system, combusted in evacuated quartz tubes in the presence of cupric oxide and metallic copper, and the resultant gases cleaned on-line before being introduced into the mass spectrometer. An internal standard, consisting of material from a \textit{Nasturtium} spp. was included in every five runs to correct for machine errors during isotopic fractionation.

The isotopic composition (\(\delta^{15}\text{N}\)) was measured according to the following relationship (Junk & Svec, 1958; Mariotti, Germon, Hubert, Kaiser, Létolle, Tardieux & Tardieux, 1981)

\[
\delta^{15}\text{N}(\%oo) = \frac{\left[^{15}\text{N}/^{14}\text{N}\right]_{\text{sample}} - \left[^{15}\text{N}/^{14}\text{N}\right]_{\text{atm}}}{\left[^{15}\text{N}/^{14}\text{N}\right]_{\text{atm}}} \times 1000
\]

Whole plant \(\delta^{15}\text{N}\) value was calculated as an average of \(\delta^{15}\text{N}\) values of all plant organs weighted by their respective N content (Robinson, Handley, Scrimgeour, Gordon, Forster & Ellis, 2000).
\[
\delta^{15}N_{\text{wholeplant}} = \sum \left( \frac{\delta^{15}N_{\text{roots}} \times N_{\text{content}}_{\text{roots}} + \delta^{15}N_{\text{shoots}} \times N_{\text{content}}_{\text{shoots}}}{(N_{\text{content}}_{\text{roots}} + N_{\text{content}}_{\text{shoots}})} \right)
\]

### 3.5.2 Nitrogen content

The N content of the plant samples were determined as the product of \( \%N \) and sample weight (Pausch, Charles, Mulchi, Lee & Meisinger, 1996):

\[
Sample N (g/\text{plant}) = \%N \times \text{sample dry mass}
\]

### 3.5.3 Percent N derived from fixation (\%Ndfa)

The proportion of N derived from N\(_2\) fixation was calculated according to the method of Shearer & Kohl (1986); and Unkovich et al (1994) as follows:

\[
\%Ndfa = \frac{\delta^{15}N_{\text{ref}} - \delta^{15}N_{\text{leg}}}{\delta^{15}N_{\text{ref}} - B} \times 100
\]

where \( \delta^{15}N_{\text{ref}} \) is the \( ^{15}\text{N} \) natural abundance of reference plant, \( \delta^{15}N_{\text{leg}} \) is the \( ^{15}\text{N} \) natural abundance of legume, and B is the \( ^{15}\text{N} \) natural abundance of soybean plants depending solely on N\(_2\) fixation for their N nutrition. The B value used for soybean shoot was -1.06 \( \% \), 0.66 \( \% \) for roots, 0.03 \( \% \) for grain and -0.0245 for whole plant.
(Chimpango & Dakora unpublished data). Maize was planted adjacent to the soybean experiment and sampled as reference plant. The mean shoots, grain and whole-plant $\delta^{15}$N values of the reference plant were 8.347 (‰), 3.66 (‰) and 6.0035 (‰) respectively at Ruace, and 7.743 (‰), 3.66 (‰) and 5.7015 (‰) respectively at Mutequelesse. At Nampula the mean shoot, root and whole-plant $\delta^{15}$N values for reference plants were 7.468 ‰, 3.0 ‰ and 5.234 ‰ respectively.

The amount of N-fixed was calculated according to the method of Maskey, Bhattarai, Peoples & Herridge (2001); Shearer & Kohl (1986) as follows.

$$N_{fixed} = \%Ndfa \times \text{legume biomass} N$$

where legume biomass N, was the plant sample N content.

The $^{13}$C value was similarly calculated according to the method of Farquhar, O'Leary & Berry (1982) as follows:

$$\delta^{13}C = \left[ \frac{^{13}C/^{12}C}_{\text{sample}} - 1 \right] \times 1000$$

where, $^{13}C/^{12}C_{\text{sample}}$ and $^{13}C/^{12}C_{\text{standard}}$ are the abundance ratios of the sample and the standard PeeDee Belemite (PDB) respectively.

The carbon concentration (%C) in the plant samples was calculated as:
\[ \%C = \frac{\alpha \cdot \text{Area}}{\text{Amount}} \]

### 3.6 \(^2\text{H}/^{1}\text{H}, ^{18}\text{O}/^{16}\text{O} \text{ and } ^{13}\text{C}/^{12}\text{C} \text{ isotopic ratio analysis} \]

The samples were analysed using a Thermo Finnigan TC/EA coupled to a Thermo Conflo III to a Thermo Delta XP Plus stable light isotope mass spectrometer. About one tenth of a micro litre 0.0001 ml of sap was injected directly into the TC/EA by a Thermo Electron AS3000 auto sampler. Repeated rinses were carried out between injections and blanks were run to check on background and memory. The samples were run against in-house reference materials and the results were normalized against and reported relative to the international standard mean ocean water (SMOW for \( \text{H}_2 \) and \( \text{O}_2 \)). In house standards used included ice from Drenning Maud Land (DML Ice), Cape Town Mountain Precipitation (CTMP), and Evian bottled water.

The isotopic composition for deuterium (\( ^2\text{H} \)) was calculated as

\[
\delta D = \left[ \frac{\left( ^2\text{H}/^{1}\text{H} \right)_{\text{sample}}}{\left( ^2\text{H}/^{1}\text{H} \right)_{\text{standard}}} - 1 \right] \times 1000
\]
where $^2\text{H}/^1\text{H}$ sample and $^2\text{H}/^1\text{H}$ std are the isotopic ratios of the sample and the standard respectively (Dawson & Ehleringer, 1991). The isotopic composition for oxygen ($\delta^{18}\text{O}$) was similarly calculated as the equation above.

### 3.7 Statistical analysis

Data collected were analyzed with either 3-way ANOVA to analyze inoculation, location and genotypic effects or 2-way ANOVA to determine inoculation and genotypic effects, using software of STATISTICA 8.0 program (Statsoft Inc., 2007). The Duncan Multiple Range test (DMRT) was used to compare treatment means at $P\leq0.05$. 
CHAPTER 4

RESULTS

4.1 Year 1: Nampula

4.1.1 Plant growth

A 2-Way ANOVA analysis of seven soybean genotypes receiving two levels of inoculation (i.e. inoculated versus uninoculated) in the 2007/2008 cropping season revealed marked differences in dry matter yield of roots, shoots and whole plants. Inoculated soybeans produced significantly \((P\leq 0.001)\) greater root, shoot, and whole-plant biomass compared to uninoculated treatment (Table 4.1). Of the seven soybean genotypes, TGx1910-14F exhibited the highest \((P\leq 0.001)\) shoot dry matter yield, and therefore showed more biomass at whole-plant level, followed by TGx1908-8F (Table 4.1). The genotype x inoculation interaction was significant \((P\leq 0.05)\) for root, shoot and whole-plant biomass (Table 4.1). A 1-Way ANOVA analysis of the interaction showed significantly \((P\leq 0.05)\) greater dry matter yield of roots, shoots and whole plants with inoculation compared to non-inoculation (Fig. 4.1 A, B, and C).

4.1.2 N concentration and content

A 2-Way ANOVA analysis of the seven inoculated and uninoculated soybean genotypes also showed significantly \((P\leq 0.001)\) higher N concentration in roots, shoots
and whole plants of inoculated compared to uninoculated soybean (Table 4.2). As a result, the N content of roots, shoots, and whole plants were also greater in inoculated relative to un-inoculated soybean plants. There were marked differences between and among the soybean genotypes with respect to N content and concentration. Whole-plant %N was highest in Solitaire and TGx1910-14F, leading to significantly ($P \leq 0.001$) greater N content in shoots and whole plants of the two genotypes (Table 4.2). The genotype x inoculation interaction was significant ($P \leq 0.05$) for root N content (Table 4.2), and analysis of this interaction revealed much higher N content of roots with inoculation compared with those of uninoculated soybean plants (Fig. 4.1D).

4.1.3 $\delta^{15}N$ and %Ndfa values

A 2-Way ANOVA analysis of the seven inoculated and uninoculated soybean genotypes also indicated significant ($P \leq 0.001$) differences in $\delta^{15}N$ and %Ndfa values (Table 4.3). Inoculating soybean genotypes with Bradyrhizobium japonicum strain WB74 decreased the $\delta^{15}N$ values of roots, shoots, and whole plants relative to uninoculated treatments (Table 4.3). As a result, the %Ndfa values were also much higher in roots, shoots, and whole plants of inoculated soybean compared with uninoculated plants (Table 4.3). Genotypes TGx1910-14F and TGx1844-4E showed the lowest root and shoot $\delta^{15}N$ values, followed by Solitaire and TGx1937-1F. As a result, the $\delta^{15}N$ of whole plants were also significantly ($P \leq 0.001$) lower in those genotypes, and this led to markedly higher %Ndfa values in TGx1910-14F and
TGx1844-4E, followed by Solitaire and TGx1937-1F (Table 4.3). The genotype x inoculation interaction was significant \((P\leq 0.001)\) for \(\delta^{15}\text{N}\) and \(%\text{Ndfa}\) of roots, shoots and whole plants. These interactions revealed markedly lower \(\delta^{15}\text{N}\) values in roots, shoots and whole plants of inoculated compared to uninoculated soybean plants, and this resulted in significantly higher \%\text{Ndfa}\) in roots, shoots and whole plants of inoculated soybean (Fig. 4.2 A, B, C, D, E and F).

### 4.1.4 Amount of N-fixed

A 2-Way ANOVA analysis showed that N-fixed (whether expressed on a per plant or per hectare basis) was higher in all organs and whole plants of soybean inoculated with *Bradyrhizobium japonicum* strain WB74. There were also marked differences in symbiotic N yield of the seven soybean genotypes. The amount of N-fixed was much higher in shoots and whole plants of TGx1910-14F and Solitaire, followed by TGx1937-1F and TGx1844-4E. As a result, each of those four genotypes fixed more than 100 N kg/ha (Table 4.4). The genotype x inoculation interaction was significant \((P\leq 0.05)\) for N-fixed in roots, shoots and whole plants (whether expressed per plant or per hectare). The data showed that the amount of N-fixed by inoculated soybean was much greater than that of uninoculated plants (Fig. 4.3). Symbiotic N yield virtually doubled in all the seven soybean genotypes inoculated with *Bradyrhizobium japonicum* strain WB74 (Fig. 4.3 A, B, C, D, E and F).
**TABLE 4.1**: Effect of bradyrhizobial inoculation on dry matter yield of field-grown soybean genotypes planted at Nampula, Mozambique, in 2008/2009 cropping season.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dry matter yield (g/plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Roots</td>
</tr>
<tr>
<td><strong>Inoculation</strong></td>
<td></td>
</tr>
<tr>
<td>+ <em>Bradyrhizobium</em></td>
<td>17.9±0.4a</td>
</tr>
<tr>
<td>- <em>Bradyrhizobium</em></td>
<td>12.5±0.5b</td>
</tr>
<tr>
<td><strong>Genotypes</strong></td>
<td></td>
</tr>
<tr>
<td>TGx 1910-14F</td>
<td>13.5±1.2c</td>
</tr>
<tr>
<td>427/5/7</td>
<td>14.4±1.1ab</td>
</tr>
<tr>
<td>TGx 1937-1F</td>
<td>13.5±1.7c</td>
</tr>
<tr>
<td>TGx 1844-4E</td>
<td>17.2±0.9a</td>
</tr>
<tr>
<td>TGx 1908-8F</td>
<td>17.3±0.8a</td>
</tr>
<tr>
<td>Ocepera</td>
<td>14.9±1.9ab</td>
</tr>
<tr>
<td>Solitaire</td>
<td>15.6±1.6b</td>
</tr>
</tbody>
</table>

**2-WAY ANOVA (F-statistics)**

<table>
<thead>
<tr>
<th></th>
<th>F-statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculation</td>
<td>181.7***</td>
</tr>
<tr>
<td>Genotype</td>
<td>199.0***</td>
</tr>
<tr>
<td>Genotype x Inoculation</td>
<td>318.2***</td>
</tr>
<tr>
<td>Genotype</td>
<td>8.9***</td>
</tr>
<tr>
<td>Genotype x Inoculation</td>
<td>12.1***</td>
</tr>
<tr>
<td>Genotype</td>
<td>6.0***</td>
</tr>
<tr>
<td>Genotype x Inoculation</td>
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</tr>
<tr>
<td>Genotype</td>
<td>5.5***</td>
</tr>
<tr>
<td>Genotype x Inoculation</td>
<td>3.3**</td>
</tr>
</tbody>
</table>

Values (Means±SE) with dissimilar letters in a column are significant at **P≤0.01, ***P≤0.001.
FIGURE 4.1: Interactive effect of genotype x inoculation on dry matter yield and N content of field-grown soybean genotypes at Nampula, Mozambique, in 2008: A) Root dry matter, B) Shoot dry matter, C) Whole-plant dry matter, and D) Root N content. Vertical lines on bars represent S.E. (n=7). Bars followed by dissimilar letters are significant at $P\leq0.05$ for each genotype.
**TABLE 4.2:** Effect of bradyrhizobial inoculation on %N and N content of field-grown soybean genotypes planted at Nampula, Mozambique, in 2008/2009 cropping season.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>%N</th>
<th>N content (g/plant)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Root</td>
<td>Shoot</td>
<td>Whole plant</td>
<td>Root</td>
<td>Shoot</td>
<td>Whole plant</td>
<td>Root</td>
<td>Shoot</td>
<td>Whole plant</td>
</tr>
<tr>
<td><strong>Inoculation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ Bradyrhizobium</td>
<td>1.54±0.03a</td>
<td>3.80±0.07a</td>
<td>2.67±0.03a</td>
<td>0.28±0.01a</td>
<td>1.28±0.04a</td>
<td>1.56±0.04a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Bradyrhizobium</td>
<td>1.34±0.04b</td>
<td>3.45±0.06b</td>
<td>2.39±0.03b</td>
<td>0.17±0.01b</td>
<td>0.84±0.04b</td>
<td>1.01±0.04b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Genotypes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TGx 1910-14F</td>
<td>1.44±0.04bc</td>
<td>3.79±0.13b</td>
<td>2.61±0.08ab</td>
<td>0.20±0.02de</td>
<td>1.32±0.09a</td>
<td>1.51±0.11a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>427/5/7</td>
<td>1.54±0.09b</td>
<td>3.43±0.13c</td>
<td>2.48±0.10bc</td>
<td>0.22±0.03bc</td>
<td>1.05±0.12cd</td>
<td>1.27±0.14b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TGx 1937-1F</td>
<td>1.32±0.03c</td>
<td>3.75±0.14b</td>
<td>2.54±0.08bc</td>
<td>0.18±0.03e</td>
<td>1.07±0.14bc</td>
<td>1.25±0.17b</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>TGx 1844-4E</td>
<td>1.42±0.04bc</td>
<td>3.38±0.06c</td>
<td>2.40±0.05c</td>
<td>0.25±0.02b</td>
<td>0.87±0.15d</td>
<td>1.12±0.16b</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>TGx 1908-8F</td>
<td>1.68±0.03a</td>
<td>3.25±0.10c</td>
<td>2.46±0.06bc</td>
<td>0.29±0.02a</td>
<td>0.96±0.10cd</td>
<td>1.25±0.12b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ocepera</td>
<td>1.33±0.06c</td>
<td>3.73±0.08b</td>
<td>2.53±0.06bc</td>
<td>0.20±0.03cde</td>
<td>0.96±0.08d</td>
<td>1.16±0.11b</td>
<td></td>
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<tr>
<td>Solitaire</td>
<td>1.35±0.10c</td>
<td>4.04±0.09a</td>
<td>2.70±0.09a</td>
<td>0.22±0.03cd</td>
<td>1.20±0.09ab</td>
<td>1.42±0.12a</td>
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<td></td>
</tr>
</tbody>
</table>

**2-WAY ANOVA (F-statistics)**

<table>
<thead>
<tr>
<th></th>
<th>F-statistics</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Inoculation</td>
<td>47.79***</td>
<td>33.53***</td>
<td>57.10***</td>
<td>262.68***</td>
<td>157.68***</td>
<td>225.95***</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genotype</td>
<td>10.87***</td>
<td>11.86***</td>
<td>4.03**</td>
<td>17.72***</td>
<td>10.74***</td>
<td>8.06***</td>
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<td></td>
</tr>
<tr>
<td>Genotype x Inoculation</td>
<td>1.44ns</td>
<td>0.22ns</td>
<td>0.33ns</td>
<td>2.95*</td>
<td>2.08ns</td>
<td>1.79ns</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values (Means±SE) with dissimilar letters in a column are significant at *P≤0.05, ** P≤0.01, *** P≤0.001 and ns = not significant.
TABLE 4.3: Effect of bradyrhizobial inoculation on $\delta^{15}$N and %Ndfa of field-grown soybean genotypes planted at Nampula, Mozambique, in 2008/2009 cropping season.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$\delta^{15}$N (%)</th>
<th>%Ndfa</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Root</td>
<td>Shoot</td>
<td>Whole plant</td>
<td>Root</td>
<td>Shoot</td>
</tr>
<tr>
<td><strong>Inoculation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ Bradyrhizobium</td>
<td>1.2±0.1b</td>
<td>1.4±0.1b</td>
<td>1.4±0.1b</td>
<td>75.4±3.9a</td>
<td>70.8±1.7a</td>
</tr>
<tr>
<td>- Bradyrhizobium</td>
<td>1.9±0.1a</td>
<td>2.3±0.2a</td>
<td>2.3±0.1a</td>
<td>47.8±4.5b</td>
<td>60.3±1.9b</td>
</tr>
<tr>
<td><strong>Genotypes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TGx 1910-14F</td>
<td>1.5±0.1c</td>
<td>1.1±0.3d</td>
<td>1.1±0.2d</td>
<td>64.7±4.1c</td>
<td>75.1±3.2b</td>
</tr>
<tr>
<td>427/5/7</td>
<td>1.9±0.3c</td>
<td>2.1±0.2b</td>
<td>2.1±0.2b</td>
<td>46.7±12.2d</td>
<td>62.4±2.5d</td>
</tr>
<tr>
<td>TGx 1937-1F</td>
<td>1.0±0.1e</td>
<td>1.9±0.3c</td>
<td>1.8±0.3d</td>
<td>83.3±5.4a</td>
<td>64.7±3.8c</td>
</tr>
<tr>
<td>TGx 1844-4E</td>
<td>1.2±0.1d</td>
<td>1.0±0.1d</td>
<td>1.1±0.1d</td>
<td>75.5±5.7c</td>
<td>75.6±1.1a</td>
</tr>
<tr>
<td>TGx 1908-8F</td>
<td>1.1±0.2de</td>
<td>2.1±0.1b</td>
<td>1.9±0.1c</td>
<td>79.4±7.4b</td>
<td>63.1±0.9cd</td>
</tr>
<tr>
<td>Ocepera</td>
<td>2.2±0.1a</td>
<td>3.1±0.3a</td>
<td>2.9±0.3a</td>
<td>35.3±5.0e</td>
<td>51.7±3.2d</td>
</tr>
<tr>
<td>Solitaire</td>
<td>1.9±0.1b</td>
<td>1.8±0.2cd</td>
<td>1.8±0.2c</td>
<td>46.3±5.4de</td>
<td>66.4±2.7b</td>
</tr>
<tr>
<td>2-WAY ANOVA (F-statistics)</td>
<td>241.02***</td>
<td>267.53***</td>
<td>379.72***</td>
<td>241.02***</td>
<td>267.53***</td>
</tr>
<tr>
<td>Inoculation</td>
<td>64.68***</td>
<td>91.61***</td>
<td>109.02***</td>
<td>64.68***</td>
<td>91.62***</td>
</tr>
<tr>
<td>Genotype x Inoculation</td>
<td>6.28***</td>
<td>8.46***</td>
<td>8.19***</td>
<td>6.28***</td>
<td>8.47***</td>
</tr>
</tbody>
</table>

Values (Means±SE) with dissimilar letters in a column are significant at *** $P \leq 0.001$. 


FIGURE 4.2: Interactive effect of genotype x inoculation on $\delta^{15}$N and %Ndfa of field-grown soybean genotypes at Nampula, Mozambique, in 2008: A) Root $\delta^{15}$N, B) Shoot $\delta^{15}$N, C) Whole-plant $\delta^{15}$N, D) Root %Ndfa, E) Shoot %Ndfa, and F) whole-plant %Ndfa. Vertical lines on bars represent S.E. (n=7). Bars followed by dissimilar letters are significant at $P \leq 0.05$ for each genotype.
**TABLE 4.4**: Effect of bradyrhizobial inoculation on fixed-N (g and kg/ha), of field-grown soybean genotypes planted at Nampula, Mozambique, in the 2008/2009 cropping season.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fixed-N (g/plant)</th>
<th></th>
<th>Fixed-N (kg N/ha)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Root</td>
<td>Shoot</td>
<td>Whole plant</td>
<td>Root</td>
</tr>
<tr>
<td><strong>Inoculation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ <em>Bradyrhizobium</em></td>
<td>0.21±0.01a</td>
<td>0.92±0.04a</td>
<td>1.15±0.06a</td>
<td>24.3±1.5a</td>
</tr>
<tr>
<td>- <em>Bradyrhizobium</em></td>
<td>0.08±0.01b</td>
<td>0.51±0.03b</td>
<td>0.57±0.04b</td>
<td>9.7±1.2b</td>
</tr>
<tr>
<td><strong>Genotypes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TGx 1910-14F</td>
<td>0.13±0.02d</td>
<td>1.00±0.11a</td>
<td>1.20±0.15a</td>
<td>15.0±2.3d</td>
</tr>
<tr>
<td>427/5/7</td>
<td>0.12±0.04e</td>
<td>0.66±0.09de</td>
<td>0.78±0.13de</td>
<td>13.8±4.4e</td>
</tr>
<tr>
<td>TGx 1937-1F</td>
<td>0.16±0.03c</td>
<td>0.72±0.13c</td>
<td>0.86±0.18cd</td>
<td>18.3±3.7c</td>
</tr>
<tr>
<td>TGx 1844-4E</td>
<td>0.19±0.03b</td>
<td>0.67±0.12cd</td>
<td>0.90±0.15c</td>
<td>22.3±3.3b</td>
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<tr>
<td>TGx 1908-8F</td>
<td>0.24±0.03a</td>
<td>0.61±0.07de</td>
<td>0.81±0.09cde</td>
<td>27.5±4.0a</td>
</tr>
<tr>
<td>Ocepera</td>
<td>0.08±0.02e</td>
<td>0.51±0.07e</td>
<td>0.54±0.10e</td>
<td>9.1±2.4e</td>
</tr>
<tr>
<td>Solitaire</td>
<td>0.11±0.03e</td>
<td>0.81±0.09b</td>
<td>0.94±0.13b</td>
<td>12.7±3.1e</td>
</tr>
<tr>
<td><strong>2-WAY ANOVA (F-statistics)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inoculation</td>
<td>440.91***</td>
<td>286.06***</td>
<td>457.26***</td>
<td>440.91***</td>
</tr>
<tr>
<td>Genotype</td>
<td>46.20***</td>
<td>24.04***</td>
<td>31.20***</td>
<td>46.20***</td>
</tr>
<tr>
<td>Genotype x Inoculation</td>
<td>3.07*</td>
<td>3.41*</td>
<td>4.08**</td>
<td>3.07*</td>
</tr>
</tbody>
</table>

Values (Means±SE) with dissimilar letters in a column are significant at * P≤0.05, ** P≤0.01, *** P≤0.001
FIGURE 4.3: Interactive effect of genotype x inoculation on fixed-N of field-grown soybean genotypes planted at Nampula, Mozambique, in 2008: A) Root per plant, B) Shoot per plant, C) Whole-plant, D) Root per hectare, E) Shoot per hectare and F) Whole-plant per hectare. Vertical lines on bars represent S.E. (n=7). Bars followed by dissimilar letters are significant at p≤0.05 for each genotype.
4.2 Year 2 Ruace and Mutequelesse

4.2.1 Plant growth

A 3-Way ANOVA analysis of seven inoculated and uninoculated soybean genotypes planted at Mutequelesse and Ruace in Mozambique during 2008/2009 cropping season revealed marked differences in shoot biomass accumulation and grain yield with respect to location, inoculation and genotypes (Table 4.5). In general, shoot and whole-plant biomass, as well as grain yield, were significantly ($P \leq 0.001$) greater at Ruace relative to Mutequelesse. Inoculating the soybean genotypes with *Bradyrhizobium* also increased shoot and whole-plant dry matter, as well as grain yield when compared to uninoculated treatments. Of the seven soybean genotypes, TGx1937-1F produced more shoot biomass, followed by TGx1844-4E and Solitaire. However, because genotypes Solitaire and 427/5/7 produced more grain yield, whole-plant biomass was markedly ($P \leq 0.05$) greater in Solitaire, TGx1937-1F and 427/5/7 (Table 4.5). Genotype x environment interaction was significant ($P \leq 0.05$) for the dry matter yield of shoots, grain and whole plants. A 1-Way ANOVA analysis of the interaction indicated a consistently increased biomass in shoots and whole plants, and much higher grain yield at Ruace compared to Mutequelesse (Fig. 4.4 A, B, and C).
4.2.2 N concentration and content

A 3-Way ANOVA analysis of N content and N concentration also showed a pattern similar to that of biomass, in that, the N level was significantly ($P \leq 0.001$) higher in shoots, grain and whole plants of soybean grown at Ruace compared to Mutequelesse (Table 4.6). *Bradyrhizobium* inoculation also increased the %N and N content of soybean shoots, grain and whole plants (Table 4.6). The soybean genotypes also exhibited differences in N content and N concentration independent of location or inoculation. Shoot and grain N concentration was significantly ($P \leq 0.001$) higher in TGx1910-14F, resulting in much greater N concentration at whole-plant level. Ocepera exhibited the lowest N concentration in shoots, grain and whole plants. The N content was similar at whole-plant level in all seven genotypes, even though the shoots and grain showed different N contents. There was a significant ($P \leq 0.05$) inoculation x environment interaction for N concentration in grain and whole plants, and for N content of shoots. Other interactions that were significant ($P \leq 0.05$) included genotype x environment for N content and N concentration in shoots, grain and whole plants; genotype x inoculation for N concentration in shoots, grain and whole plants, as well as for N content of shoots and whole plants; and genotype x inoculation x environment for %N in shoots, grain and whole plants, and for N content of shoots (Table 4.6). One-way ANOVA analysis for inoculation x environment showed higher N concentration in grain and whole plants of inoculated and uninoculated soybean plants at Ruace compared to Mutequelesse (Fig. 4.5A, and B). N content was also higher in inoculated and uninoculated soybean at Ruace relative to Mutequelesse
Similarly, analysis of the genotype x environment interaction revealed significantly ($P \leq 0.01$) higher N content in all genotypes planted at Ruace compared to Mutequelesse due to the markedly greater %N of grain and whole plants but less so in shoots (Fig. 4.6). The genotype x inoculation interaction data also showed consistently higher N concentration and N content in shoots, grain and whole plants of all seven soybean genotypes inoculated with *Bradyrhizobium* (Fig. 4.7). Furthermore, analysis of the genotype x inoculation x environment interaction for each genotype showed that shoot %N was always significantly ($P \leq 0.05$) higher with inoculation at Ruace, generally followed by inoculated plants at Mutequelesse, except for 427/5/7 and Solitaire (Fig. 4.8A). The data for grain %N were similar to those of shoots, except that uninoculated soybean plants at Ruace were the next higher in grain %N relative to both inoculated and uninoculated plants at Mutequelesse (Fig. 4.8B). Inoculated plants of all genotypes also exhibited higher N concentration over uninoculated at Mutequelesse (Fig. 4.8B). Whole-plant N concentration and N content were also always higher in inoculated soybean genotypes at Ruace, followed by uninoculated plants, except for N content of TGx1937-1F and Ocepera (Fig. 4.9 A and B). Inoculated plants of all genotypes exhibited higher N concentrations and N content compared to uninoculated at Mutequelesse (Fig. 4.9 A and B).

4.2.3 $\delta^{15}$N and %Ndfa values

Analysis of $\delta^{15}$N and %Ndfa values (using 3-Way ANOVA) revealed significantly ($P \leq 0.001$) lower $\delta^{15}$N values in shoots, grain and whole plants at Ruace relative to
Mutequelesse, and this resulted in markedly higher %Ndfa values for shoots, grain and whole plants at Ruace (Table 4.7). Inoculation with *Bradyrhizobium* also decreased $\delta^{15}N$ values in shoots, grain and whole plants, leading to significantly ($P \leq 0.001$) higher %Ndfa values (Table 4.7). Although shoot $\delta^{15}N$ values were similar for all seven genotypes, those of grain and whole plants differed significantly ($P \leq 0.05$), with TGx1937-1F exhibiting the lowest grain and whole-plant $\delta^{15}N$ values, and hence the highest % Ndfa, followed by Ocepera and 427/5/7 (Table 4.7). The inoculation x environment interaction was significant ($P \leq 0.001$) for $\delta^{15}N$ and %Ndfa of shoots, grain and whole plants. Other interactions that were significant ($P \leq 0.05$) included genotype x environment for $\delta^{15}N$ and %Ndfa of shoots, grain and whole plants; genotype x inoculation for $\delta^{15}N$ and %Ndfa of grain and whole plants; and genotype x inoculation x environment for $\delta^{15}N$ and %Ndfa of grain. A 1-Way ANOVA analysis of the inoculation x environment interaction indicated significantly ($P \leq 0.001$) lower $\delta^{15}N$ values for shoots, grain and whole-plants of inoculated and uninoculated soybean at Ruace relative to same treatments at Mutequelesse (Fig. 4.10 A B and C). As a result, %Ndfa of shoots, grain and whole plants of inoculated and uninoculated soybean were markedly higher at Ruace compared to those of Mutequelesse (Fig. 4.10 D, E and F). Genotype x environment analysis also showed significantly ($P \leq 0.05$) lower $\delta^{15}N$ values for shoots, grain and whole plants at Ruace relative to Mutequelesse, resulting in markedly greater %Ndfa values for shoots, grain and whole plants at Ruace (Fig. 4.11 A, B, C, D, E, and F). A 1-Way ANOVA analysis of genotype x inoculation interaction showed significantly ($P \leq 0.05$) lower $\delta^{15}N$ values for grain and whole plants of all inoculated plants relative to uninoculated counterparts.
(Fig. 4.12 A and B). As a result, %Ndfa values were also markedly higher in grain and whole plants of all inoculated treatments (Fig. 4.12 C and D). Analysis of the genotype x inoculation x environment interaction also revealed consistently lower $\delta^{15}$N values for grain of all inoculated and uninoculated soybean genotypes at Ruace, followed by inoculated, and then uninoculated plants at Mutequelesse (Fig. 4.13A). As a result, the %Ndfa of grain was highest in the inoculated and uninoculated soybean plants at Ruace, followed by inoculated, and then uninoculated plants at Mutequelesse (Fig. 4.13B).

### 4.2.4 Amount of N-fixed

A 3-Way ANOVA analysis of the seven soybean genotypes planted with and without inoculation showed much larger amounts of N-fixed in shoots, grain and whole plants at Ruace compared to Mutequelesse (Table 4.8). Soybean inoculation with *Bradyrhizobium japonicum* strain WB74 also increased the amount of N-fixed in shoots, grain and whole plants relative to uninoculated (Table 4.8). Independent of location and inoculation, there were marked differences between and among the seven soybean genotypes in the amounts of N-fixed in shoots, grain and whole plants. Because TGx1937-1F showed more N-fixed in shoots, followed by Solitaire, and the two genotypes also had high grain content of N-fixed, they emerged as the genotypes with the highest N$_2$ fixation at whole-plant level (Table 4.8). Inoculation x environment interaction was significant ($P \leq 0.05$) for N-fixed in shoots and whole plants, just as genotype x environment interaction was significant ($P \leq 0.01$) for N-fixed
in shoots, grain and whole plants (Table 4.8). Similarly, the genotype x inoculation, and genotype x inoculation x environment interactions were significant ($P \leq 0.05$) for N-fixed in shoots and whole plants (Table 4.8). One-way ANOVA analysis of the interactions showed that N-fixed in shoots and whole plants (expressed on a per plant basis) was always higher at Ruace irrespective of inoculation level, and lower at Mutequelesse (Fig. 4.14 A and B). As a result, the amount of N-fixed per hectare in shoots and whole plants exhibited the same pattern, with higher $N_2$ fixation recorded in inoculated and uninoculated plants at Ruace compared to Mutequelesse (Fig. 4.14 C and D). Data from analysis of genotype x environment interaction also revealed consistently higher N-fixed in all genotypes at Ruace relative to Mutequelesse whether expressed as g/plant or kg/ha (Fig. 4.15 A, B, C, D, E and F). Similarly, the genotype x inoculation interaction showed significantly ($P \leq 0.05$) higher amounts of N-fixed (g/plant or kg/ha) in all seven genotypes with inoculation compared to uninoculated treatments (Fig. 4.16 A, B, C and D), while the data from genotype x inoculation x environment interaction indicated consistently greater amounts of N-fixed (g/plant or kg/ha) by all inoculated plants at Ruace, followed by uninoculated plants at Ruace, then the inoculated genotypes at Mutequelesse, and least, the uninoculated plants at Mutequelesse (Fig. 4.17 A and B).
### TABLE 4.5: Effect of bradyrhizobial inoculation on dry matter yield of field-grown soybean genotypes, planted at Ruace and Mutequelesse, Mozambique in the 2008/2009 cropping season.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dry matter yield (g/plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoot</td>
</tr>
<tr>
<td><strong>Location</strong></td>
<td></td>
</tr>
<tr>
<td>Ruace</td>
<td>20.0±1.1a</td>
</tr>
<tr>
<td>Mutequelesse</td>
<td>9.2±0.5b</td>
</tr>
<tr>
<td><strong>Inoculation</strong></td>
<td></td>
</tr>
<tr>
<td>+ <em>Bradyrhizobium</em></td>
<td>16.4±1.3a</td>
</tr>
<tr>
<td>- <em>Bradyrhizobium</em></td>
<td>12.8±1.0b</td>
</tr>
<tr>
<td><strong>Genotypes</strong></td>
<td></td>
</tr>
<tr>
<td>TGx 1910-14F</td>
<td>14.7±2.2bc</td>
</tr>
<tr>
<td>427/5/7</td>
<td>12.9±1.9bc</td>
</tr>
<tr>
<td>TGx 1937-1F</td>
<td>18.7±3.2a</td>
</tr>
<tr>
<td>TGx 1844-4E</td>
<td>15.7±2.0abc</td>
</tr>
<tr>
<td>TGx 1908-8F</td>
<td>13.5±1.0bc</td>
</tr>
<tr>
<td>Ocepera</td>
<td>11.4±1.3c</td>
</tr>
<tr>
<td>Solitaire</td>
<td>15.2±3.1abc</td>
</tr>
<tr>
<td><strong>3-WAY ANOVA (F-statistics)</strong></td>
<td></td>
</tr>
<tr>
<td>Location</td>
<td>130.6***</td>
</tr>
<tr>
<td>Inoculation</td>
<td>14.6***</td>
</tr>
<tr>
<td>Genotype</td>
<td>3.5**</td>
</tr>
<tr>
<td>Inoculation x Environment</td>
<td>0.5&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>Genotype x environment</td>
<td>3.8**</td>
</tr>
<tr>
<td>Genotype x inoculation</td>
<td>1.8&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>Genotype x inoc x environ</td>
<td>2.0&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values (Means±SE) with dissimilar letters in a column are significant at *P*≤0.05, **P*≤0.01, ***P*≤0.001 and ns= not significant
**FIGURE 4.4:** Interactive effect of genotype x environment on shoot and whole-plant dry matter and on grain yield in seven soybean genotypes planted in Mozambique: A) Shoot dry matter, B) Grain dry matter, and C) Whole-plant dry matter. Vertical lines on bars represent S.E. (n=7). Bars followed by dissimilar letters are significant at p≤0.05 for each genotype.
TABLE 4.6: Effect of bradyrhizobial inoculation on %N and N content of field-grown soybean genotypes, planted at Ruace and Mutequelesse, Mozambique in 2008/2009 cropping season.

<table>
<thead>
<tr>
<th>Location</th>
<th>%N</th>
<th>N content (g/plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoots</td>
<td>Grain</td>
</tr>
<tr>
<td>Ruace</td>
<td>4.8±0.1a</td>
<td>7.5±0.1a</td>
</tr>
<tr>
<td>Mutequelesse</td>
<td>4.0±0.1b</td>
<td>4.7±0.1b</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Inoculation</th>
<th>%N</th>
<th>N content (g/plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ Bradyrhizobium</td>
<td>4.8±0.1a</td>
<td>6.5±0.2a</td>
</tr>
<tr>
<td>- Bradyrhizobium</td>
<td>4.0±0.1b</td>
<td>5.7±0.3b</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>%N</th>
<th>N content (g/plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGx 1910-14F</td>
<td>4.8±0.2b</td>
<td>6.8±0.7a</td>
</tr>
<tr>
<td>427/5/7</td>
<td>4.3±0.3c</td>
<td>6.1±0.4c</td>
</tr>
<tr>
<td>TGx 1937-1F</td>
<td>4.3±0.3c</td>
<td>5.9±0.4d</td>
</tr>
<tr>
<td>TGx 1844-4E</td>
<td>4.8±0.1a</td>
<td>5.9±0.4d</td>
</tr>
<tr>
<td>TGx 1908-8F</td>
<td>4.8±0.2b</td>
<td>6.5±0.5b</td>
</tr>
<tr>
<td>Ocepera</td>
<td>3.3±0.2c</td>
<td>5.5±0.4d</td>
</tr>
<tr>
<td>Solitaire</td>
<td>4.6±0.1c</td>
<td>6.0±0.5d</td>
</tr>
</tbody>
</table>

3-WAY ANOVA (F-statistics)

<table>
<thead>
<tr>
<th></th>
<th>Location</th>
<th>Inoculation</th>
<th>Genotype</th>
<th>Inoculation x Environment</th>
<th>Genotype x environment</th>
<th>Genotype x inoculation</th>
<th>Genotype x inoc x environ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>139.5***</td>
<td>195.2***</td>
<td>41.3***</td>
<td>0.6* ns</td>
<td>5.9***</td>
<td>6.5***</td>
<td>3.1*</td>
</tr>
<tr>
<td></td>
<td>1185.7***</td>
<td>99.5***</td>
<td>15.6***</td>
<td>11.6**</td>
<td>12.3***</td>
<td>4.5**</td>
<td>3.0*</td>
</tr>
<tr>
<td></td>
<td>1288.9***</td>
<td>292.3***</td>
<td>32.4***</td>
<td>5.4*</td>
<td>5.4***</td>
<td>8.9***</td>
<td>16.1***</td>
</tr>
<tr>
<td></td>
<td>139.4***</td>
<td>34.3***</td>
<td>4.9***</td>
<td>3.8*</td>
<td>3.2**</td>
<td>2.9*</td>
<td>2.4*</td>
</tr>
<tr>
<td></td>
<td>109.8***</td>
<td>7.4**</td>
<td>3.5**</td>
<td>0.1 ns</td>
<td>4.2**</td>
<td>0.8* ns</td>
<td>0.5* ns</td>
</tr>
<tr>
<td></td>
<td>223.2***</td>
<td>30.4***</td>
<td>1.9 ns</td>
<td>1.3 ns</td>
<td>3.2**</td>
<td>2.7*</td>
<td>1.9 ns</td>
</tr>
</tbody>
</table>

Values (Means±SE) with dissimilar letters in a column are significant at *P≤0.05, ** P≤0.01, *** P≤0.001 and ns= not significant.
FIGURE 4.5: Interactive effect of Bradyrhizobial inoculation x environment on %N and N content in seven soybean genotypes planted at Ruace and Mutequelesse, Mozambique: A) Grain %N, B) Whole-plant %N and C) Shoot N. Vertical lines on bars represent S.E. (n=7). Bars followed by dissimilar letters are significant at $P \leq 0.05$ for each Bradyrhizobium level.
FIGURE 4.6: Interactive effect of genotype x environment on organ and whole plant %N and N content in seven soybean genotypes, planted in Mozambique: A) Shoot %N, B) Grain %N, C) Whole-plant %N, D) Shoot N, E) Grain N and F) Whole-plant N. Vertical lines on bars represent S.E. (n=7). Bars followed by dissimilar letters are significant at $P \leq 0.05$ for each genotype.
FIGURE 4.7: Interactive effect of genotype x inoculation on shoot, grain and whole plant %N and N content in seven soybean genotypes planted at two locations in Mozambique: A) Shoot %N, B) Grain %N, C) Whole-plant %N, D) Shoot N and E) Whole-plant N. Vertical lines on bars represent S.E. (n=7). Bars followed by dissimilar letters are significant at $P \leq 0.05$ for each genotype.
FIGURE 4.8: Interactive effect of genotype x inoculation x environment on shoot and grain %N in seven soybean genotypes planted at two locations in Mozambique. A) Shoot %N, and B) Grain %N. Vertical lines on bars represent S.E. (n=7). Bars followed by dissimilar letters are significant at $P \leq 0.05$ for each genotype.
FIGURE 4.9: Interactive effect of genotype x inoculation x environment on whole-plant %N and shoots N content in seven soybean genotypes planted at two locations in Mozambique: A) Whole-plant %N and B) Shoot N content. Vertical lines on bars represent S.E. (n=7). Bars followed by dissimilar letters are significant at $P \leq 0.05$ for each genotype.
TABLE 4.7: Effect of bradyrhizobial inoculation on $\delta^{15}\text{N}$ and %Ndfa of field-grown soybean genotypes, planted at Ruace and Mutequelesse, Mozambique in the 2008/2009 cropping season.

| Treatment | δ$^{15}\text{N}$ (%) | %Ndfa | | | |
|-----------|----------------------|--------|--------|--------|
|           | Shoots              | Grain  | Whole plant | Shoots | Grain  | Whole plant |
| Location  |                      |        |            |        |        |            |
| Ruace     | 0.3±0.02b           | 0.4±0.02b | 0.8±0.04b | 85.1±0.22a | 90.9±0.67a | 83.6±0.79a |
| Mutequelesse | 3.4±0.05a     | 1.9±0.08a | 2.7±0.06a | 49.7±0.61b | 46.8±2.15b | 52.7±1.07b |
| Inoculation |                      |        |            |        |        |            |
| + Bradyrhizobium | 1.7±0.23b     | 0.9±0.10b | 1.5±0.14b | 69.1±2.64a | 75.7±2.84a | 72.4±2.24a |
| - Bradyrhizobium | 2.0±0.25a      | 1.4±0.15a | 2.0±0.17a | 65.7±2.94b | 62.1±4.29b | 63.9±2.75b |
| Genotypes |                      |        |            |        |        |            |
| TGx 1910-14F | 1.9±0.44a    | 1.3±0.28a | 1.8±0.34b | 67.2±5.13a | 65.2±7.75d | 67.9±5.62c |
| 427/5/7 | 1.8±0.44a        | 1.3±0.29a | 1.7±0.30bc | 68.2±5.20a | 65.1±8.13d | 68.3±4.94bc |
| TGx 1937-1F | 1.8±0.48a   | 1.0±0.23d | 1.6±0.33c | 68.2±5.61a | 74.1±6.33a | 71.5±5.59a |
| TGx 1844-4E | 1.8±0.47a     | 1.0±0.24cd | 1.9±0.28a | 67.6±5.44a | 71.8±6.79ab | 65.3±4.52c |
| TGx 1908-8F | 1.8±0.44a   | 1.2±0.24b | 1.7±0.28bc | 67.9±5.19a | 67.3±6.80cd | 67.7±4.53c |
| Ocepera | 1.9±0.44a       | 1.1±0.24bcd | 1.7±0.26c | 66.6±5.13a | 69.6±6.81bc | 68.4±4.13b |
| Solitaire | 2.0±0.53a     | 1.1±0.29bc | 1.7±0.33c | 66.0±6.11a | 69.0±8.03bc | 67.9±5.36c |

3-WAY ANOVA (F-statistics)

| Location | 5762.3*** | 2067.1*** | 2867.7*** | 6116.7*** | 2200.8*** | 2235.2*** |
| Inoculation | 56.2*** | 209.9*** | 170.57*** | 54.5*** | 209.9*** | 171.6*** |
| Genotype | 2.1 ns | 7.2*** | 3.77** | 1.9 ns | 7.2*** | 4.4*** |
| Inoculation x Environment | 13.7*** | 96.0*** | 29.4*** | 14.7*** | 94.7*** | 20.4*** |
| Genotype x environment | 2.7* | 3.6** | 5.3*** | 2.6* | 3.6** | 6.1*** |
| Genotype x inoculation | 1.8 ns | 3.6** | 2.8* | 1.7 ns | 3.6** | 3.0* |
| Genotype x inoc x environ | 1.1 ns | 3.9** | 1.9 ns | 1.1 ns | 3.9** | 1.9 ns |

Values (Means±SE) with dissimilar letters in a column are significant at *P≤0.05, ** P≤0.01, ***P≤0.001 and ns= not significant.
FIGURE 4.10: Interactive effect of Bradyrhizobial inoculation x environment on δ¹⁵N and %Ndfa in seven soybean genotypes planted at Ruace and Mutequelesse, Mozambique: A) Shoot δ¹⁵N, B) Grain δ¹⁵N, C) Whole-plant δ¹⁵N D) Shoot %Ndfa, E) Grain %Ndfa and F) Whole-plant %Ndfa. Vertical lines on bars represent S.E. (n=7). Bars followed by dissimilar letters are significant at $P \leq 0.05$ for each *Bradyrhizobium* level.
FIGURE 4.11: Interactive effect of genotype x environment on organ and whole plant $\delta^{15}$N and %Ndfa in seven soybean genotypes, planted in Mozambique: A) Shoot $\delta^{15}$N, B) Grain $\delta^{15}$N, C) Whole-plant $\delta^{15}$N D) Shoots %Ndfa, E) Grain %Ndfa and F) Whole-plant %Ndfa. Vertical lines on bars represent S.E. (n=7). Bars followed by dissimilar letters are significant at $P \leq 0.05$ for each genotype.
FIGURE 4.12: Interactive effect of genotype x inoculation on grain and whole plant $\delta^{15}$N and %Ndfa values in seven soybean genotypes planted at two locations in Mozambique: A) Grain $\delta^{15}$N, B) Whole-plant $\delta^{15}$N, C) Grain %Ndfa and D) Whole-plant %Ndfa. Vertical lines on bars represent S.E. (n=7). Bars followed by dissimilar letters are significant at $P\leq0.05$ for each genotype.
FIGURE 4.13: Interactive effect of Genotype x inoculation x environment on grain $\delta^{15}$N and grain %Ndfa in seven soybean genotypes planted at two locations in Mozambique: A) Grain $\delta^{15}$N and B) Grain %Ndfa. Vertical lines on bars represent S.E. (n=7). Bars followed by dissimilar letters are significant at $P \leq 0.05$ for each genotype.
**TABLE 4.8**: Amount of N-fixed in field-grown soybean genotypes, planted at Ruace and Mutequelesse, Mozambique, in the 2008/2009 cropping season.

<table>
<thead>
<tr>
<th>Location</th>
<th>Shoots N-fixed (g/plant)</th>
<th>Grain N-fixed (g/plant)</th>
<th>Whole plant N-fixed (g/plant)</th>
<th>Shoots N-fixed (kg/ha)</th>
<th>Grain N-fixed (kg/ha)</th>
<th>Whole plant N-fixed (kg/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ruace</td>
<td>0.8±0.06a</td>
<td>0.9±0.07a</td>
<td>1.7±0.10a</td>
<td>97.0±6.94a</td>
<td>103.7±7.67a</td>
<td>199.2±11.8a</td>
</tr>
<tr>
<td>Mutequelesse</td>
<td>0.2±0.02b</td>
<td>0.2±0.02b</td>
<td>0.4±0.03b</td>
<td>22.3±1.76b</td>
<td>19.5±1.82b</td>
<td>45.2±3.3b</td>
</tr>
</tbody>
</table>

**Inoculation**

+ *Bradyrhizobium*

<table>
<thead>
<tr>
<th>Shoots N-fixed (g/plant)</th>
<th>Grain N-fixed (g/plant)</th>
<th>Whole plant N-fixed (g/plant)</th>
<th>Shoots N-fixed (kg/ha)</th>
<th>Grain N-fixed (kg/ha)</th>
<th>Whole plant N-fixed (kg/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.6±0.08a</td>
<td>0.6±0.08a</td>
<td>1.3±0.14a</td>
<td>73.3±8.89a</td>
<td>71.6±8.83a</td>
<td>148.3±16.7a</td>
</tr>
</tbody>
</table>

- *Bradyrhizobium*

<table>
<thead>
<tr>
<th>Shoots N-fixed (g/plant)</th>
<th>Grain N-fixed (g/plant)</th>
<th>Whole plant N-fixed (g/plant)</th>
<th>Shoots N-fixed (kg/ha)</th>
<th>Grain N-fixed (kg/ha)</th>
<th>Whole plant N-fixed (kg/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4±0.05b</td>
<td>0.4±0.07b</td>
<td>0.8±0.10b</td>
<td>46.0±5.57b</td>
<td>51.6±8.10b</td>
<td>96.1±11.35b</td>
</tr>
</tbody>
</table>

**Genotypes**

<table>
<thead>
<tr>
<th>Shoots N-fixed (g/plant)</th>
<th>Grain N-fixed (g/plant)</th>
<th>Whole plant N-fixed (g/plant)</th>
<th>Shoots N-fixed (kg/ha)</th>
<th>Grain N-fixed (kg/ha)</th>
<th>Whole plant N-fixed (kg/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGx 1910-14F</td>
<td>0.5±0.12bc</td>
<td>0.5±0.13ab</td>
<td>1.1±0.26abc</td>
<td>62.5±13.45bc</td>
<td>56.2±15.11ab</td>
</tr>
<tr>
<td>4275/5/7</td>
<td>0.5±0.11bc</td>
<td>0.7±0.19a</td>
<td>1.1±0.27abc</td>
<td>54.4±12.87bc</td>
<td>82.4±21.71a</td>
</tr>
<tr>
<td>TGx 1937-1F</td>
<td>0.7±0.19a</td>
<td>0.5±0.16ab</td>
<td>1.3±0.33a</td>
<td>77.4±22.46a</td>
<td>63.0±18.26ab</td>
</tr>
<tr>
<td>TGx 1844-4E</td>
<td>0.6±0.11bc</td>
<td>0.3±0.06b</td>
<td>0.9±0.16bc</td>
<td>67.0±12.62bc</td>
<td>38.1±7.29b</td>
</tr>
<tr>
<td>TGx 1908-8F</td>
<td>0.5±0.07c</td>
<td>0.4±0.08b</td>
<td>0.9±0.13bc</td>
<td>53.8±8.42c</td>
<td>51.6±9.01b</td>
</tr>
<tr>
<td>Ocepera</td>
<td>0.3±0.07c</td>
<td>0.5±0.13ab</td>
<td>0.8±0.17c</td>
<td>34.8±7.59c</td>
<td>58.5±4.60ab</td>
</tr>
<tr>
<td>Solitaire</td>
<td>0.6±0.15b</td>
<td>0.7±0.18a</td>
<td>1.2±0.29ab</td>
<td>67.7±17.70b</td>
<td>81.7±20.45a</td>
</tr>
</tbody>
</table>

**3-WAY ANOVA (F-statistics)**

<table>
<thead>
<tr>
<th>Location</th>
<th>Shoots N-fixed (g/plant)</th>
<th>Grain N-fixed (g/plant)</th>
<th>Whole plant N-fixed (g/plant)</th>
<th>Shoots N-fixed (kg/ha)</th>
<th>Grain N-fixed (kg/ha)</th>
<th>Whole plant N-fixed (kg/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location</td>
<td>240.0***</td>
<td>165.5***</td>
<td>310.0***</td>
<td>240.0***</td>
<td>165.5***</td>
<td>310.0***</td>
</tr>
<tr>
<td>Inoculation</td>
<td>32.0***</td>
<td>9.4**</td>
<td>35.5***</td>
<td>32.0***</td>
<td>9.4**</td>
<td>35.5***</td>
</tr>
<tr>
<td>Genotype</td>
<td>4.6***</td>
<td>3.4**</td>
<td>2.7*</td>
<td>4.6***</td>
<td>3.4**</td>
<td>2.7*</td>
</tr>
<tr>
<td>Inoculation x Environment</td>
<td>8.0**</td>
<td>0.2ns**</td>
<td>4.8*</td>
<td>8.0**</td>
<td>0.2ns**</td>
<td>4.8*</td>
</tr>
<tr>
<td>Genotype x environment</td>
<td>3.6**</td>
<td>4.2**</td>
<td>3.5**</td>
<td>3.6**</td>
<td>4.2**</td>
<td>3.5**</td>
</tr>
<tr>
<td>Genotype x inoculation</td>
<td>3.2**</td>
<td>0.7ns**</td>
<td>2.6*</td>
<td>3.2**</td>
<td>0.7ns**</td>
<td>2.6*</td>
</tr>
<tr>
<td>Genotype x inoc x environ</td>
<td>2.8*</td>
<td>0.7ns**</td>
<td>2.0ns</td>
<td>2.8*</td>
<td>0.7ns**</td>
<td>2.0ns</td>
</tr>
</tbody>
</table>

Values (Means±SE) with dissimilar letters in a column are significant at \(*P \leq 0.05, **P \leq 0.01, ***P \leq 0.001\) and ns= not significant.
FIGURE 4.14: Interactive effect of Bradyrhizobial inoculation x environment on fixed-N in seven soybean genotypes planted at Ruace and Mutequelesse, Mozambique: A) Shoot N-fixed, B) Whole-plant N-fixed, C) Shoot N-fixed, D) Whole-plant N-fixed. Vertical lines on bars represent S.E. (n=7). Bars followed by dissimilar letters are significant at $P \leq 0.05$ for each *Bradyrhizobium* level.
FIGURE 4.15: Interactive effect of genotype x environment on shoot, grain and whole-plant fixed-N in seven soybean genotypes, planted in Mozambique: A) Shoot fixed-N (g/plant), B) Grain fixed-N(g/plant), C) Whole-plant fixed-N(g/plant), D) Shoot -N (kg/ha), E) Grain fixed-N(kg/ha), F) Whole-plant fixed-N(kg/ha). Vertical lines on bars represent S.E. (n=7). Bars followed by dissimilar letters are significant at $P \leq 0.05$ for each genotype.
FIGURE 4.16: Interactive effect of genotype x inoculation on shoots and whole plant N-fixed in seven soybean genotypes planted at two locations in Mozambique: A) Shoot N-fixed (g/plant), B) Whole-plant N-fixed (g/plant), C) Shoot N-fixed (kg/ha), D) Whole-plant N-fixed (kg/ha). Vertical lines on bars represent S.E. (n=7). Bars followed by dissimilar letters are significant at $P \leq 0.05$ for each genotype.
FIGURE 4.17: Interactive effect of genotype x inoculation x environment on shoot N-fixed in seven soybean genotypes planted at two locations in Mozambique: A) Shoot N-fixed (g/plant) and B) Shoot N-fixed (kg/ha). Vertical lines on bars represent S.E. (n=7). Bars followed by dissimilar letters are significant at $P \leq 0.05$ for each genotype.
4.3 Year 2: Pot experiment

4.3.1 Plant growth and nodulation

A 2-Way ANOVA analysis of data on dry matter yield and nodulation of seven soybean genotypes grown in potted soil in South Africa and inoculated with *Bradyrhizobium japonicum* strain WB74 showed marked differences and genotypic effects. Nodule number and nodule mass, as well as root and shoot biomass and whole-plant dry matter were significantly greater with *Bradyrhizobium* inoculation compared to uninoculated soybean plants (Table 4.9). Although TGx1910-14F exhibited higher nodule number and mass than the other genotypes, TGx1844-4E emerged as the genotype with the biggest growth because of its increased shoot and root biomass (Table 4.9). The genotype x inoculation interaction was significant ($P \leq 0.05$) for nodule number, nodule mass, as well as for shoot and whole-plant dry matter yield (Table 4.9). In all instances, inoculation significantly increased nodule number, nodule mass, shoot biomass, and whole-plant dry matter over uninoculated controls (Fig. 4.18 A, B, C and D).

4.3.2 Nitrogen concentration and content

Similar analysis, using 2-Way ANOVA, revealed marked increases in N concentration and N content of nodules, roots, shoots and whole plants with *Bradyrhizobium* inoculation (Table 4.10 and 4.11). Although shoot N concentrations were similar in the
seven genotypes, the differences in root and nodule %N resulted in significantly 
\( P \leq 0.001 \) higher N concentration in whole-plants of TGx1910-14F, and lower %N in 
TGx1937-1F and Ocepera (Table 4.10). Similarly, because of the greater root N 
content of Solitaire and TGx1844-4E, the two genotypes showed the highest N 
content at whole-plant level (Table 4.11). The genotype x inoculation interaction was 
significant \( P \leq 0.05 \) for %N in shoots, roots, nodules and whole plants (Table 4.10), 
as well as for N content of shoots, roots and whole plants (Table 4.11). One-way 
analysis of this interaction showed that inoculation consistently increased %N in 
nodules, roots, shoots and whole plants over their un-inoculated counterparts (Fig. 
3.19 A, B, C and D). As a result, N content of roots shoots and whole plants were also 
much higher in inoculated relative to un-inoculated soybean genotypes (Fig. 3.20 A, B 
and C).

4.3.3 \( \delta^{15}\text{N} \) and %Ndfa values

A 2-way ANOVA analysis indicated significantly \( P \leq 0.001 \) lower \( \delta^{15}\text{N} \) values for 
shoots, roots, nodules and whole plants of inoculated compared to uninoculated 
soybean (Table 4.12). As a result, the %Ndfa values of these components were 
markedly higher in inoculated than un-inoculated controls (Table 4.12). Of the seven 
soybean genotypes, TGx1908-8F showed very low \( \delta^{15}\text{N} \) values for shoots and roots, 
and the lowest for whole plants, followed by TGx1910-14F (Table 4.12). The 
genotype x inoculation interaction was significant \( P \leq 0.001 \) for \( \delta^{15}\text{N} \) of shoots, roots, 
nodules and whole plants, as well as for %Ndfa of shoots, roots and whole plants
(Table 4.12). Analysis of these interactions showed consistently lower $\delta^{15}$N values for nodules, roots, shoots and whole plants of inoculated soybean relative to un-inoculated controls (Fig. 3.21 A, B, C and D). As a result, the %Ndfa values of organs and whole plants were also generally higher for the inoculated treatments, except for those of roots, shoots and whole plants of TGx1937-1F, 427/5/7 and TGx1937-1F respectively (Fig. 3.22 A, B and C).

4.3.4 N-fixed

Analysis of N-fixed (using 2-WayANOVA) further confirmed the superior symbiotic performance of inoculated compared to un-inoculated soybean plants. As shown in Table 3.13, plant inoculation increased the amounts of N-fixed in shoots and roots, leading to much greater symbiotic N yield whole plants (Table 4.13). Independent of inoculation, the genotypes also differed in their levels of N contribution with TGx1908-8F and TGx1910-14F showing higher content of N-fixed in shoots and roots compared to the other genotypes. As a result, the symbiotic N yield was much higher in the two genotypes at whole-plant level, followed by TGx1937-1F and TGx1844-4E, and least in Ocepera (Table 4.13). The genotype x inoculation interaction was significant ($P \leq 0.001$) for only N-fixed in roots. As shown in Fig. 4.22 D, the amount of N-fixed in roots increased significantly in inoculated plants compared with their uninoculated counterparts.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Nodule number</th>
<th>Nodules</th>
<th>Roots</th>
<th>Shoots</th>
<th>Whole plant</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inoculation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ <em>Bradyrhizobium</em></td>
<td>110.5±17.7a</td>
<td>2.4±0.1a</td>
<td>10.4±0.2a</td>
<td>22.6±0.3a</td>
<td>35.4±0.4a</td>
</tr>
<tr>
<td>- <em>Bradyrhizobium</em></td>
<td>47.1±5.6b</td>
<td>1.9±0.1b</td>
<td>9.4±0.1b</td>
<td>14.2±0.6b</td>
<td>25.5±0.6b</td>
</tr>
<tr>
<td><strong>Genotypes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TGx 1910-14F</td>
<td>143.3±27.7a</td>
<td>2.4±0.2a</td>
<td>9.3±0.2c</td>
<td>17.4±1.6b</td>
<td>29.0±1.8b</td>
</tr>
<tr>
<td>427/5/7</td>
<td>93.0±8.7bc</td>
<td>2.2±0.2ab</td>
<td>9.5±0.3bc</td>
<td>17.6±2.3b</td>
<td>29.4±2.7b</td>
</tr>
<tr>
<td>TGx 1937-1F</td>
<td>50.5±4.6cd</td>
<td>2.1±0.2ab</td>
<td>9.1±0.3c</td>
<td>18.7±2.7b</td>
<td>30.0±3.1b</td>
</tr>
<tr>
<td>TGx 1844-4E</td>
<td>48.8±10.1cd</td>
<td>2.0±0.1b</td>
<td>10.3±0.4ab</td>
<td>21.8±1.0a</td>
<td>34.0±1.4a</td>
</tr>
<tr>
<td>TGx 1908-8F</td>
<td>35.0±9.1d</td>
<td>2.2±0.1ab</td>
<td>10.6±0.2a</td>
<td>17.6±1.9b</td>
<td>30.4±2.0b</td>
</tr>
<tr>
<td>Ocepera</td>
<td>46.0±7.9d</td>
<td>2.0±0.1b</td>
<td>9.6±0.3bc</td>
<td>17.5±2.0b</td>
<td>29.1±2.3b</td>
</tr>
<tr>
<td>Solitaire</td>
<td>135.3±51.5ab</td>
<td>2.4±0.2a</td>
<td>10.7±0.6a</td>
<td>17.9±2.1b</td>
<td>31.0±2.8b</td>
</tr>
</tbody>
</table>

2-WAY ANOVA (F-statistics)

<table>
<thead>
<tr>
<th>Factor</th>
<th>F-statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculation</td>
<td>31.6***</td>
</tr>
<tr>
<td>Genotype</td>
<td>9.2***</td>
</tr>
<tr>
<td>Genotype x Inoculation</td>
<td>4.2***</td>
</tr>
</tbody>
</table>

Values (Means±SE) with dissimilar letters in a column are significant at ** $P \leq 0.01$, *** $P \leq 0.001$
FIGURE 4.18: Interactive effect of genotype x inoculation on nodulation and dry matter yield of seven soybean genotypes grown in pot experiments in 2008, in South Africa: A) Nodule number, B) Nodule biomass, C) Shoot dry matter, and D) Whole-plant biomass. Vertical lines on bars represent S.E. (n=7). Bars followed by dissimilar letters are significant at $P \leq 0.05$ for each genotype.
**TABLE 4.10:** Effect of bradyrhizobial inoculation on %N of seven soybean genotypes planted in pot experiments in South Africa, in the 2008/2009 cropping season.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>%N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nodules</td>
</tr>
<tr>
<td><strong>Inoculation</strong></td>
<td></td>
</tr>
<tr>
<td>+ <em>Bradyrhizobium</em></td>
<td>4.1±0.2a</td>
</tr>
<tr>
<td>- <em>Bradyrhizobium</em></td>
<td>2.8±0.1b</td>
</tr>
<tr>
<td><strong>Genotypes</strong></td>
<td></td>
</tr>
<tr>
<td>TGx 1910-14F</td>
<td>4.4±0.5a</td>
</tr>
<tr>
<td>427/5/7</td>
<td>3.4±0.3b</td>
</tr>
<tr>
<td>TGx 1937-1F</td>
<td>2.9±0.1c</td>
</tr>
<tr>
<td>TGx 1844-4E</td>
<td>3.4±0.3bc</td>
</tr>
<tr>
<td>TGx 1908-8F</td>
<td>3.4±0.5bc</td>
</tr>
<tr>
<td>Ocepera</td>
<td>3.3±0.3bc</td>
</tr>
<tr>
<td>Solitaire</td>
<td>3.5±0.3b</td>
</tr>
</tbody>
</table>

**2-WAYS ANOVA (F-statistics)**

<table>
<thead>
<tr>
<th>Source</th>
<th>F-statistic</th>
<th>df</th>
<th>P-value</th>
</tr>
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<tbody>
<tr>
<td>Inoculation</td>
<td>92.100***</td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>Genotype</td>
<td>6.684***</td>
<td></td>
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<tr>
<td>Genotype x Inoculation</td>
<td>3.114**</td>
<td></td>
<td>**</td>
</tr>
</tbody>
</table>

Values (Means±SE) with dissimilar letters in a column are significant at ** *P*≤0.01, *** *P*≤0.001 and ns= not significant.
FIGURE 4.19: Interactive effect of genotype x inoculation on %N of seven soybean genotypes grown in pot experiments in 2008, in South Africa: A) Nodule %N, B) Root %N, C) Shoot %N, and D) Whole-plant %N. Vertical lines on bars represent S.E. (n=7). Bars followed by dissimilar letters are significant at $P \leq 0.05$ for each genotype.
TABLE 4.11: Effect of bradyrhizobial inoculation on N content of seven soybean genotypes planted in pot experiments in South Africa, in the 2008/2009 cropping season.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N content (g/plant)</th>
<th>Nodules</th>
<th>Roots</th>
<th>Shoots</th>
<th>Whole plant</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inoculation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ Bradyrhizobium</td>
<td>0.10±0.01a</td>
<td>0.16±0.01a</td>
<td>0.69±0.02a</td>
<td>0.95±0.03a</td>
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</tr>
<tr>
<td>- Bradyrhizobium</td>
<td>0.05±0.00b</td>
<td>0.10±0.01b</td>
<td>0.26±0.02b</td>
<td>0.42±0.02b</td>
<td></td>
</tr>
<tr>
<td><strong>Genotypes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TGx 1910-14F</td>
<td>0.11±0.02a</td>
<td>0.13±0.01bcd</td>
<td>0.47±0.10a</td>
<td>0.70±0.12b</td>
<td></td>
</tr>
<tr>
<td>427/5/7</td>
<td>0.08±0.01bc</td>
<td>0.12±0.02cd</td>
<td>0.51±0.13a</td>
<td>0.71±0.16b</td>
<td></td>
</tr>
<tr>
<td>TGx 1937-1F</td>
<td>0.06±0.01c</td>
<td>0.11±0.01cd</td>
<td>0.46±0.11a</td>
<td>0.63±0.12b</td>
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</tr>
<tr>
<td>TGx 1844-4E</td>
<td>0.07±0.01bc</td>
<td>0.15±0.02ab</td>
<td>0.50±0.05a</td>
<td>0.71±0.08b</td>
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</tr>
<tr>
<td>TGx 1908-8F</td>
<td>0.07±0.01bc</td>
<td>0.13±0.01b</td>
<td>0.51±0.11a</td>
<td>0.72±0.13ab</td>
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</tr>
<tr>
<td>Ocepera</td>
<td>0.07±0.01b</td>
<td>0.10±0.02d</td>
<td>0.42±0.09a</td>
<td>0.59±0.11b</td>
<td></td>
</tr>
<tr>
<td>Solitaire</td>
<td>0.09±0.01b</td>
<td>0.16±0.01a</td>
<td>0.48±0.12a</td>
<td>0.73±0.14a</td>
<td></td>
</tr>
</tbody>
</table>

2-WAYS ANOVA (F-statistics)

<table>
<thead>
<tr>
<th></th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculation</td>
<td>97.624***</td>
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</tr>
<tr>
<td>Genotype</td>
<td>6.116***</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Genotype x Inoculation</td>
<td>1.517ns</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td></td>
<td>113.481***</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>334.847***</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>450.643***</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>7.334***</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>1.048ns</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td></td>
<td>2.460**</td>
<td>&lt;0.01</td>
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<tr>
<td></td>
<td>3.520*</td>
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<tr>
<td></td>
<td>2.735**</td>
<td>&lt;0.01</td>
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</table>

Values (Means±SE) with dissimilar letters in a column are significant at *P≤0.05, ** P≤0.01, *** P≤0.001 and ns= not significant.
FIGURE 4.20: Interactive effect of genotype x inoculation on N content of soybean grown in pot experiments in 2008, in South Africa: A) Root N, B) Shoot N and C) Whole-plant N. Vertical lines on bars represent S.E. (n=7). Bars followed by dissimilar letters are significant at $P \leq 0.05$ for each genotype.
**TABLE 4.12**: Effect of bradyrhizobial inoculation on $\delta^{15}$N and %Ndfa of seven soybean genotypes planted in pot experiments in South Africa, in the 2008/2009 cropping season.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Nodules</th>
<th>Roots</th>
<th>Shoots</th>
<th>Whole plant</th>
<th>Roots</th>
<th>Shoots</th>
<th>Whole plant</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inoculation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ <em>Bradyrhizobium</em></td>
<td>6.2±0.3b</td>
<td>1.3±0.1b</td>
<td>1.9±0.3b</td>
<td>2.3±0.2b</td>
<td>66.4±1.7a</td>
<td>67.0±3.0a</td>
<td>67.1±2.7a</td>
</tr>
<tr>
<td>- <em>Bradyrhizobium</em></td>
<td>9.0±0.3a</td>
<td>2.1±0.2a</td>
<td>3.0±0.3a</td>
<td>3.5±0.2a</td>
<td>56.1±2.2b</td>
<td>55.1±3.9b</td>
<td>49.1±3.4b</td>
</tr>
<tr>
<td><strong>Genotypes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TGx 1910-14F</td>
<td>8.8±0.6a</td>
<td>0.8±0.0e</td>
<td>1.1±0.1d</td>
<td>2.2±0.2d</td>
<td>74.0±0.2a</td>
<td>76.4±1.5b</td>
<td>68.3±3.0c</td>
</tr>
<tr>
<td>427/5/7</td>
<td>6.9±1.3d</td>
<td>1.6±0.1d</td>
<td>4.0±0.2a</td>
<td>3.9±0.3a</td>
<td>62.8±1.3c</td>
<td>44.1±2.4d</td>
<td>43.9±4.2d</td>
</tr>
<tr>
<td>TGx 1937-1F</td>
<td>5.3±0.6d</td>
<td>2.6±0.2a</td>
<td>1.1±0.1d</td>
<td>1.8±0.2d</td>
<td>47.9±3.0e</td>
<td>76.4±1.2bc</td>
<td>73.4±3.2b</td>
</tr>
<tr>
<td>TGx 1844-4E</td>
<td>8.6±0.6b</td>
<td>1.7±0.3d</td>
<td>2.6±0.6c</td>
<td>3.0±0.6c</td>
<td>61.8±3.7d</td>
<td>59.4±6.8c</td>
<td>56.9±8.0c</td>
</tr>
<tr>
<td>TGx 1908-8F</td>
<td>8.5±0.5b</td>
<td>1.1±0.2e</td>
<td>0.9±0.1d</td>
<td>1.8±0.2d</td>
<td>69.2±3.0b</td>
<td>78.3±0.7a</td>
<td>74.3±2.6a</td>
</tr>
<tr>
<td>Ocepera</td>
<td>7.4±0.4cd</td>
<td>2.0±0.3b</td>
<td>3.6±0.4bc</td>
<td>3.8±0.4c</td>
<td>56.5±4.0e</td>
<td>47.5±4.3d</td>
<td>45.3±5.6d</td>
</tr>
<tr>
<td>Solitaire</td>
<td>7.8±0.5c</td>
<td>2.0±0.2c</td>
<td>3.8±0.3b</td>
<td>3.8±0.2b</td>
<td>56.8±2.6d</td>
<td>45.4±3.1d</td>
<td>44.7±3.1d</td>
</tr>
</tbody>
</table>

**2-WAY ANOVA (F-statistics)**

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>$\delta^{15}$N (‰)</th>
<th>%Ndfa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculation</td>
<td>331.84*** 116.69*** 201.20*** 291.93***</td>
<td>116.70*** 201.20*** 291.93***</td>
</tr>
<tr>
<td>Genotype</td>
<td>37.89*** 46.52*** 203.63*** 97.95***</td>
<td>46.52*** 203.63*** 97.95***</td>
</tr>
<tr>
<td>Genotype x Inoculation</td>
<td>11.32*** 5.20*** 19.62*** 10.33***</td>
<td>5.20*** 19.63*** 10.33***</td>
</tr>
</tbody>
</table>

Values (Means±SE) with dissimilar letters in a column are significant at *** $P<0.001$. 
FIGURE 4.21: Interactive effect of genotype x inoculation on $\delta^{15}$N of seven soybean genotypes grown in pot experiments in 2008, in South Africa: A) Nodule $\delta^{15}$N, B) Root $\delta^{15}$N, C) Shoot $\delta^{15}$N and D) Whole-plant $\delta^{15}$N. Vertical lines on bars represent S.E. (n=7). Bars followed by dissimilar letters are significant at $P \leq 0.05$ for each genotype.
FIGURE 4.22: Interactive effect of genotype x inoculation on %Ndfa and fixed-N of seven soybean genotypes grown in pot experiments in 2008, in South Africa: A) Root %Ndfa, B) Shoot %Ndfa, C) Whole-plant %Ndfa and D) Root fixed-N. Vertical lines on bars represent S.E. (n=7). Bars followed by dissimilar letters are significant at $P \leq 0.05$ for each genotype.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fixed-N (g/plant)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Roots</td>
<td>Shoots</td>
</tr>
<tr>
<td>Inoculation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ Bradyrhizobium</td>
<td>0.11±0.02a</td>
<td>0.46±0.02a</td>
</tr>
<tr>
<td>- Bradyrhizobium</td>
<td>0.06±0.01b</td>
<td>0.15±0.01b</td>
</tr>
<tr>
<td>Genotypes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TGx 1910-14F</td>
<td>0.10±0.01a</td>
<td>0.36±0.08ab</td>
</tr>
<tr>
<td>427/5/7</td>
<td>0.08±0.01c</td>
<td>0.24±0.07c</td>
</tr>
<tr>
<td>TGx 1937-1F</td>
<td>0.05±0.01c</td>
<td>0.36±0.09ab</td>
</tr>
<tr>
<td>TGx 1844-4E</td>
<td>0.09±0.02b</td>
<td>0.31±0.06b</td>
</tr>
<tr>
<td>TGx 1908-8F</td>
<td>0.09±0.01ab</td>
<td>0.40±0.09a</td>
</tr>
<tr>
<td>Ocepera</td>
<td>0.06±0.01c</td>
<td>0.22±0.06c</td>
</tr>
<tr>
<td>Solitaire</td>
<td>0.09±0.01c</td>
<td>0.23±0.07c</td>
</tr>
</tbody>
</table>

2-WAY ANOVA (F-statistics)

<table>
<thead>
<tr>
<th>Source</th>
<th>F-statistics</th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculation</td>
<td>175.768***</td>
<td>358.904***</td>
<td>531.410***</td>
</tr>
<tr>
<td>Genotype</td>
<td>12.593***</td>
<td>11.351***</td>
<td>14.811***</td>
</tr>
<tr>
<td>Genotype x Inoculation</td>
<td>3.997***</td>
<td>1.040ns</td>
<td>0.927ns</td>
</tr>
</tbody>
</table>

Values (Means±SE) with dissimilar letters in a column are significant at *** $P\leq0.001$ and ns= not significant.
4.4 Year 1: Nampula

4.4.1 Carbon concentration, C content and $\delta^{13}C$

A 2-Way ANOVA analysis of C concentration, C content and $\delta^{13}C$ revealed marked increases in %C of roots, shoots and whole plants of soybean inoculated with *Bradyrhizobium japonicum* strain WB74 (Table 4.14). As a result, the C content of those organs and whole plants also rose with inoculation of soybean plants (Table 4.14). Independent of inoculation, the seven soybean genotypes exhibited marked differences in C concentration and content. Because TGx1910-14F showed the highest C concentration of shoots, it also recorded the highest C accumulation in shoots, as well as at whole-plant level, followed by Solitaire (Table 4.14). The genotype x inoculation interaction was significant ($P\leq0.05$) for %C of roots and whole plants, as well as for C content of roots and shoots. Analysis of these interactions revealed a consistently greater C concentration and C accumulation in roots and whole plants of inoculated soybean (Fig. 4.23 A, B, C and D).

The $\delta^{13}C$ values were also significantly greater (i.e. less negative) in roots, shoots and whole plants of inoculated soybean (Table 4.15). Solitaire had the highest (or less negative) $\delta^{13}C$ value, followed by Ocepera and TGx1908-8F (Table 4.15). Root and whole-plant $\delta^{13}C$ values were however not different for all seven soybean genotypes (Table 4.15).
TABLE 4.14: Effect of bradyrhizobial inoculation on %C and C content of field-grown soybean genotypes planted at Nampula, Mozambique, in the 2007/2008 cropping season.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>%C</th>
<th>C content (g/plant)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Root</td>
<td>shoots</td>
<td>Whole-</td>
<td>Roots</td>
</tr>
<tr>
<td><strong>Inoculation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ <em>Bradyrhizobium</em></td>
<td>41.5±0.6a</td>
<td>44.6±0.7a</td>
<td>43.0±0.3a</td>
<td>7.4±0.2a</td>
<td>15.0±0.4a</td>
</tr>
<tr>
<td>- <em>Bradyrhizobium</em></td>
<td>36.3±0.8b</td>
<td>39.5±0.5b</td>
<td>37.9±0.5b</td>
<td>4.5±0.2b</td>
<td>9.6±0.4b</td>
</tr>
<tr>
<td><strong>Genotypes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TGx 1910-14F</td>
<td>36.7±0.9b</td>
<td>45.6±1.0a</td>
<td>41.1±1.0a</td>
<td>5.4±0.6bcd</td>
<td>14.9±0.9a</td>
</tr>
<tr>
<td>427/5/7</td>
<td>39.4±1.0b</td>
<td>42.6±1.5bc</td>
<td>41.0±1.2a</td>
<td>5.9±0.6bc</td>
<td>13.1±1.5bc</td>
</tr>
<tr>
<td>TGx 1937-1F</td>
<td>35.9±2.8b</td>
<td>42.0±0.7c</td>
<td>39.0±2.0a</td>
<td>5.4±0.8cd</td>
<td>12.0±1.4cd</td>
</tr>
<tr>
<td>TGx 1844-4E</td>
<td>40.3±1.7b</td>
<td>40.0±1.1c</td>
<td>40.2±1.7a</td>
<td>6.9±0.5a</td>
<td>10.6±1.8</td>
</tr>
<tr>
<td>TGx 1908-8F</td>
<td>40.1±1.2b</td>
<td>42.0±1.6c</td>
<td>41.1±0.9a</td>
<td>6.9±0.5a</td>
<td>11.4±1.1de</td>
</tr>
<tr>
<td>Ocepera</td>
<td>39.4±1.4b</td>
<td>40.1±1.7c</td>
<td>39.8±0.9a</td>
<td>5.1±0.9d</td>
<td>10.7±1.0de</td>
</tr>
<tr>
<td>Solitaire</td>
<td>40.5±1.5a</td>
<td>42.0±1.8c</td>
<td>41.2±1.0a</td>
<td>6.4±0.8b</td>
<td>13.6±1.2b</td>
</tr>
<tr>
<td><strong>2-WAY ANOVA (F-statistics)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inoculation</td>
<td>51.60***</td>
<td>59.84***</td>
<td>138.44***</td>
<td>287.45***</td>
<td>273.51***</td>
</tr>
<tr>
<td>Genotype</td>
<td>3.76**</td>
<td>4.65**</td>
<td>2.31ns</td>
<td>11.30***</td>
<td>13.96***</td>
</tr>
<tr>
<td>Genotype x Inoculation</td>
<td>3.41*</td>
<td>2.27ns</td>
<td>2.81*</td>
<td>2.59*</td>
<td>2.93*</td>
</tr>
</tbody>
</table>

Values (Means±SE) with dissimilar letters in a column are significant at * $P\leq0.05$, ** $P\leq0.01$, *** $P\leq0.001$ and ns= not significant.
FIGURE 4.23: Interactive genotype x inoculation effect on roots and whole-plant %C and roots and shoots C content of soybean genotypes planted in the field in Mozambique: A) Root %C, B) Whole-plant %C, C) Root C, and D) Shoots C. Vertical lines on bars represent S.E. (n=7). Bars followed by dissimilar letters are significant at $p \leq 0.05$ for each genotype. Vertical lines on bars represent S.E. (n=7). Bars followed by dissimilar letters are significant at $P \leq 0.05$ for each genotype.
**TABLE 4.15**: Effect of bradyrhizobial inoculation on δ^{13}C of field-grown soybean genotypes planted at Nampula, Mozambique, in the 2007/2008 cropping season.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>δ^{13}C (%)</th>
<th>Root</th>
<th>Shoot</th>
<th>Whole-plant</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inoculation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ <em>Bradyrhizobium</em></td>
<td>-27.7±0.2a</td>
<td>-28.2±0.1a</td>
<td>-28.0±0.1a</td>
<td></td>
</tr>
<tr>
<td>- <em>Bradyrhizobium</em></td>
<td>-28.0±0.1b</td>
<td>-28.5±0.1b</td>
<td>-28.2±0.1b</td>
<td></td>
</tr>
<tr>
<td><strong>Genotypes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TGx 1910-14F</td>
<td>-27.9±0.1a</td>
<td>-28.5±0.1bc</td>
<td>-28.2±0.1a</td>
<td></td>
</tr>
<tr>
<td>427/5/7</td>
<td>-28.0±0.1a</td>
<td>-28.3±0.2bc</td>
<td>-28.1±0.1a</td>
<td></td>
</tr>
<tr>
<td>TGx 1937-1F</td>
<td>-27.7±0.2a</td>
<td>-28.5±0.1bc</td>
<td>-28.1±0.1a</td>
<td></td>
</tr>
<tr>
<td>TGx 1844-4E</td>
<td>-27.7±0.1a</td>
<td>-28.8±0.1c</td>
<td>-28.2±0.1a</td>
<td></td>
</tr>
<tr>
<td>TGx 1908-8F</td>
<td>-27.8±0.1a</td>
<td>-28.2±0.2ab</td>
<td>-28.0±0.1a</td>
<td></td>
</tr>
<tr>
<td>Ocepera</td>
<td>-27.9±0.2a</td>
<td>-28.2±0.1ab</td>
<td>-28.0±0.1a</td>
<td></td>
</tr>
<tr>
<td>Solitaire</td>
<td>-27.8±0.1a</td>
<td>-28.0±0.1a</td>
<td>-27.9±0.1a</td>
<td></td>
</tr>
</tbody>
</table>

**2-WAY ANOVA (F-statistics)**

<table>
<thead>
<tr>
<th></th>
<th>F-statistic</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculation</td>
<td>5.36*</td>
<td>5.62*</td>
<td>10.37**</td>
<td></td>
</tr>
<tr>
<td>Genotype</td>
<td>0.81&lt;sub&gt;ns&lt;/sub&gt;</td>
<td>4.39**</td>
<td>1.74&lt;sub&gt;ns&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>Genotype x Inoculation</td>
<td>0.38&lt;sub&gt;ns&lt;/sub&gt;</td>
<td>1.23&lt;sub&gt;ns&lt;/sub&gt;</td>
<td>1.13&lt;sub&gt;ns&lt;/sub&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Values (Means±SE) with dissimilar letters in a column are significant at * P≤0.05, ** P≤0.01, and ns= not significant
4.5 Year 2: Ruace and Mutequelesse interaction

4.5.1 Carbon concentration, C content and δ\textsuperscript{13}C

A 3-Way ANOVA analysis of seven soybean genotypes planted at Ruace and Mutequelesse and inoculated with and without *Bradyrhizobium japonicum* WB74 revealed significant differences in location and inoculation effects. Soybean plants showed significantly \((P\leq0.001)\) greater C concentration in shoots, grain and whole plants at Ruace when compared to Mutequelesse. As a result, the C content of shoots, grain and whole plants of soybean was markedly higher at Ruace relative to Mutequelesse (Table 4.16). Inoculation with *Bradyrhizobium* increased both C concentration and accumulation in shoots, grain and whole plants of soybean (Table 4.16). Independent of location and inoculation, the seven soybeans differed in grain %C, but similar in %C of shoots and whole soybean plants (Table 4.16). The inoculation x environment interaction was significant \((P\leq0.01)\) for %C of shoots and whole plants. Genotype x environment interaction was also significant \((P\leq0.001)\) for %C of shoots, grain and whole plants, just as genotype x inoculation was significant \((P\leq0.05)\) for %C of shoots (Table 4.16). One-way ANOVA analysis of these interactions revealed consistently higher C concentration of shoots and whole plants of inoculated and uninoculated soybean grown at Ruace compared to same treatments at Mutequelesse (Fig. 4.24 A and B). Analysis of genotype x environment interaction also indicated markedly elevated C concentration and C accumulation in shoots, grain and whole plants of all seven soybean genotypes grown at Ruace
compared to those at Mutequelesse (Fig. 4.25 A, B, C, D, E, and F). The data further indicated greater C concentration in shoots of all soybean genotypes inoculated with *Bradyrhizobium*, except for 427/5/7 and Ocepera (Fig. 3.26A).

A 3-Way ANOVA analysis showed that carbon to nitrogen ratio (C/N ratio) values were consistently greater for shoots, grain and whole plants at Ruace compared to Mutequelesse. However, the C/N-fixed values were lower for shoots, grain and whole plants at Ruace relative to Mutequelesse (Table 4.17). Shoot and whole-plant C/N ratios were similar for the seven soybean genotypes. Except for Ocepera, shoot, grain and whole-plant C/N-fixed values were also similar for the seven soybean genotypes (Table 4.17). The data however showed significantly ($P \leq 0.001$) higher C/N ratios in shoots and whole plants of the seven soybean genotypes grown at Ruace (Fig. 4.28 A and B). Inoculation x environment interaction was significant ($P \leq 0.05$) for shoot C/N ratio, as well as the C/N-fixed values of shoots, grains and whole plants (Fig. 4.27 A, B, C and D). Also, genotype x environment interaction was significant ($P \leq 0.001$) for C/N ratios of shoots and whole plants, and C/N-fixed values of shoots, grain and whole plants (Fig. 4.28 A, B, C, D and E). Furthermore, genotype x inoculation interaction was significant ($P \leq 0.05$) for the C/N ratio of whole plants and C/N-fixed values of shoots and grain. However, whole-plant C/N ratios, as well as the C/N-fixed values of shoots and grain were lower in inoculated plants relative to their uninoculated counterparts (Fig. 4.29 A, B, and C). Analysis of the genotype x inoculation x environment interaction also showed that the C/N ratio of grain was generally greater in uninoculated soybean at Ruace, followed by inoculated plants at
Ruace, and least, in the inoculated and uninoculated soybean at Mutequelesse (Fig. 4.30A). The data further revealed much higher C/N-fixed values for grain and whole plants of soybean grown at Mutequelesse without inoculation, followed by those with inoculation at Mutequelesse, then uninoculated at Ruace and lowest for those inoculated at Ruace (Fig. 4.30 B and C).

A 3-Way analysis showed significant differences in location and inoculation effects (Table 4.18). The δ¹³C of shoots and whole plants were much higher (i.e. less negative) at Mutequelesse, while for δ¹³C of grain was higher at Ruace (Table 4.18). With inoculation of soybean, however, the δ¹³C of shoots, grain and whole plants were all significantly higher (i.e. less negative) than uninoculated controls (Table 4.18). But there were no differences in δ¹³C between the seven soybean genotypes. The inoculation x environment interaction was significant for δ¹³C of grain and whole plants. Analysis of these interactions of those interactions revealed much more negative δ¹³C values of inoculated and uninoculated plants at Ruace compared to Mutequelesse (Fig. 4.31A). The δ¹³C of grain was less negative with inoculation at Ruace than Mutequelesse and more negative without inoculation at Ruace compared to Mutequelesse (Fig. 4.31).
TABLE 4.16: Effect of bradyrhizobial inoculation on %C and C content of seven field grown soybean genotypes planted at Ruace and Mutequelesse in Mozambique.

<table>
<thead>
<tr>
<th>Location</th>
<th>%C Shoots</th>
<th>%C Grain</th>
<th>%C Whole-plant</th>
<th>C content (g/plant) Shoots</th>
<th>C content (g/plant) Grain</th>
<th>C content (g/plant) Whole-plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ruace</td>
<td>48.4±0.4a</td>
<td>51.7±0.2a</td>
<td>50.1±0.3a</td>
<td>9.7±0.5a</td>
<td>6.8±0.5a</td>
<td>16.6±0.8a</td>
</tr>
<tr>
<td>Mutequelesse</td>
<td>27.8±0.4b</td>
<td>35.5±0.1b</td>
<td>31.6±0.2b</td>
<td>4.9±0.3b</td>
<td>3.5±0.2b</td>
<td>8.3±0.4b</td>
</tr>
<tr>
<td><strong>Inoculation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ Bradyrhizobium</td>
<td>39.3±1.8a</td>
<td>44.0±1.3a</td>
<td>41.6±1.5a</td>
<td>8.5±0.6a</td>
<td>5.6±0.5a</td>
<td>14.1±1.0a</td>
</tr>
<tr>
<td>- Bradyrhizobium</td>
<td>36.9±1.5b</td>
<td>43.2±1.3b</td>
<td>40.0±1.4b</td>
<td>6.1±0.4b</td>
<td>4.6±0.4b</td>
<td>10.8±0.8b</td>
</tr>
<tr>
<td><strong>Genotypes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TGx 1910-14F</td>
<td>38.3±3.3a</td>
<td>43.3±2.3c</td>
<td>40.8±2.8a</td>
<td>7.4±1.1ab</td>
<td>4.1±0.6c</td>
<td>11.5±1.6a</td>
</tr>
<tr>
<td>427/5/7</td>
<td>37.7±3.5a</td>
<td>43.4±2.3c</td>
<td>40.6±2.9a</td>
<td>6.9±1.0b</td>
<td>6.5±1.1ab</td>
<td>13.3±2.0a</td>
</tr>
<tr>
<td>TGx 1937-1F</td>
<td>38.2±2.5a</td>
<td>43.0±2.3c</td>
<td>40.6±2.4a</td>
<td>9.0±1.5a</td>
<td>5.1±1.0abc</td>
<td>14.2±2.2a</td>
</tr>
<tr>
<td>TGx 1844-4E</td>
<td>37.8±2.9a</td>
<td>43.6±2.4bc</td>
<td>40.7±2.6a</td>
<td>7.6±0.9ab</td>
<td>3.4±0.4c</td>
<td>11.2±1.1a</td>
</tr>
<tr>
<td>TGx 1908-8F</td>
<td>38.9±3.3a</td>
<td>43.4±2.4c</td>
<td>41.1±2.8a</td>
<td>6.8±0.5b</td>
<td>4.7±0.3bc</td>
<td>11.5±0.6a</td>
</tr>
<tr>
<td>Ocepera</td>
<td>37.5±3.5a</td>
<td>44.3±2.7a</td>
<td>40.9±3.1a</td>
<td>5.8±0.6b</td>
<td>5.5±0.9abc</td>
<td>11.3±1.3a</td>
</tr>
<tr>
<td>Solitaire</td>
<td>38.2±3.3a</td>
<td>44.1±2.7ab</td>
<td>41.2±3.0a</td>
<td>7.7±1.5ab</td>
<td>6.5±1.0a</td>
<td>14.2±2.5a</td>
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</tbody>
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**3-WAY ANOVA (F-statistics)**

<table>
<thead>
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<th>Source</th>
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<tbody>
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<td>Location</td>
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<tr>
<td>Inoculation</td>
<td>32.9***</td>
</tr>
<tr>
<td>Genotype</td>
<td>0.7*ns</td>
</tr>
<tr>
<td>Inoculation x Env.</td>
<td>8.4**</td>
</tr>
<tr>
<td>Genotype x Env.</td>
<td>6.0***</td>
</tr>
<tr>
<td>Genotype x inoc</td>
<td>2.2*</td>
</tr>
<tr>
<td>Genotype x inoc x Env</td>
<td>0.3*ns</td>
</tr>
</tbody>
</table>

Values (Means±SE) with dissimilar letters in the same column are significant at *P≤0.05, **P≤0.01, ***P≤0.001 and ns= not significant.
**FIGURE 4.24**: Interactive effect of inoculation x environment on %C in shoots and Whole-plants of field-grown soybean genotypes planted at Mozambique: A) Shoot %C, and B) Whole-plant %C. Vertical lines on bars represent S.E. (n=7). Bars followed by dissimilar letters are significant at $P\leq0.05$ for each *Bradyrhizobium* level.
FIGURE 4.25: Interactive effect of genotype x environment on %C and C content in shoots, grain and Whole-plants of field-grown soybean genotypes planted at Mozambique: A) Shoot %C, B) Grain %C, C) Whole-plant %C, D) Shoot C content, E) Grain C content and F) Whole-plant C content. Vertical lines on bars represent S.E. (n=7). Bars followed by dissimilar letters are significant at $P \leq 0.05$ for each genotype.
FIGURE 4.26: Interactive effect of genotype x inoculation on %C in shoots of field-grown soybean genotypes planted at Mozambique: A) Shoot %C. Vertical lines on bars represent S.E. (n=7). Bars followed by dissimilar letters are significant at $P \leq 0.05$ for each genotype.
TABLE 4.17: Effect of bradyrhizobial inoculation on C/N ratio and C/N-fixed ratio of seven field-grown soybean genotypes planted at Ruace and Mutequelesse in Mozambique.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>C/N ratio</th>
<th>C/N-fixed ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoots</td>
<td>Grain</td>
</tr>
<tr>
<td>Location</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ruace</td>
<td>12.5±0.6a</td>
<td>11.3±0.3a</td>
</tr>
<tr>
<td>Mutequelesse</td>
<td>7.2±0.3b</td>
<td>7.8±0.2b</td>
</tr>
<tr>
<td>Inoculation</td>
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<tr>
<td>Bradyrhizobium</td>
<td>10.4±0.8a</td>
<td>8.5±0.3b</td>
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<td>- Bradyrhizobium</td>
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<td>10.6±0.4a</td>
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<tr>
<td>Genotypes</td>
<td></td>
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</tr>
<tr>
<td>TGx 1910-14F</td>
<td>8.1±0.7b</td>
<td>9.9±0.8a</td>
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<td>427/5/7</td>
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<tr>
<td>TGx 1937-1F</td>
<td>9.3±0.7b</td>
<td>9.1±0.7a</td>
</tr>
<tr>
<td>TGx 1844-4E</td>
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<td>9.8±0.7a</td>
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<tr>
<td>TGx 1908-8F</td>
<td>9.6±1.1b</td>
<td>9.3±0.8a</td>
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<tr>
<td>Ocepera</td>
<td>12.1±1.3a</td>
<td>9.4±0.6a</td>
</tr>
<tr>
<td>Solitaire</td>
<td>12.2±2.0a</td>
<td>10.3±0.9a</td>
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3-WAY ANOVA (F-statistics)

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<td>2.79*</td>
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<td>Location</td>
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<td>6.11***</td>
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<td>Genotype x inoculation</td>
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<td>5.71***</td>
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</table>

Values (Means±SE) with dissimilar letters in the same column are significant at *P≤0.05, **P≤0.01, *** P≤0.001 and ns= not significant.
FIGURE 4.27: Interactive effect of inoculation x environment on C/N ratio and C/N-fixed ratio in shoots, grain and Whole-plants of field-grown soybean genotypes planted at Mozambique: A) Shoot C/N ratio, and B) Shoot C/N-fixed ratio, C) Grain C/N-fixed ratio and D) Whole-plant C/N-fixed ratio. Vertical lines on bars represent S.E. (n=7). Bars followed by dissimilar letters are significant at $P \leq 0.05$ for each genotype.
FIGURE 4.28: Interactive effect of genotype x environment on shoots and whole-plant C/N ratio and C/N-fixed ratio of shoots, grain and whole-plants of field-grown soybean genotypes planted at Mozambique: A) Shoot C/N ratio, B) Whole-plant C/N ratio, C) Shoot C/N-fixed ratio, D) Grain C/N-fixed ratio and E) Whole-plant C/N-fixed ratio. Vertical lines on bars represent S.E. (n=7). Bars followed by dissimilar letters are significant at $P \leq 0.05$ for each genotype.
**FIGURE 4.29**: Interactive effect of genotype x inoculation on whole-plant C/N ratio and C/N-fixed ratio of shoots and grain of field-grown soybean genotypes planted at Mozambique: A) Whole-plant C/N ratio, B) Shoot C/N ratio and C) Grain C/N ratio. Vertical lines on bars represent S.E. (n=7). Bars followed by dissimilar letters are significant at $P \leq 0.05$ for each genotype.
FIGURE 4.30: Interactive effect of genotype x inoculation x environment on grain C/N ratio and C/N-fixed ratio of grain and whole-plant of field-grown soybean genotypes planted at Mozambique: A) Grain C/N ratio, B) Grain C/N-fixed ratio and C) Whole-plant C/N-fixed ratio. Vertical lines on bars represent S.E. (n=7). Bars followed by dissimilar letters are significant at $P \leq 0.05$ for each genotype.
**TABLE 4.18**: Effect of bradyrhizobial inoculation on $\delta^{13}$C of seven field grown soybean genotypes planted at Ruace and Mutequelesse in Mozambique.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Shoots</th>
<th>Grain</th>
<th>Whole-plant</th>
</tr>
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<td>-26.2±0.1b</td>
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<tr>
<td>Mutequelesse</td>
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<td>-26.3±0.1b</td>
<td>-25.9±0.1a</td>
</tr>
<tr>
<td><strong>Inoculation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ <em>Bradyrhizobium</em></td>
<td>-25.9±0.1a</td>
<td>-26.0±0.1a</td>
<td>-26.0±0.1a</td>
</tr>
<tr>
<td>- <em>Bradyrhizobium</em></td>
<td>-26.1±0.1b</td>
<td>-26.3±0.1b</td>
<td>-26.2±0.1b</td>
</tr>
<tr>
<td><strong>Genotypes</strong></td>
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<tr>
<td>TGx 1910-14F</td>
<td>-26.1±0.2a</td>
<td>-26.1±0.1a</td>
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<tr>
<td>427/5/7</td>
<td>-26.0±0.1a</td>
<td>-26.2±0.2a</td>
<td>-26.1±0.1a</td>
</tr>
<tr>
<td>TGx 1937-1F</td>
<td>-26.1±0.2a</td>
<td>-26.2±0.1a</td>
<td>-26.1±0.1a</td>
</tr>
<tr>
<td>TGx 1844-4E</td>
<td>-26.1±0.1a</td>
<td>-26.0±0.1a</td>
<td>-26.0±0.1a</td>
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<tr>
<td>TGx 1908-8F</td>
<td>-26.1±0.2a</td>
<td>-26.3±0.1a</td>
<td>-26.2±0.1a</td>
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<tr>
<td>Ocepera</td>
<td>-26.0±0.1a</td>
<td>-26.1±0.1a</td>
<td>-26.1±0.1a</td>
</tr>
<tr>
<td>Solitaire</td>
<td>-25.9±0.1a</td>
<td>-26.2±0.1a</td>
<td>-26.0±0.1a</td>
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**3-WAY ANOVA (F-statistics)**

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<tr>
<th>Source of Variability</th>
<th>F-statistic</th>
<th>df</th>
<th>P-value</th>
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<td>1</td>
<td>0.0001</td>
</tr>
<tr>
<td>Inoculation</td>
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<td>0.01</td>
</tr>
<tr>
<td>Genotype</td>
<td>0.9 ns</td>
<td>1</td>
<td>0.36</td>
</tr>
<tr>
<td>Inoculation x Environment</td>
<td>0.8 ns</td>
<td>1</td>
<td>0.36</td>
</tr>
<tr>
<td>Genotype x environment</td>
<td>1.0 ns</td>
<td>1</td>
<td>0.36</td>
</tr>
<tr>
<td>Genotype x inoculation</td>
<td>0.4 ns</td>
<td>1</td>
<td>0.36</td>
</tr>
<tr>
<td>Genotype x inoc x environ</td>
<td>0.5 ns</td>
<td>1</td>
<td>0.36</td>
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</tbody>
</table>

Values (Means±SE) with dissimilar letters in the same column are significant at **$P \leq 0.01$, ***$P \leq 0.001$ and ns= not significant.
FIGURE 4.31: Interactive effect of inoculation x environment on δ\textsuperscript{13}C of grain and whole-plants of soybean genotypes planted in the field in Mozambique. A) Grain δ\textsuperscript{13}C and B) Whole-plant δ\textsuperscript{13}C. Horizontal lines on bars represent S.E. (n=7). Bars followed by dissimilar letters are significant at P≤0.05 for each genotype.
4.6 Year 2: Pot experiment

4.6.1 Carbon concentration, C content and δ^{13}C

A 2-Way ANOVA analysis of data from pot experiments showed significantly ($P\leq0.001$) higher C concentration in nodules, roots, shoots and whole soybean plants inoculated with *Bradyrhizobium japonicum* strain WB74. Uninoculated control plants consistently exhibited low C concentration in organs and whole plants (Table 4.19). As a result of these differences in C concentration of inoculated plants, the C content of nodules, roots, shoots and whole plants were also much greater. In general, TGx1844-4E and Solitaire showed the highest C concentration in plant organs and at whole-plant level (Table 4.19). As a result, they also had the highest C content of organs and in whole plants (Table 4.19). The genotype x inoculation interaction was significant ($P\leq0.05$) for C concentration and content in nodules, roots and whole plants (Table 4.19). A 1-Way ANOVA analysis of the interaction revealed consistently significant ($P\leq0.05$) C concentration in nodules, roots and whole plants with bradyrhizobial inoculation (Fig. 4.32 A, B, and C). Similarly, C accumulation was always greater in inoculated than uninoculated controls (Fig. 4.29 D, and E).

A 2-Way ANOVA analysis of C/N ratios also showed significant ($P\leq0.001$) inoculation effects (Table 4.20). The C/N ratios of roots, shoots and whole plants were much lower in inoculated compared to uninoculated plants (Table 4.20). Of the seven soybean genotypes, Ocepera exhibited the highest C/N ratio at whole-plant level and
TGx1910-14F the lowest due to the greater C/N values of nodules, roots, and shoots (Table 4.20).

δ¹³C Values of nodules, roots, shoots and whole plants were significantly ($P \leq 0.001$) higher (i.e. less negative) in inoculated soybean plants relative to their uninoculated counterparts, which were consistently more negative (Table 4.20). Independent of inoculation, the seven soybean genotypes also showed differences in δ¹³C of organs. Solitaire for example, showed the highest δ¹³C values (less negative) for nodules, roots and shoots, and therefore had the highest δ¹³C at whole-plant level. In contrast, TGx1937-1F, which exhibited the lowest δ¹³C values (i.e. more negative) for nodules, roots and shoots, also recorded the lowest δ¹³C value at whole-plant level (Table 4.20).

Genotype x inoculation interaction was significant ($P \leq 0.05$) for the C/N ratio of roots and shoots, as well as for δ¹³C of nodules, shoots and whole plants (Table 4.20). Analysis revealed consistently lower C/N ratios of shoots and roots of inoculated compared to uninoculated soybean plants except for TGx1910-14F and TGx1908-8F, which had similar C/N ratios for inoculated and uninoculated treatments (Fig. 4.33 A and B). A 1-Way ANOVA analysis also showed that the δ¹³C of nodules, shoots and whole plants was always higher (i.e. less negative ) in inoculated than uninoculated plants of each genotype, except for TGx1937-1F, where the values were either similar or lower with *Bradyrhizobium* inoculation (Fig. 4.34 A and B).
### TABLE 4.19: Effect of bradyrhizobial inoculation on C concentration and content of seven soybean genotypes planted in potted soil in South Africa.

<table>
<thead>
<tr>
<th>Inoculation</th>
<th>C concentration</th>
<th>C content</th>
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<tbody>
<tr>
<td></td>
<td>Nodules</td>
<td>Roots</td>
</tr>
<tr>
<td>+ Bradyrhizobium</td>
<td>40.3±1.3a</td>
<td>38.1±0.7a</td>
</tr>
<tr>
<td>- Bradyrhizobium</td>
<td>28.8±1.2b</td>
<td>30.2±0.9b</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Nodules</th>
<th>Roots</th>
<th>Shoots</th>
<th>Whole-plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGx 1910-14F</td>
<td>32.3±2.6cde</td>
<td>37.9±2.2b</td>
<td>41.1±1.5c</td>
<td>55.6±3.0b</td>
</tr>
<tr>
<td>427/5/7</td>
<td>28.9±2.7e</td>
<td>33.5±1.4c</td>
<td>42.4±1.4bc</td>
<td>52.4±2.5c</td>
</tr>
<tr>
<td>TGx 1937-1F</td>
<td>35.7±2.8bc</td>
<td>33.1±2.1d</td>
<td>42.1±1.5bc</td>
<td>55.5±2.9bc</td>
</tr>
<tr>
<td>TGx 1844-4E</td>
<td>31.2±1.2de</td>
<td>38.1±1.2a</td>
<td>47.7±2.2a</td>
<td>58.5±2.1b</td>
</tr>
<tr>
<td>TGx 1908-8F</td>
<td>34.4±5.7cd</td>
<td>27.8±2.2d</td>
<td>47.0±2.2a</td>
<td>54.6±4.9c</td>
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<tr>
<td>Ocepera</td>
<td>41.1±2.9a</td>
<td>36.0±1.4b</td>
<td>45.0±1.0bc</td>
<td>61.1±2.3a</td>
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<tr>
<td>Solitaire</td>
<td>38.6±2.4ab</td>
<td>32.8±2.4d</td>
<td>47.6±2.0a</td>
<td>59.5±3.1a</td>
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<th>Genotype</th>
<th>Genotype x Inoculation</th>
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</tr>
<tr>
<td></td>
<td>337.5***</td>
<td>39.0***</td>
<td>3.7**</td>
</tr>
<tr>
<td></td>
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<td>7.1***</td>
<td>1.3* ns</td>
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<tr>
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<td>5.5***</td>
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<tr>
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<td>6.4***</td>
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<td>1.4* ns</td>
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<tr>
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<td>710.2***</td>
<td>10.0***</td>
<td>3.7**</td>
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</table>

Values (Means±SE) with dissimilar letters in the same column are significant at * $P\leq0.05$, ** $P\leq0.01$, *** $P\leq0.001$ and ns= not significant.
FIGURE 4.32: Interactive effect of genotype x inoculation on %C and C content in nodules, roots and whole-plants of seven soybean genotypes planted in potted soil in South Africa: A) Nodule %C, B) Root %C, C) Whole-plant %C, D) Nodule C content and E) Whole-plant C content. Vertical lines on bars represent S.E. (n=7). Bars followed by dissimilar letters are significant at $P \leq 0.05$ for each genotype.
TABLE 4.20: Effect of bradyrhizobial inoculation on C/N ratio and $\delta^{13}C$ of seven soybean genotypes planted in potted soil in South Africa.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>C/N ratios</th>
<th>$\delta^{13}C$ (%)</th>
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</thead>
<tbody>
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<td>Roots</td>
</tr>
<tr>
<td>Inoculation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ Bradyrhizobium</td>
<td>10.2±0.5a</td>
<td>25.4±0.8b</td>
</tr>
<tr>
<td>- Bradyrhizobium</td>
<td>10.4±0.5a</td>
<td>30.2±2.1a</td>
</tr>
<tr>
<td>Genotypes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TGx 1910-14F</td>
<td>7.5±0.5d</td>
<td>27.4±0.7bc</td>
</tr>
<tr>
<td>427/5/7</td>
<td>8.7±0.6d</td>
<td>27.0±1.8c</td>
</tr>
<tr>
<td>TGx 1937-1F</td>
<td>12.4±0.5ab</td>
<td>28.4±2.0b</td>
</tr>
<tr>
<td>TGx 1844-4E</td>
<td>9.4±0.7cd</td>
<td>29.6±3.4b</td>
</tr>
<tr>
<td>TGx 1908-8F</td>
<td>10.1±0.2c</td>
<td>22.2±0.9c</td>
</tr>
<tr>
<td>Ocepera</td>
<td>12.9±0.9a</td>
<td>37.7±4.7a</td>
</tr>
<tr>
<td>Solitaire</td>
<td>11.3±0.5bc</td>
<td>22.2±1.5c</td>
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</tbody>
</table>

2-WAY ANOVA (F-stats)

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<th>Roots</th>
<th>Shoots</th>
<th>Whole-plant</th>
<th>Nodules</th>
<th>Roots</th>
<th>Shoots</th>
<th>Whole-plant</th>
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</thead>
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<td>53.9***</td>
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<td>59.6***</td>
<td>113.3***</td>
<td>142.5***</td>
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<tr>
<td>Genotype</td>
<td>11.6***</td>
<td>10.4***</td>
<td>2.8*</td>
<td>11.4***</td>
<td>19.2***</td>
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<td>Genotype x Inoculation</td>
<td>1.7ns</td>
<td>6.9***</td>
<td>2.5*</td>
<td>2.0ns</td>
<td>3.6**</td>
<td>2.2ns</td>
<td>6.5***</td>
<td>3.0*</td>
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</tbody>
</table>

Values (Means±SE) with dissimilar letters in the same column are significant at * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$ and ns= not significant.
FIGURE 4.33: Interactive effect of genotype x inoculation on C/N ratio of roots and shoots of seven soybean genotypes planted in potted soil in South Africa: A) Root C/N ratio, and B) Shoot C/N ratio. Vertical lines on bars represent S.E. (n=7). Bars followed by dissimilar letters are significant at $P \leq 0.05$ for each genotype.
FIGURE 4.34: Interactive effect of genotype x inoculation on $\delta^{13}\text{C}$ of nodules, shoots and whole-plants of seven soybean genotypes planted in potted soil in South Africa: A) Nodule $\delta^{13}\text{C}$, B) Shoot $\delta^{13}\text{C}$, and C) Whole-plant $\delta^{13}\text{C}$. Horizontal lines on bars represent S.E. (n=7). Bars followed by dissimilar letters are significant at $P \leq 0.05$ for each genotype.
4.7 Xylem sap analysis for δD, δ¹⁸O and δ¹³C

4.7.1 Nampula

A 2-Way ANOVA analysis of xylem sap revealed an effect of inoculation on δD and δ¹³C values. Inoculated soybean plants showed a significantly (P≤0.01) more negative δ²D value of xylem sap compared to uninoculated controls (Table 4.21). However, the opposite was obtained for δ¹³C, in that bradyrhizobial inoculation yielded significantly (P≤0.05) less negative δ¹³C values (Table 4.21). But the δ¹⁸O of xylem sap was unaffected by inoculation. Of the three soybean genotypes that produced adequate xylem sap for isotopic analysis, TGx1937-1F had a higher δD (less negative) of xylem sap, followed by TGx1844-4E, with TGx1910-14F exhibiting the lowest (more negative) δD value (Table 4.21). The highest (less negative) δ¹³C was also found in the xylem sap of TGx1937-1F, followed by TGx1910-14F and TGx1844-4E, which showed the lowest (more negative) δ¹³C value of xylem sap (Table 4.21). However, the δ¹⁸O values of xylem sap were similar in all three soybean genotypes.

The genotype x inoculation interaction was significant (P≤0.05) for δD and δ¹³C, but not δ¹⁸O. Analysis of this interaction revealed significantly (P≤0.05) more negative δD values for the xylem sap of inoculated plants of TGx1910-14F and TGx1844-4E. But similar δD values were obtained for both inoculated and uninoculated plants of TGx1937-1F (Fig. 35A). The data also showed that xylem δ¹³C of inoculated TGx1910-14F and TGx1937-1F was much lower than that of uninoculated controls;
the xylem $\delta^{13}$C values of TGx1844-4E were similar for both inoculated and uninoculated plants (Fig. 4.35B).

**TABLE 4.21**: Effect of bradyrhizobial inoculation on $\delta$D, $\delta^{18}$O and $\delta^{13}$C in field-grown soybean genotypes planted at Nampula, Mozambique in the 2007/2008 cropping season.

<table>
<thead>
<tr>
<th>Inoculation</th>
<th>$\delta$D ($\pm$SE)</th>
<th>$\delta^{18}$O ($\pm$SE)</th>
<th>$\delta^{13}$C ($\pm$SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ Bradyrhizobium</td>
<td>-8.4±0.3b</td>
<td>-5.1±0.1a</td>
<td>6.5±0.1b</td>
</tr>
<tr>
<td>- Bradyrhizobium</td>
<td>-7.6±0.1a</td>
<td>-5.0±0.2a</td>
<td>6.6±0.1a</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>$\delta$D ($\pm$SE)</th>
<th>$\delta^{18}$O ($\pm$SE)</th>
<th>$\delta^{13}$C ($\pm$SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGx 1910-14F</td>
<td>-8.4±0.3b</td>
<td>-5.3±0.1a</td>
<td>6.5±0.1b</td>
</tr>
<tr>
<td>TGx 1937-1F</td>
<td>-7.6±0.1a</td>
<td>-5.1±0.2a</td>
<td>6.4±0.1b</td>
</tr>
<tr>
<td>TGx 1844-4E</td>
<td>-8.0±0.3ab</td>
<td>-4.8±0.2a</td>
<td>6.6±0.1a</td>
</tr>
</tbody>
</table>

2-WAY ANOVA (F-statistics)

<table>
<thead>
<tr>
<th>Source</th>
<th>F-value</th>
<th>P-value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculation</td>
<td>15.2**</td>
<td>0.2ns</td>
<td>6.1*</td>
</tr>
<tr>
<td>Genotype</td>
<td>5.0*</td>
<td>3.0ns</td>
<td>5.5*</td>
</tr>
<tr>
<td>Genotype x Inoculation</td>
<td>5.6*</td>
<td>1.1ns</td>
<td>5.2*</td>
</tr>
</tbody>
</table>

Values (Means±SE) in a column are significant at *$P$≤0.05, **$P$≤0.01 and ns = not significant.
**FIGURE 4.35**: Interactive effect of genotype x inoculation on δD and δ^{13}C in xylem sap collected from seven soybean genotypes planted at Nampula: A) δD and B) δ^{13}C. Vertical and Horizontal lines on bars represent S.E. (n=7). Bars followed by dissimilar letters are significant at $P \leq 0.05$ for each genotype.
4.8 Xylem sap analysis for δD, δ¹⁸O and δ¹³C

4.8.1 Ruace

A 2-Way ANOVA analysis of xylem sap collected from inoculated and uninoculated plants of seven soybean genotypes showed no inoculation effect on δD, δ¹⁸O or δ¹³C (Table 4.19). The genotypes also had similar δ¹³C values, which was independent of *Bradyrhizobium* inoculation. There were however marked differences in δD and δ¹⁸O values between and among the soybean genotypes. For example, TGx1937-1F showed the highest (less negative) δD value, followed by Ocepera and TGx1844-4E, while TGx1908-8F exhibited the lowest (more negative) δD value (Table 4.19). Similarly, TGx1937-1F recorded the highest (less negative) δ¹⁸O value, followed by Ocepera, while Solitaire showed the lowest (more negative) δ¹⁸O value (Table 4.22). But the δ¹³C values were similar for all seven soybean genotypes.

The genotype x inoculation interaction was significant ($P \leq 0.05$) for δD and δ¹⁸O, but not δ¹³C of xylem sap (Table 4.22). A 1-Way ANOVA analysis of the interaction revealed significantly ($P \leq 0.001$) lower (more negative) δD values of xylem sap collected from inoculated plants of TGx1910-14F, TGx1908-8F and Ocepera, while with 427/5/7, TGx1937-1F, TGx1844-4E and Solitaire, it was the uninoculated plants that showed markedly lower (more negative) δD values of xylem sap (Fig. 4.36A). With δ¹⁸O, however, inoculated TGx1910-14F, Ocepera and Solitaire, showed significantly ($P \leq 0.01$) much lower (more negative) values compared to uninoculated controls. The reverse situation was obtained with 427/5/7, TGx1844-4E and
TGx1908-8F, which showed very low (more negative) $\delta^{18}O$ values of xylem sap from uninoculated soybean plants (Fig. 4.33B). But the $\delta^{18}O$ of xylem sap from both inoculated and uninoculated plants of TGx1937-1F were similar (Fig. 4.36B).

**TABLE 4.22**: Effect of bradyrhizobial inoculation on $\delta D$, $\delta^{18}O$ and $\delta^{13}C$ in field-grown soybean genotypes planted at Ruace, Mozambique in the 2007/2008 cropping season.

<table>
<thead>
<tr>
<th>Inoculation</th>
<th>$\delta D$</th>
<th>$\delta^{18}O$</th>
<th>$\delta^{13}C$</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ Bradyrhizobium</td>
<td>-16.3±1.79a</td>
<td>-2.7±0.13a</td>
<td>6.6±0.03a</td>
</tr>
<tr>
<td>- Bradyrhizobium</td>
<td>-15.3±1.02a</td>
<td>-2.4±0.18a</td>
<td>6.6±0.03a</td>
</tr>
</tbody>
</table>

**Genotypes**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>$\delta D$</th>
<th>$\delta^{18}O$</th>
<th>$\delta^{13}C$</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGx 1910-14F</td>
<td>-14.9±1.65c</td>
<td>-2.4±0.31bc</td>
<td>6.6±0.07a</td>
</tr>
<tr>
<td>427/5/7</td>
<td>-16.0±0.90c</td>
<td>-2.4±0.22bc</td>
<td>6.6±0.03a</td>
</tr>
<tr>
<td>TGx 1937-1F</td>
<td>-11.0±1.43a</td>
<td>-2.0±0.22a</td>
<td>6.7±0.09a</td>
</tr>
<tr>
<td>TGx 1844-4E</td>
<td>-13.2±2.50b</td>
<td>-3.0±0.32bc</td>
<td>6.6±0.05a</td>
</tr>
<tr>
<td>TGx 1908-8F</td>
<td>-23.6±4.15d</td>
<td>-2.5±0.22bc</td>
<td>6.5±0.05a</td>
</tr>
<tr>
<td>Ocepera</td>
<td>-13.3±2.73b</td>
<td>-2.4±0.36ab</td>
<td>6.6±0.05a</td>
</tr>
<tr>
<td>Solitaire</td>
<td>-18.5±1.57d</td>
<td>-3.2±0.21c</td>
<td>6.5±0.06a</td>
</tr>
</tbody>
</table>

2-WAY ANOVA (F-statistics)

<table>
<thead>
<tr>
<th>Source</th>
<th>$F$-value</th>
<th>Significant?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculation</td>
<td>1.7ns</td>
<td></td>
</tr>
<tr>
<td>Genotype</td>
<td>18.8***</td>
<td>**</td>
</tr>
<tr>
<td>Genotype x Inoculation</td>
<td>30.1***</td>
<td>***</td>
</tr>
</tbody>
</table>

Values (Means±SE) in a column are significant at *$P \leq 0.05$, **$P \leq 0.01$and ns = not significant.
FIGURE 4.36: Interactive effect of genotype x inoculation on δD and δ^{18}O in xylem sap collected from seven soybean genotypes planted at Ruace: A) δD and B) δ^{18}O. Horizontal lines on bars represent S.E. (n=7). Bars followed by dissimilar letters are significant at P≤0.05 for each genotype.
4.9 Xylem sap analysis for δD, δ^{18}O and δ^{13}C.

4.9.1 Ruace and Nampula comparison

A 3-Way ANOVA analysis of xylem sap collected from the seven soybean genotypes planted at both Ruace and Nampula and inoculated with and without *Bradyrhizobium japonicum* strain WB74 revealed significant (P≤0.001) differences in δD, δ^{18}O and δ^{13}C values with regards to location and inoculation. There were significantly (P≤0.001) much lower δD (more negative) values obtained at Ruace relative to Nampula, and higher δ^{18}O (less negative) values at Ruace compared to Nampula (Table 4.23). Inoculating soybean with *Bradyrhizobium* resulted in greater δD (less negative) values of xylem sap relative to uninoculated controls. The seven genotypes also differed significantly (P≤0.001) in δD, but were similar in δ^{18}O and δ^{13}C values (Table 4.23).

The inoculation x environment was significant (P≤0.05) for δD and δ^{13}C; genotype x environment interaction was significant (P≤0.05) for δD and δ^{18}O, while genotype x inoculation interaction and genotype x inoculation x environment interaction were each significant (P≤0.001) for only δD. The inoculation x environment interaction revealed greater δD values (less negative) at Nampula than Ruace, and higher δ^{13}C values at Ruace than Nampula (Fig. 4.36). All the soybean genotypes showed consistently higher δD values at Nampula relative to Ruace (Fig. 4.37). Except for TGx1910-14F, the other two genotypes showed greater δD values of xylem sap from uninoculated plants (Fig. 4.38). The genotype x inoculation x environment interaction,
revealed greater δD values following inoculation and uninoculation of soybean genotypes grown at Nampula when compared to those with and without inoculation at Ruace (Fig. 4.39).

**TABLE 4.23**: Effect of bradyrhizobial inoculation on δD, δ18O and δ13C in field-grown soybean genotypes planted at Ruace, Mozambique in the 2007/2008 cropping season.

<table>
<thead>
<tr>
<th>Location</th>
<th>δD</th>
<th>δ18O</th>
<th>δ13C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ruace</td>
<td>-13.0±1.1b</td>
<td>-2.5±0.2a</td>
<td>6.6±0.1a</td>
</tr>
<tr>
<td>Nampula</td>
<td>-8.0±0.2a</td>
<td>-5.1±0.1b</td>
<td>6.5±0.1a</td>
</tr>
</tbody>
</table>

**Inoculation**

+ *Bradyrhizobium*
- *Bradyrhizobium*

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>δD</th>
<th>δ18O</th>
<th>δ13C</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGx 1910-14F</td>
<td>-11.6±1.3c</td>
<td>-3.8±0.5a</td>
<td>6.6±0.1a</td>
</tr>
<tr>
<td>TGx 1937-1F</td>
<td>-9.3±0.9a</td>
<td>-3.6±0.5a</td>
<td>6.6±0.1a</td>
</tr>
<tr>
<td>TGx 1844-4E</td>
<td>-10.6±1.4b</td>
<td>-3.9±0.3a</td>
<td>6.6±0.1a</td>
</tr>
</tbody>
</table>

**3-WAY ANOVA (F-statistics)**

<table>
<thead>
<tr>
<th>Source</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location</td>
<td>918.41***</td>
<td>3.04ns</td>
</tr>
<tr>
<td>Inoculation</td>
<td>65.87***</td>
<td>0.06ns</td>
</tr>
<tr>
<td>Genotype</td>
<td>64.49***</td>
<td>0.08ns</td>
</tr>
<tr>
<td>Inoculation x Environment</td>
<td>155.80***</td>
<td>4.43*</td>
</tr>
<tr>
<td>Genotype x environment</td>
<td>30.70***</td>
<td>2.21ns</td>
</tr>
<tr>
<td>Genotype x inoculation</td>
<td>304.09***</td>
<td>1.66ns</td>
</tr>
<tr>
<td>Genotype x inoc x environ</td>
<td>243.71***</td>
<td>0.22ns</td>
</tr>
</tbody>
</table>

Values (Means±SE) in a column are significant at *P* ≤ 0.05, **P* ≤ 0.01, ***P* ≤ 0.001 and ns = not significant.
FIGURE 4.37: Interactive effect of inoculation x environment on $\delta D$ and $\delta^{13}C$ in xylem sap collected from three soybean genotypes planted at Ruace and Nampula: A) $\delta D$ and B) $\delta^{13}C$. Vertical and horizontal lines on bars represent S.E. (n=7). Bars followed by dissimilar letters are significant at $P \leq 0.05$ for each genotype.
FIGURE 4.38: Interactive effect of genotype x environment on δD in xylem sap collected from three soybean genotypes planted at Ruace and Nampula. Horizontal lines on bars represent S.E. (n=7). Bars followed by dissimilar letters are significant at $P \leq 0.05$ for each genotype.
**FIGURE 4.39**: Interactive effect of genotype x inoculation on δD of xylem sap collected from three soybean genotypes. Horizontal lines on bars represent S.E. (n=7). Bars followed by dissimilar letters are significant at $P \leq 0.05$ for each genotype.

**FIGURE 4.40**: Interactive effect of genotype x inoculation x environment on δD in xylem sap collected from three soybean genotypes planted at Ruace and Nampula: A)  δD. Horizontal lines on bars represent S.E. (n=7). Bars followed by dissimilar letters are significant at $P \leq 0.05$ for each genotype.
CHAPTER 5

DISCUSSION

5.1 Inoculation and location response

The study evaluated the effect of inoculation on four promiscuous-nodulating soybean genotypes and three commercial varieties (non-promiscuous) with *Bradyrhizobium japonicum* strain WB74 on plant growth, symbiotic N nutrition, C accumulation and xylem sap isotope composition in a pot experiment in South Africa, and at three field sites in Mozambique. Whether in the field or pot experiments, the results consistently showed increased plant growth (measured as biomass) and N\textsubscript{2} fixation (measured as $\delta^{15}$N, %Nd\textsubscript{fa} and N-fixed) with bradyrhizobial inoculation. Because inoculation resulted in significantly lower $\delta^{15}$N values for all seven soybean genotypes, the %Nd\textsubscript{fa} and actual amounts of N-fixed were also greater in inoculated plants grown at Nampula, Ruace and Mutequelesse. This positive symbiotic response to inoculation of promiscuous-nodulating soybean has been observed by Pule-Meulenburg *et al.* (2010). Similar findings have also been obtained by Koutroubas *et al.* (1998) and Osunde *et al.* (2003). However, where the inoculant strain was outcompeted by native rhizobia, there was no inoculation effect (Okogun & Sanginga, 2003).

Independent of inoculation, the seven genotypes showed marked differences in symbiotic performance. At Nampula, genotypes TGx1910-14F, TGx1844-8F and Solitaire showed superior symbiotic performance compared to the rest, whiles at
Ruace and Mutequelesse, TGx1937-1F, Solitaire and 427/5/7 emerged as the genotypes with superior symbiosis. These genotypic differences in symbiotic performance have been reported for a range of legumes including cowpea (Belane & Dakora, 2010; Makoi, Chimphango & Dakora, 2009). Interestingly, TGx1908-8F, TGx1937-1F and TGx1910-14F showed higher N$_2$ fixation in the pot experiment, a finding similar to that of Nampula.

A 2-Way ANOVA analysis of data collected from sites in 2009 in Mozambique revealed better plant growth and symbiotic performance at Ruace compared to Mutequelesse. This indicates that there was also a location effect, in addition to the inoculation effect. The higher symbiotic fixation at Ruace over Mutequelesse with inoculation clearly indicates the efficiency and/or population size of the introduced inoculant strain compared to indigenous strains of local bradyrhizobia populations at the Ruace site. Abaidoo et al. (2000) observed ineffective nodulation and N$_2$ fixation from indigenous *Bradyrhizobium* spp. isolated from nodules of TGx soybean. The Ruace experimental site had been planted with soybean and prior to that, cowpea, unlike the Mutequelesse site which had no history of crop cultivation. This could have increased the populations of the indigenous rhizobia in the soils of Ruace significant enough to result in more than 50% dependence on symbiotic fixation for N nutrition of the soybean plants without inoculation. Similar crop rotation effects on N$_2$ fixation in soybean (Peoples, Ladha & Herridge, 1995) and other dry land legume crops have also been reported (Herridge & Holland, 1992). Genotypes TGx1937-1F, 427/5/7, TGx1910-14F and Solitaire generally performed better at Ruace and accumulated the
highest biomass and fixed more than 200 kg N/ha in whole plants at Ruace compared to Mutequelesse. The high amounts of N$_2$-fixed at Ruace by both promiscuous and non-promiscuous soybeans genotypes following inoculation therefore gives an indication that the soil N levels at these sites was not sufficient enough for utilization by the plants resulting in higher N$_2$-fixed. This could also give an indication of the symbiotic efficacy of the introduced strain to out compete the indigenous rhizobia at both sites. Earlier studies (Okereke & Eaglesham, 1992; Sanginga, Abaidoo, Dashiell, Carsky & Okogun, 1996; Sanginga, Thottappilly & Dashiell, 2000) have also reported of the response of promiscuous genotypes to inoculation.

5.2 Carbon accumulation, $\delta^{13}$C and photosynthetic N-use efficiency

As with symbiotic N nutrition, photosynthetic C accumulation in soybean plants grown at Nampula, Ruace, Mutequelesse, and in the pot experiment was affected by both inoculation and location. At Nampula, C concentration and accumulation in organs was much greater in inoculated compared to uninoculated plants. A similar result was obtained in the pot experiment in South Africa. As a result, whole-plant C content was significantly higher with bradyrhizobial inoculation of soybean. The C accumulation closely mirrored the dry matter yield of soybean plants inoculated with or without Bradyrhizobium in the field and in pot experiments. This was not surprising as photosynthesis accounts for over 90 % of the biomass in land plants (Taiz & Zeiger, 2002; Zelitch, 1982). The relationship between C accumulation and inoculation is not unexpected as inoculated plants fixed more N and likely synthesized more Rubisco
which would result in higher photosynthetic rates and increased C accumulation. Uninoculated controls, on the other hand, fixed less N and synthesized less Rubisco and chlorophyll (Makoi, Chimphango & Dakora, 2010), which in turn would lead to lower photosynthetic rates and decreased C accumulation. These observations were consistent with Pule-Meulenberg et al. (2010) who also found increased C accumulation in inoculated promiscuous-nodulating soybean plants.

There was also location effect on C accumulation in soybean plants. As shown in Table 4.16, C concentration and accumulation was greater in soybean plants grown at Ruace than Mutequelesse. Again, this was more likely due to the superior symbiotic performance of soybean plants grown at Ruace. Although there were significant differences in organ C concentration and content between and among the soybean genotypes, whole-plant C was unaffected, indicating lack of overall genotypic variation in photosynthetic C nutrition in the seven promiscuous nodulating soybean genotypes. However, with the better plant growth by inoculated soybean grown at Ruace, the C/N ratios of these materials were higher in magnitude relative those of Mutequelesse. However, because the N-fixed by inoculated plants were very high, their C/N ratios were significantly lower than those of uninoculated plants. However, when organ or whole-plant C was expressed per unit N-fixed, the data showed lower C/N-fixed values for inoculated plants grown at Ruace due to high level of N₂ fixation at Ruace. Independent of the parameter measured, the interactive effects of genotype x inoculation, genotype x environment, genotype x inoculation or genotype x inoculation x environment consistently revealed superior inoculation response at all sites. Greater plant growth and development as well as symbiotic
response were observed in both inoculated and uninoculated plants at Ruace compared to Mutequelesse. Both the field studies showed a location effect as well as revealed differences in $\delta^{13}C$ values with inoculation, similar to the pot experiment. In all instances, the inoculated soybean plants consistently showed lower discrimination (or less negative values) indicating greater water-use efficiency, while the uninoculated soybean plants exhibited higher discrimination (i.e. more negative values), which suggests lower water use efficiency. A recent study (Pule-Meulenberg et al., 2010) found *Bradyrhizobium* induced decrease in stomatal conductance in some promiscuous-nodulating soybean genotypes, which led to increased water-use efficiency from reduced water loss by transpiration, and therefore lower $^{13}C$ discrimination. Thus, the inconsistently lower $^{13}C$ discrimination with *Bradyrhizobium* inoculation in this study suggests enhanced water-use efficiency by these nodulated plants. The decrease in stomatal conductance and increase in water-use efficiency from *Bradyrhizobium* application to soybean rhizosphere is now known to be caused by microbial metabolites such as abscisic acid and lumichrome (Matiru & Dakora, 2005). Clearly, these findings reveal a previously unrecognized benefit of inoculation.

In the study at Nampula, a consistently significant accumulation of root and shoot %C among the seven soybean genotypes was recorded. A 1-way analysis revealed a higher %C accumulation in roots of Solitaire following inoculation with *Bradyrhizobium japonicum*. Whole-plant %C was consistently higher for all the genotypes with inoculation indicating efficient photosynthesis relative to uninoculated controls. Consistent significant $\delta^{13}C$ values were also recorded for shoots of the seven
genotypes. According to Farquhar et al. (1989), $\delta^{13}C$ values can be used as an indicator of water use efficiency of C3 plants, where the more negative (higher discrimination) $\delta^{13}C$ values indicate low water use efficiency and less negative (lower discrimination) $\delta^{13}C$ values gives an indication of high water use efficiency. In this regard, Solitaire recorded less $^{13}C$ discrimination (less negative $\delta^{13}C$ value) at Nampula indicating a better water use efficiency relative to the remaining genotypes.

In the Ruace and Mutequelesse location experiment, whole-plants accumulated higher %C values at Ruace relative to lower %C values accumulated by the seven soybean genotypes planted at Mutequelesse. Inoculation and uninoculation at Ruace led to greater %C accumulation compared to Mutequelesse indicating increased C supply from photosynthate at Ruace relative to Mutequelesse. Consistent increase in %C accumulation of all the genotypes was recorded at Ruace relative to Mutequelesse. This resulted in higher biomass accumulation and better plant development at Ruace relative to Mutequelesse. However, higher whole-plants $^{13}C$ discrimination was recorded at Ruace indicating lower water use efficiency of the genotypes compared to lower $^{13}C$ discrimination at Mutequelesse indicating higher water use efficiency of the genotypes (Table 4.17). Water use efficiency is reported to be generally higher in areas where plants experience inadequate water supply for growth and development. Ruace generally records a higher and longer duration of rainfall (2000 to 2500mm annually from November to April) compared to Mutequelesse (<2000mm annually) usually from November to March. The shorter duration of rainfall at Mutequelesse compared to Ruace could explain why the
genotypes at Mutequelesse exhibited higher water use efficiency compared to Ruace. C/N ratio is generally a good measure of plant N nutrition. Where C/N ratio values are smaller, it gives an indication of high plant tissue N, and where the values tends to be higher, plant N content is low. In general, very low C/N ratio values were recorded at both locations with Mutequelesse recording the lowest shoots and whole-plants C/N ratio values. However, inoculation with *Bradyrhizobium* led to very low whole-plants C/N ratio values, with genotype TGx1910-14F recording the lowest. Legumes are reported to have C/N ratio values of < 24, and non legumes >24 (Hobbie et al., 1998), findings consistent with this study. The C/N ratio values at Mutequelesse were generally lower than Ruace but however recorded higher δ¹⁵N values, giving an indication that the plants were utilizing soil N. Interactive genotype x inoculation effect revealed inoculation generally led to decreased δ¹⁵N values and lower C/N ratio values of all seven soybean genotypes with TGx1910-14F recording the lowest. This gives an indication that the plants following inoculation depended on atmospheric fixation for their N nutrition. C/N-fixed ratio values in whole plants were generally higher at Mutequelesse without inoculation, followed by inoculation at Mutequelesse, then uninoculation at Ruace and least of all inoculation at Ruace.

The pot experiment showed very high %C values in both organs and whole plants of the seven soybean genotypes inoculated with *Bradyrhizobium japonicum* relative to uninoculated controls. Inoculation with *Bradyrhizobium japonicum* also resulted in lower ¹³C discrimination (less negative) in nodules, shoots and whole-plants of the seven soybean genotypes compared to uninoculated controls. Inoculation with
Bradyrhizobium japonicum also reduced C/N ratio values in shoots of all the seven soybean genotypes, with genotypes TGx1910-14F and 427/5/7 recording the lowest C/N ratio values.

5.3 Natural isotope composition of xylem sap from soybean

Measurement of stable isotopes of hydrogen and oxygen can provide an indication of the source of water taken up by plants. Water sources such as sub-surface water, soil water and deep groundwater are usually isotopically different, thus offering an opportunity for the use of isotopes to differentiate the sources of water taken up by plants (Dawson, 1996; Flanagan & Ehleringer, 1991). Depending on whether the plant is sourcing water from sub-surface soil using lateral roots, or from deep water table using tap roots, the xylem sap is bound to show different isotopic compositions. Additionally, variation in isotopic composition of xylem sap could also occur due to discrimination at the level of uptake by plant roots and transport in the xylem stream. However, earlier findings (Dawson, 1993; Dawson & Ehleringer, 1991; Thorburn, Hatton & Walker, 1993) have shown that water is not altered isotopically during uptake by roots. Of the seven genotypes tested at Nampula, only three produced adequate xylem sap for isotopic analysis. The data obtained showed more negative δD with bradyrhizobial inoculation, a finding similar to that of Ruace (Tables. 4.21 and 4.22). It is generally argued that differences in δD and δ¹⁸O of plant tissues relate to their rates of transpiration and photosynthesis (Sternberg, Deniro & Johnson, 1986). Thus the variation in δD and δ¹⁸O of xylem sap also probably reflect genotypic
differences in the rates of transpiration, a process that controls the ionic strength of xylem stream. Apparently, tissue water from tissue C₃ plants become more enriched (less negative) in ¹⁸O and δD when they are transpiring at higher rates (Sternberg et al., 1986), implying a stronger pull in the xylem stream. The δD values obtained for soybean sap ranged from -7.6 to -8.4 ‰ at Nampula and -11.0 to -23.6 ‰ at Ruace. These values are within the range found for xylem sap of Banksia and Eucalyptus species (-11 to -21 ‰) in Australia (Dawson & Pate, 1996). That study recorded δD values of -1.0 to -7.5 ‰ for rainwater, -33.0 to -35.0 ‰ for deep groundwater, and -14.0 to -19.0 ‰ for lateral root sap (Dawson & Pate, 1996). Compared to those values, the δD of soybean xylem sap is close to rainwater and lateral root sap, indicating that the soybean plants were obtaining their water from shallow sources supplied by lateral roots in the sub-surface soil.
CHAPTER 6
CONCLUSIONS

From the findings of this study, the promiscuous varieties exhibited a greater response to inoculation with *Bradyrhizobium japonicum* strain WB74 and recorded a higher plant growth and symbiotic performance. This led to higher amounts of N-fixed. These findings agree with Singleton *et al.* (1992) that the notion that it is unnecessary to inoculate promiscuous legumes in tropical soils is flawed. The findings in this study therefore suggest that inoculation of promiscuous soybean is necessary for plant growth and development especially in soils where they are being introduced for the first time or soils without history of soybean cultivation. In locations where resource poor farmers lack inoculants, these genotypes have the potential for cultivation rather than producing the strict nodulating genotypes without inoculation. However, where farmers have access to both seeds of promiscuous genotypes and inoculants, it will be advisable to inoculate to obtain the optimum N$_2$ fixation and nutrition benefits. Also, because the genotypes differed in their symbiotic performance at the different locations, wide spread testing of these genotypes is advisable to ascertain which genotypes are suitable for which location.
REFERENCES


PAUSCH, R. C., CHARLES, L., MULCHI, C. L., LEE, E. H. & MEISINGER, J. J. 1996. Use of \textsuperscript{13}C and \textsuperscript{15}N isotopes to investigate O3 effects on C and N metabolism in


