ISOLATION AND CHARACTERIZATION OF *VIGNA UNGUICULATA* NODULE SYMBIONTS AND EVALUATING COWPEA YIELD IN RESPONSE TO BIOINOCULANTS IN THE KAVANGO REGION.

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Abstract

The Kavango region is extensively involved in agriculture and is also known to be dominated by sandy arenosols. The bad soils in the region, which have poor nutrients and water holding capacity, combined with climate change have contributed to the reduction in yield of most crops grown in the area. One of the aims of this study was to isolate and identify cowpea nodule symbionts from soils in the Kavango Region while the other was to determine cowpeas' response to bio-inoculants by assessing yield of the pulse. Six different cultivars of *Vigna unguiculata* (cowpea) were subjected to 3 different treatments. One with chemical fertilizer, another with two *Bradyrhizobium* spp. bio-inoculants and a third which was a negative control with no treatment. The nodule isolates were grown on Modified Arabinose Gluconate agar and PCR used to amplify the DNA. ITS Sequence analysis of the obtained nodule isolates from the GenBank nucleotide database revealed 5 isolates namely *Bradyrhizobium americanum* strain CMVU44, *Bradyrhizobium vignae* strain 7-2, *Bradyrhizobium kavangense* strain 14-3, *Bradyrhizobium ferriligni* strain CCBAU 51502. The cowpeas that were subjected to the bio-inoculant treatments yielded a lager grain yield in kg per hectare as compared to the negative control and the fertilizer treatments. The outcome of this study therefore provided the local subsistence farmers with a potential cheaper eco-friendly alternative to mineral fertilizers.

Key Words: Arenosols, cultivars, *Vigna unguiculata*, Cowpea, bio-inoculants, *Bradyrhizobium*.

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This paper was presented at the Namibia University of Science and Technology Institutional Research Week.

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List of Abbreviations

DNA	Deoxyribonucleic acid
GC	Guanine- Cytosine
GDP	Gross Domestic Product
ITS	Internal Transcribed Spacer
MAG	Modified Arabinose Gluconate
Nif	Nitrogenase encoding gene
NifD	Gene encoding dinitrogenase alpha subunit
NifH	Gene encoding dinitrogenase reductase
NifK	Gene encoding dinitrogenase beta subunit
PCR	Polymerase Chain Reaction
PGP	Plant Growth Promoting
PVP	Polyvinylpyrrolidone
sp.	Singular form of species
spp.	Plural form of species
v/v	% volume per volume

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Declaration

I, **Chaluma Charlie Luchen**, declare hereby that this study is a true reflection of my own research, and that this work, or part thereof has not been submitted for a degree in any other institution of higher education.

……. Date……

Chaluma Charlie Luchen

Dedication

I dedicate this dissertation to the Luchen family. Mr. Luchen Snr and Mrs. Luchen have been a constant pillar of support during my dissertation and they always gave me a reason to continue and finish my research when I felt like the pressure was too much. This dedication extends to my siblings and extended family at large for the support rendered in every way possible throughout this journey.

CHAPTER 1. Introduction

1.1 Agriculture in the Kavango Region

There is a general notion that the Kavango Region could be the "Breadbasket" of Namibia if the fields in the area were productive (Mendelsohn, 2009). This belief is met with challenges due to the harsh climate and the Kalahari sands (arenosols) that dominate most of the area (Figure 1). *Terminalia sericea* species are dominant in this area and these trees have been known to be an indication of poor sandy soils in an area (Strohbach and Petersen, 2007).

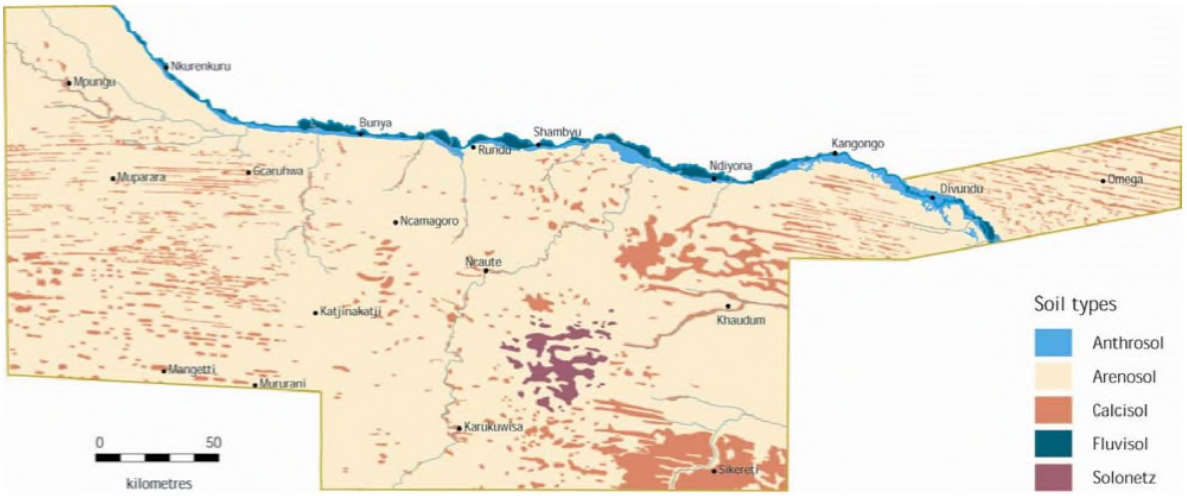


Figure 1. Soil types found in the Okavango region. The figure shows a dominance of Aresonols in the region. Source (Mendelsohn, 2009).

The Kavango region is divided into East and West, with the study site being in the Eastern Region. The Eastern region generally has undulating plains of sands that are unconsolidating and tend to slope down eastwards and towards the Kavango region and

even go down towards the lower parts of the Kavango river that enters Botswana (Ministry Of Lands and Resettlements, 2015).

Small scale subsistence farming or household level crops farming dominates most of the region as farming is the main source of income of the local inhabitants (Mendelsohn, 2009). This mainly relies on rainfall due to the expenses of setting up irrigation facilities. This type of farming is mainly carried out along the rivers or near them for easy irrigation from the river. Most of the farming land averages 1.9 hectares in size with mainly three types of cereals being cultivated (pearl millet, sorghum and maize) while some vegetables are produced solely for domestic consumption (Mendelsohn, 2009).

The constraints that affect agriculture in the Kavango are similar across the entire Northern part of Namibia (Mendelsohn, 2009). They are characterized by erratic rainfalls and nutritionally poor sandy soils with low water retention. Consequently, the poor crop production for domestic consumption for small scale farmers has become endemic (Mendelsohn & Obeid, 2003). Hence the need for new resilient crops that can grow and improve soils in drought prone areas such as Kavango with the goal to increase agricultural productivity.

1.2 Cowpea and importance of bio-inoculation

Research suggests that the world population and its current pace of growth versus the food production balance is increasingly becoming unsustainable. This has led to food crises particularly in semi-arid countries where soils are poor and water resources are scarce. It is of uttermost importance to change this trend to a long term food security. As the world's population is on the verge of a dramatic increase that will threaten food security, there is

an important need of looking for a long-term food security solution by selection of crops that are highly nutritious and are high-yielding (Moray et al. 2014). Therefore, plant breeders and scientists at large are looking for a crop that can be enhanced or is already adapted to the foreseeable biotic and abiotic environmental changes (Moray et al. 2014). With Southern Africa having the highest population of undernourished people in the world (Moray et al. 2014), cowpea, which is one of the major grain legumes in the region (Saima et al. 2014), is favorable to be explored to counter food insecurity.

Vigna unguiculata L. Walp (cowpea) also known as black eye pea, crowder pea and Southern pea belongs to the *Fabaceae* family. It has been documented to be native to Africa where it is grown and consumed in most sub-tropical and tropical parts of Africa, Asia and Latin America (Saima, et al. 2014). Cowpea is the most widely produced pulse grain behind chickpea (*Cicer arietenum*) and the common bean (*Phaseolus vulgaris*). West Africa is the world's largest producer of the pulse accounting for more than 87% of world production as of 2014.

Legumes such as cowpea are known to be raw materials that are important in the balancing of the human diet (Kiim et al. 2018). Its seeds represent a chief source of protein, carbohydrates, vitamins and minerals according to Kiim et al. (2018) and has been used in folk medicine. It is has been suggested that Cowpea can be referred to as a functional food with multiple physiological effects such as the prevention of metabolic diseases like colon cancer and diabetics (Hoover et al. 2010). *Vigna unguiculata* has been reported to have a high amount of organic matter and general multiuse properties hence its use by farmers as fodder to feed their animals (Phiri et al. 2017).

Cowpea is of wide occurrence in traditional crop settings where legumes are deliberately used to manage soil fertility by most small scale subsistence farmers (Kermah et al. 2018). Legumes do this by fixing atmospheric nitrogen and then into ammonia with the help of the nitrogenase enzyme, resulting in increased soil fertility as was demonstrated in Kermah et al. (2018). Nitrogen fixation is an important process for life forms on earth that, fixing about 60% of the world's nitrogen (Kermah et al. 2018.) Therefore, this process is a major source of fixed nitrogen into soils especially in arid environments. Leite et al. (2017) states that nitrogen fixation by rhizobia on soy bean production in Brazil resulted in an estimated save of about US$10 billion annually instead of the use of chemical fertilizer, this was by using *Bradyrhizobium* as a bio-inoculant.

Bio-inoculants are defined as a consortium of microorganisms or individual microbial strains with potential plant growth promotion characteristics (Owen et al. 2015). They are either added as a seed coat when seeding or are added directly to the soils before planting. Owen et al. (2015) states that bio-inoculants have been reported to improve plant characteristics such as an increase in crop yield, plant growth and plant rooting. They possess other secondary properties to the primary properties of increasing soil fertility, such as production of antibiotics and phytohormones which inhibit other microbes that could be pathogenic to the crop (Owen et al. 2015).

1.2 Statement of the Problem

The main constraints to agricultural development in the Kavango regions are attributed to the predominance of sandy poor soils, and low annual rainfall that results in low crop productivity (Gronemeyer et al. 2016). Many soil microbes have been shown to alleviate

drought stress effects and increase yield in plants (Guimaraes et al. 2012). However, in the Kavango, there has not been any survey of soil bacteria with the ability to survive and colonize crop species which are naturally adapted to drought.

1.3 Objectives

The main aim of the study was to determine cowpeas response to bio-inoculants by assessing yield of the pulse.

The specific aims are as follows:

a) To isolate and identify cowpea nodule symbionts from soils in the Kavango region.

b) To investigate cowpea plants response to bio-inoculant and the ability to promote growth and yield under natural conditions.

c) To compare the different cowpea cultivars yield in terms of root,grain and plant biomass

1.4 Significance of the study

The outcome of this study will contribute to formulating a potential cowpea bio-inoculant for the subsistence farmers in the Kavango regions. This bio inoculant could in turn increase crop productivity. In addition to animal husbandry, the increased yield of cowpea can be an alternative source of income to the local communities.

1.5 Limitations of the Study

Sampling will be restricted to the Kavango due to the constriction by the available funds and the time that will be allocated for the study to be carried out. Results obtained in this study can only be applicable to these very regions.

1.6 Delimitation of the study

The study will be carried out in Mashare which is about 40 km from Rundu Town which is situated in Northern Namibia, with the data being obtained from here used as a representative of the Kavango region at large due to time and resources being limiting factors. Furthermore, only cowpea will be used as the legume of choice to isolate rhizobia because it has been shown to be well adapted to arid conditions.

Chapter 2. Literature Review

2.1 The Kavango Region

Montle and Teweldemedhin, (2014) state that agriculture is one of the most important sectors in Namibia, although it only contributes about 5.9% towards the national gross domestic product. The authors however insist that an approximated 40% of commodities exported by Namibia are based on Agricultural commodities. Therefore, there is need to boost the agriculture sector into a sustainable one (Montle & Teweldemedhin, 2014). On the other hand, Mushendani et al. (2008), indicated that approximately 27% of Namibia's workforce is based in the agricultural sector and of this, about 57% of the rural based workforce is in Agriculture. Montle and Teweldemedhin, (2014) went on to state that about 70% of Namibia's population has a direct dependency on agriculture.

The Kavango regions' long-term ecological and agricultural sustainability is often neglected due to the poor population's urgent need to secure short-term food supplies (Hoffman et al. 2010). The population in this region exclusively depends on agriculture (Mendelsohn, 2009) with approximately 24,000 households in the region living primarily from agricultural produce (Hoffman et al. 2010).

Information on the soils and vegetation type in the Kavango is limited but a few surveys that were conducted concluded that the soils lacked the necessary nutrients to sustain agriculture in the area for a long period (Mendelsohn, 2009). The region is dominated by arenosols (Strohbach & Petersen, 2007) which are porous and sandy and are notoriously known to leave little moisture in the soils and to only be able to hold a few nutrients. Therefore, people in the area depend largely on ground water due to the poor ability of the

sands to hold any water making surface water less. Most of the agricultural practices in the region are based along the Kavango River, although Likuwa (2005) states that people are constantly forced to relocate away from the flood plains due to frequent floods.

Practices such as the traditional slush and burn, which are common in the area, were discouraged as they resulted in the degradation of the natural biota and contributed to high levels of climate change experienced in the Kavango region (Strohbach & Petersen, 2007). Although the effect of fire in the Kavango is debatable, as Geldenhuys (1977) stated that fires are essential in maintaining the woodlands ecosystem based his conclusion on the long term study on trial plots.

On the contrary, Mendelsohn and Obeid (2003) stated that fires are detrimental to the environment and vegetation in the Kavango region and elsewhere. Therefore, the introduction of bio inoculants which allows for the restoration of soil fertility and in the long run foster sustainable land use and management and prevent environmental degradation and are considered “eco-friendly” agricultural practices (Leite et al. 2017).

The Kavango region in the North-Western region of Namibia reports less annual rainfall almost every year with soil temperatures having been reported to reach as high as 50 ˚C (Strohbach & Petersen, 2007). The region is expected to be extensively affected by climate change according to (Roder et al. 2015) (Figure 2). Thus, the need to carry out studies on drought-tolerant bacteria associated with crop species which are naturally adapted to drought, such as cowpea.

This is of uttermost importance for regions that are involved in extensive crop development in Namibia (Strohbach & Petersen, 2007). This study will evaluate the potential of drought-tolerant cowpea symbiont to increase yield in these semi-arid regions.

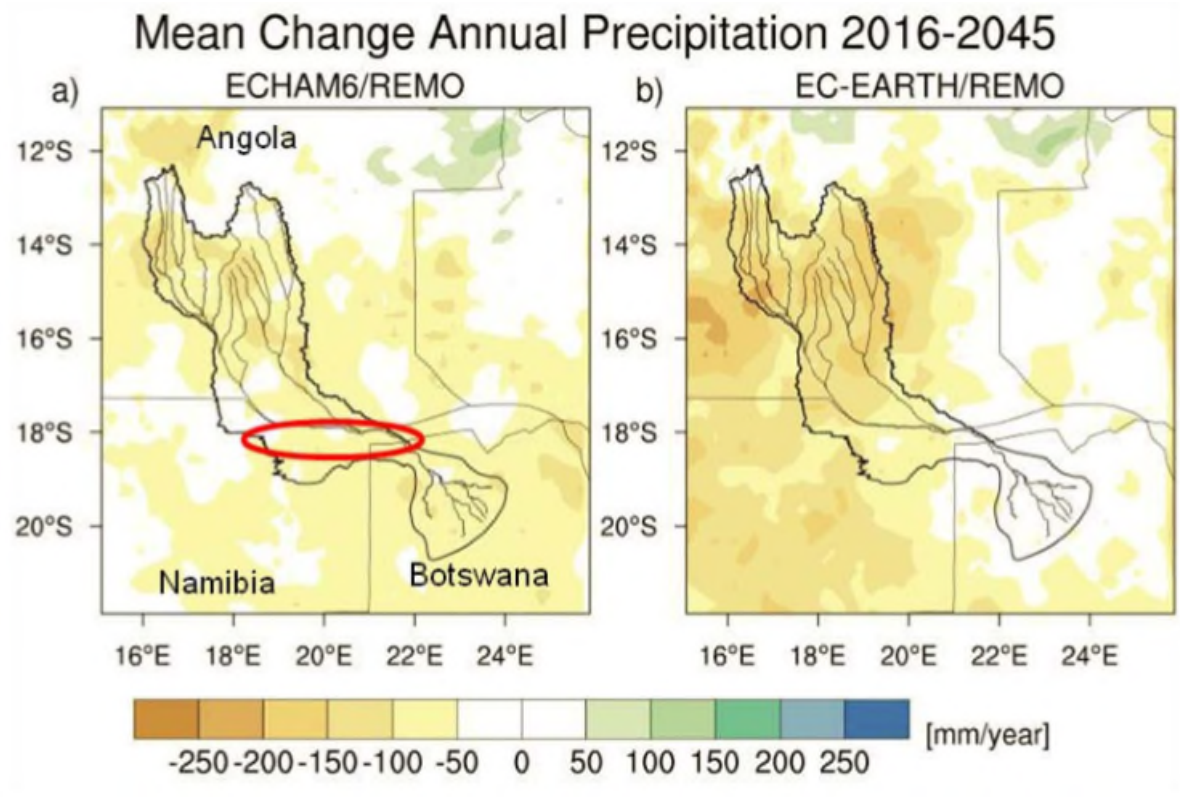


Figure 2. Map showing the climatic change prediction of the Kavango region between the years 2016-2045. The map shows a drop of about 150 mm of precipitation per year for that time. Modified from (Roder et al. 2015).

The Kavango soils have been a source of novel bacterial strains that are promising in the use as bio-inoculants (Grönemeyer & Reinhold-Hurek, 2018). Montanez (2000) states that information about the type of microbes found in an area in the ecosystem is important because the introduced bacterial strains will have to compete with the local microbes found in the area for nutrients, therefore the competitiveness of the chosen strain with respect to the local ones should be high.

2.2 Bio-inoculants

The ‘Green Revolution’ brought along agricultural practices such as the use of fertilizer, pesticides and herbicides derived from chemical origins (Martinez-Hidalgo et al. 2014). These agricultural practices brought about by the revolution resulted in an increase in agricultural produce. Years later after the introduction of these practices, the costs that come about from their use resulted in the quest of a new agricultural revolution, with the use of microorganisms turning out to be the better solution in terms of cost and being environmentally friendly (Martinez-Hidalgo et al. 2014).

Due to the green revolution, there has been a worldwide global demand for nitrogen fertilizers over the years (Abi-Ghanem et al. 2012). Abi-Ghanem et al (2012) predicted an increase of demand by 1.4% annually. There has been a loss of millions of dollars in farm profit due to the sky rocketing prices of nitrogen fertilizers (Abi-Ghanem *et al.* 2012) hence the need for an alternative and cheaper source of nitrogen especially in areas that are drought stricken.

The high cost of nitrogen fertilizer and their harmful impacts on the environment (Hamza & Alebejo, 2017) has led to the search of microbial strains (bio-inoculants) that can effectively fix atmospheric nitrogen in cowpea and other legumes with the intention of developing them into inoculants. Incorporation of bio fertilizers into the agricultural farming systems could have a positive impact on food security and agricultural productivity (Hamza & Alebejo, 2017).

Cowpea is able to form root nodules that fix nitrogen by being in a symbiotic relationship with a diverse number of bacteria, therefore it is considered as being promiscuous

(Tampakaki et al. 2017) as it capable of forming functional nodules with bacteria from other genera other than *Bradyrhizobium*. Cowpea nodule microbial flora have been studied and their use to promote growth and increase yield holds promises. Cowpea is mostly nodulated by bacteria belonging to the genus *Bradyrhizobium* (Guimaraes et al. 2012). This heterogeneous is collectively called "cowpea miscellany" (Grönemeyer & Reinhold-Hurek, 2018). The genus *Bradyrhizobium* had thirty-eight type strains (Tampakaki et al. 2017) including the novel *Bradyrhizobium kavangense* 14-3^{T} which was first isolated from the region were this study was carried out by Gronemeyer et al. (2016). There are some rare cases where cowpea has been nodulated by rhizobia from the genera *Rhizobium, Mesorhizobium* and *Sinorhizobium,* which are fast growing (Gronemeyer et al. 2016). *Bradyrhizobium* is a very diverse group that is capable of nodulating most legumes but has also been reported to nodulate non-legume plants such as *Parasponia* (Steenkamp et al. 2008).

For rhizobia to efficiently establish itself on the legume seeds, it has to be attached to a carrier material, which should possess properties such as non-toxicity, eco-friendly and high-water holding capacity (Albareda et al. 2008). Albareda et al. (2008) assessed the survival of rhizobia in different carrier materials and liquid formulations. The study tested inorganic carriers such as perlite and sepiolite as well as organic carries such as cork and grape, with peat being used as a reference. Of the tested carriers, Albareda et al. (2008) reports that cork compost had the largest bacterial survival data followed by peat and then perlite hence Albareda et al. (2008) recommends the use of alternative organic carriers to peat depending on their availability and cost. However, the inorganic carriers had the lowest viable cell counts. Kishore et al. (2008) favored the use of peat formulations as the

author reported large viable cell counts associated with peat as a carrier as some bacteria had a good shelf life in peat of up to 180 days after inoculation. Abd El-Fattah et al. (2013) wrote that although peat is the widely used carrier, it is not universally available.

Two parameters, rhizobia specificity and effectivity are always key in selecting the best rhizobial inocula for legumes (Batista et al. 2015). Most bio inoculation studies tend to not be successful because the ecological origin of the inoculant and its climatic adaptation are rarely put into consideration while soil pH, carbon content, mineral soil availability and many other biotic and abiotic factors tend to hinder successful inoculation (Ndungu et al. 2018).

Ndungu et al. (2018) further stated that to obtain a strain of rhizobia that will not only establish itself in the host but persist after inoculation, it is of utmost importance to know its geographical as well as ecological distribution and also its physiochemical soil requirements. Several factors are required for a bio-inoculation to be successful, as shown in Figure 3.

Most soils already have bacterial strains that are able to form nodules but these are ineffective rhizobia as they produce nodules that cannot fix atmospheric nitrogen (Montanez, 2000). Montanez, (2000) emphasized that in such soils, the inoculum introduced should be highly effective in nitrogen fixation and highly competitive so that it is able to replace the local microbes as the competition could reduce the efficacy of the inoculum. In soils that have a deficiency in phosphorus, Albareda et al. (2008) reported an increase in P intake by cowpea shoots that had been grown with bio fertilizers as compared to those that received nitrogen, phosphorus, potassium fertilizer.

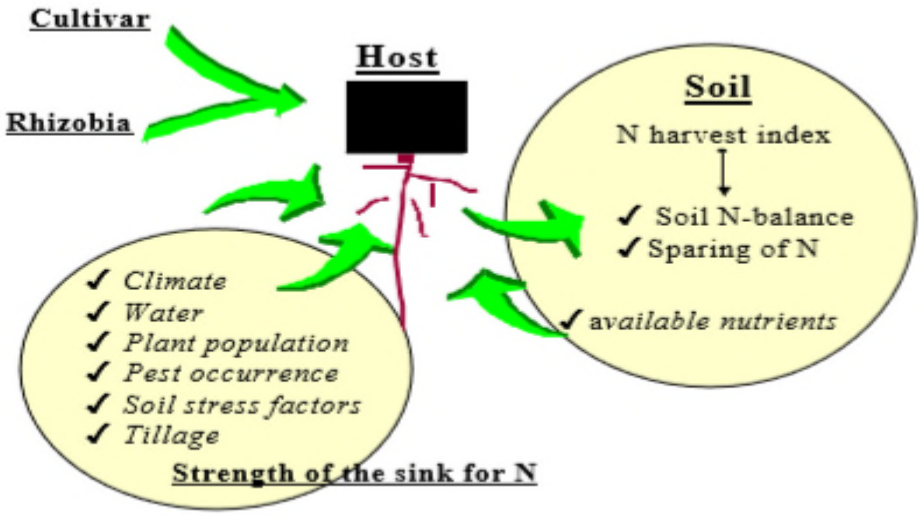


Figure 3. A conceptual diagram of some of the factors required and to be considered for a successful nitrogen fixation study using rhizobia. Adapted from Montanez (2000).

According to a study carried out on Kenyan soils, Ndugu et al. (2018) reported that dry soils house a variety of rhizobia compared to soils that have more moisture. This was supported by Grönemeyer et al. (2014) who observed that rhizobia obtained from semi-arid areas were highly diverse than those obtained from humid areas in Namibia. Therefore, the Kavango region being an area of low rainfall is expected to house diverse rhizobial species.

There have been cases where co-inoculation of *Rhizobium* with other strains of bacteria with plant growth promoting characteristics such as the *Bacillus* and *Pseudomonas* genera resulted in improved growth of the legumes compared to just inoculating with *Rhizobium* alone (Rodrigues et al. 2013). In a study carried out by Rodrigues et al. (2013), it was observed that co-inoculation of cowpea with plant growth-promoting bacteria (*Pseudomonas graminis* and *P. durus*) and *Bradyrhizobium* sp. led to the delay of the

deleterious effects of senescence. This conclusion was reached upon after the co-inoculated cowpeas showed better results for biochemical indicators that are related to antioxidant metabolism than those that were just inoculated with *Bradyrhizobium*.

Most of the strains that are used as bio-inoculants and can fix atmospheric nitrogen have been shown to have plant growth promoting characteristics such as improved plant development and increased yield (Hamza & Alebejo, 2017). Dutta and Bandyopadhyay (2009) are in support of co-inoculation yielding more as compared to inoculation with one bacterial species and no inoculation at all. Their study revealed that co-inoculation with *Rhizobium* and phosphobacterin yielded more grains per hectare than the legumes inoculated with only *Rhizobium* or phosphobacterin (*Pseudomanas striata*).

2.2 Nitrogen Fixation

Atmospheric air contains more nitrogen than any other element (Swain & Abhijita, 2013), therefore plants and diazotrophs can utilize this abundant nitrogen into its more usable forms of either nitrates or ammonia (Swain & Abhijita, 2013). The nitrogenase complex enzyme (Figure 4) plays a crucial role in nitrogen fixation. The enzyme is a complex metalloenzyme consisting of two proteins, a reductase which provides electrons and a nitrogenase which turns nitrogen to ammonia (Meena et al. 2017).

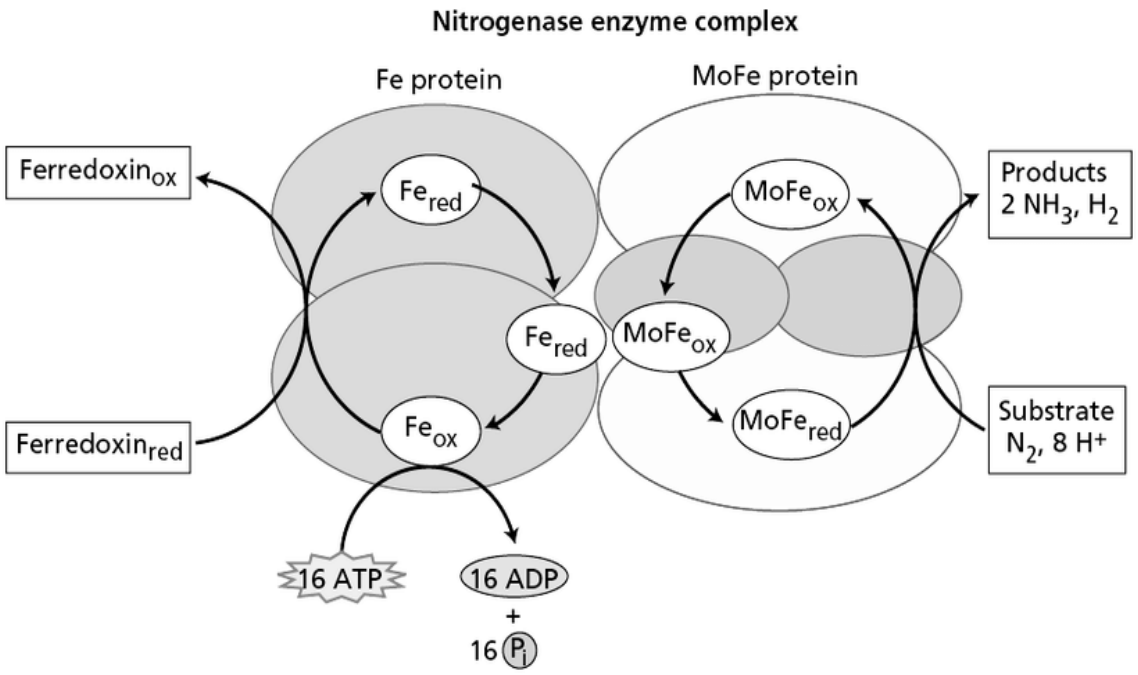


Figure 4. A representation of the nitrogenase complex with two components, the Fe Protein which is called dinitrogenase reductase and the MoFe Protein called dinitrogenase. Adapted from (Taiz & Zeiger, 2003).

In order for a successful symbiotic relationship to take place between the host plant and its rhizobia partner, there has to be some level of deficiency in nitrogen in the soil (Montanez, 2000). It has been observed that if the soils have nitrogen in abundance then the legume symbiont will not form an infection thread that is required for it to colonize the host plant as this is an energy costly process (Montanez, 2000).

For the process of nitrogen fixation to take place, Isidoro and Messier (2009) reported that *Rhizobium* should be able to occupy and colonize the root hairs of the host for it to be able to form an infection thread. Variable metabolites, for example flavonoids, initiate the formation of the infection thread hence its necessary that the host plant secrets them, and they are specific for a target rhizobia (Taiz & Zeiger, 2003).

Flavonoids bind and activate the NodD proteins that would initiate the transcription process. Nod proteins will in turn construct and secret Nod factors (Isidoro & Messier, 2009). Rhizobia nodulation, at the genetic level, is controlled by the nodulation genes *nol*, *noe* and *nod* (Steenkamp et al. 2008). These nodulation genes determine the structure and formation of Nod factors (Steenkamp et al. 2008). The Nod factors that represent the primary signal molecules are involved in nodule organogenesis and infection of the host.

The infection thread assists the nodules to reach the primordium cells. While travelling down the infection thread, rhizobia extend inwards towards the root hairs (Taiz & Zeiger, 2003). A symbiosome, formed via the infection of adjacent cells by the infection thread, is a compartment that is membrane bound that has its metabolic parts in the cytoplasm. When released into the cytoplasm, the bacteria are transformed into bacteriods. Taiz and Zeiger (2003) indicated that the formed bacteriods are then surrounded by plant membranes forming structures called symbiosomes which are the site of nitrogen fixation and facilitate the production of leghemoglobin which is needed to reduce the amount of oxygen in the nodules.

The production of an infection thread is not always an indication of nitrogen fixation. In most cases the bacteriod is reddish in colour which is an indication of the presence of leghemoglobin which is in most cases associated with nitrogen fixation (Isidoro & Messier, 2009). Guimaraes et al. (2012) reported that dark colored nodules are normally disregarded due to the assumption that they do not have leghemoglobin. However, they are important for studying nodule competitiveness with pink-red colored nodules. The host legume plant can abort symbiosis with the bacteria if the relationship becomes parasitic in either direction (Taiz & Zeiger, 2003).

Symbiotic genes, *nifH* (Nitrogenase) and *nodC* (N-acetylglucosaminyltransferase) genes are used for phylogenetic analysis with the *nodC* gene (Donate-Correa et al. 2007), and are necessary for determining host promiscuity and host range.

The ^{15}N natural abundance method is needed to determine how much nitrogen is derived from nitrogen fixation in legumes as was first reported by McNeil et al. (1994). The amount of nitrogen that is derived from nitrogen fixation can be calculated in terms of percentage using the equation of McNeil et al. (1994) as stated in Rasmussen et al. (2012):

$$\%\text{Ndfa (Nitrogen fixation)} = \left(1 - \frac{legume\ atom\%excess}{grass\ atom\%excess}\right) \times 100$$

Another form of the equation used to measure the percentage of nitrogen derived from the nitrogen fixation (Ndfa %) is according to Mathu et al. (2012):

$$\text{Ndfa} = (\delta 15\text{Nnf} - \delta^{15}\text{Nf}/\delta^{15}\text{Nnf} - \delta^{15}\text{Na}) \times 100$$

Where according to Mathu et al. (2012), δ15Nnf is the natural isotopic abundance value of the reference plants that is interpreted as the value of the amount of nitrogen from other sources other than the atmosphere. $\delta\ ^{15}Nf$ is the value of the isotopic abundance of the nitrogen fixing legume grown under conditions where atmospheric dinitrogen and N from other sources are available. $\delta^{15}Na$ is the $\delta^{15}N$ value of the nitrogen fixing legume dependent only on fixed nitrogen grown in a nitrogen free medium (Mathu et al. 2012).

2.3 Bio-inoculants and yield in Cowpea

Cowpea yield is affected mostly by water stress coupled with nutrient availability. In a study by XIE et al. (2015) who grew pot experiments of alfalfa (*Medicago sativa* L.) and

smooth brome grass, it was reported that the dry matter of brome grass increased when grown as a mixture with alfalfa which is known to be a nitrogen fixing legume. On the other hand there was a decrease in dry matter yield of the brome grass when it was grown alone XIE et al. (2015).

To add on Stamford et al. (2013) reported that there was no significant difference between the treatments of bio fertilizers and mineral nitrogen in the total nitrogen accumulation of the shoot dry biomass yield. Andrade et al. (2013) reported similar results when a diazotrophic bacterium was used as a bio fertilizers on cowpea. The author stated that the highest rate of bio fertilizers applied on cowpea seeds did not show a difference in shoot dry biomass yield compared to the application of minimal amount of chemical fertilizer.

According to Ronner et al. (2016), to conclude whether a treatment with inoculant or fertilizer works, farmers need to observe a substantial increase in yield, with a 10% increment in grain yield being one of the indicators of a treatment working. Dekhane et al (2011) recorded the highest grain yield of 1,441 kg/ha on cowpea inoculated by *Rhizobium* compared to the cowpea that was not inoculated which was at par with the fertilizer treatment.

A high yield of the legume, measured in terms of dry matter biomass is also of importance in the dairy industry. Obtaining quality forage that possesses appreciable agro-qualitative attributes can go an extra mile by sustainably increasing a ruminant's productivity and this in turn contributes to ensuring the food security of the world's skyrocketing population (Iqbal et al. 2018). Sontag et al. (2016) as cited in Iqbal et al. (2018) stated that the widely used cereal forages are poor in nutrients and have a low digestibility hence their value is decreased in qualitative terms. Iqbal et al. (2018) also stated that, although

these cereal forages yield substantial quantities of green forage for ruminants, costly additives that are associated with their use result in high costs and a decrease in profits. Hence cowpea could be a good substitute to cereal forages as it is rich in nutrients and does not require additives.

Stamford et al. (2013) reported a reduction in soil pH values after the addition of bio fertilizers. However, although there was a reduction in the soil pH during the study by Stamford et al. (2013), the authors reported an increase in the yield of cowpea with increase in number of nodules and plant biomass after the biofertilizers applications. Andrade et al. (2013) concluded that bio fertilizers composed of some diazotrophic bacteria applied on cowpea had significant effects on nodulation by increasing the nodule biomass when the biofertilizers is applied at the highest rate.

Hogh-Jensen (2006) supported the hypothesis that fertilizer treatment on legumes decreases the amount of nitrogen derived from the atmosphere. On the contrary, Rasmussen et al. (2012) while studying Alfalfa and bird's foot trefoil reported data that did not support this hypothesis. Although (Kai-yun et al. 2015), who also carried out a study with alfalfa reported that the application of fertilizer resulted in a reduced %Ndfa of the legume which are findings in line with Hogh-Jensen (2006). Montanez, (2000) reported that nitrogen fixation can be quantified using plant dry weight measurements. This is due to plant dry matter being correlated to effectiveness of nitrogen fixation provided N is the only limiting growth factor.

2.4. Identification, Taxonomy and Sequencing

Most of the leguminous bacterial species are characterized by an alkaline reaction in culture media that contains mannitol hence most of them are grown on mannitol salt agar medium or mannitol salt broth (Menna et al. 2009: Parker, 1999). On the other hand, Grönemeyer et al. (2013) used modified arabinose gluconate medium, which also gave good reliable results. These and other ecological characterization techniques such as hydrolytic activities towards starch, casein; strains production of indole; and growth under exposure to different environmental conditions have been used by Martinez-Hidalgo et al. (2014) to characterize rhizobia obtained from *Medicago sativa*.

Grönemeyer et al. (2014) tested the rhizobial isolates for drought tolerance and temperature tolerance by supplementing the growth media such as MAG agar with PEG (polyethylene glycol) and measuring the bacterial density after growing the cultures on a rotary shaker at 28 °C. Temperature tolerance was tested by incubating the isolates at different temperatures or using an agitating water bath for higher temperatures as was performed in Grönemeyer et al. (2014).

Several molecular techniques have been employed to identify legume rhizobia, especially *Bradyrhizobium*, with the use of the 16S rRNA for phylogeny and taxonomy being the most favored one due to it being simple and easy to conduct (Jaiswal et al. 2017). Since rhizobia is taxonomically highly diverse, using 16s rRNA alone as marker of phylogeny so as to be able to differentiate related species and strains in rhizobia has not been the most ideal due to some bacteria having multiple copies in their genome Jaiswal et al. (2017), 16s rRNAs low divergence towards closely related species so as to define and

delineate them (Azevedo et al. (2015).Lastly its high susceptibility to horizontal gene transfer and genetic recommendation (Jaiswal et al. 2017).

Jaiswal et al. (2017) recommended using housekeeping genes and intergenic spacers as markers for phylogeny in rhizobia and stated that the 16S-23S rRNA sequences gave results that were more coherent. Azevedo et al. (2015) also concurred with Jaiswal et al. (2017) in that the authors recommended the approach of using housekeeping genes that were conserved but had a higher evolution rate as well as the use of the multilocus sequencing analysis methodology to precisely detect diversity within the genus *Bradyrhizobium*.

Delamuta et al. (2012) reported that, in order to detect high diversity in several rhizobia genera, ribosomal genes such as the 23S rRNA and the intergenic transcribed spacer (ITS) regions should be chosen over the most commonly used 16S rRNA since they evolve at a faster rate than the 16S rRNA. Grönemeyer et al. (2014) amplified the 16S-23S rRNA gene using a primer pair of FGPS1490/FGPS132 with an annealing temperature of 58 ^{o}C and amplified the *nifH* gene with a primer pair of FGPH19/Po1R.

Nitrogen fixing organisms have been shown to share the exact same operon in which the Fe protein subunit of the nitrogenase enzyme (Figure 4) is encoded by the *nifH* gene (Poly et al. 2001). The authors also stated that since phylogeny based on the *nifH* gene seems to correspond with that of the 16S rRNA phylogeny of nitrogen fixers then a diversity of the *nifH* gene is representative of the diversity of nitrogen fixing organisms. Therefore, this permits for polymerase chain reaction (PCR) amplification of this segment to construct a phylogeny of rhizobia (Poly et al. 2001). On the other hand, Gaby and Buckley (2012) reported that there are disparities in the sequence coverage of *nifH* primers for

PCR, the authors reported a variation in the specificity and coverage of the *nifH* primers. This variation having been possibly caused by primer design requiring the use of a sequence database that represents the entire sequence diversity that is to be targeted by the primer (Gaby & Buckley, 2012). Therefore based on these various findings ITS is more ideal for accurate phylogeny.

Chapter 3. Methodology

3.1 Research Design

The research design consisted of two sections, one that provided the molecular data for taxonomy and phylogeny and the other that provided data for assessing the yield (Figure 5).

Figure 5. A systematic overview of the study

3.1 Study area

This study was carried out at Mashare Government Research Center based in Rundu North, Eastern Namibia. It is situated about 40 Km East of Rundu Town. This study is part of a bigger project called “The Future of the Kavango.” This site was chosen based on the existence of an anthropological pre-study of the area.

Figure 6. Google Earth screenshot of Mashare with coordinates showing Mashare Irrigation Scheme Training Center (Namibia, S 17,89º, E 20.18º).

3.2 Seed Sample

The cowpea cultivars used in this study were obtained from Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ). These seeds were for the following cultivars: Nakare (a), Lutembwe (b), I2 (c), Bira (d), Shindimba (e), and Silwana (f) (Figure 7). Cowpea strains that are resistant to the ‘witch weed’ *Alectra vogelii* were obtained from

The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and were named I2. *Alectra vogelii*, according to Mwaipopo (2014), is a parasitic weed that is known to cause major constraints on legumes and most especially cowpea. Seeds were carefully selected and those that showed signs of weevil infestation or holes were avoided.

Figure 7. The 6 different cowpea cultivars that were used during the study.

Figure 8 that follows shows the layout of the plot that was used to conduct the study. As shown on the figure the plot was demarcated into three potions of no treatment, bio-inoculant and nitrogen fertilizer. The individual subplots were numbers as in Figure 8 for easy data collection.

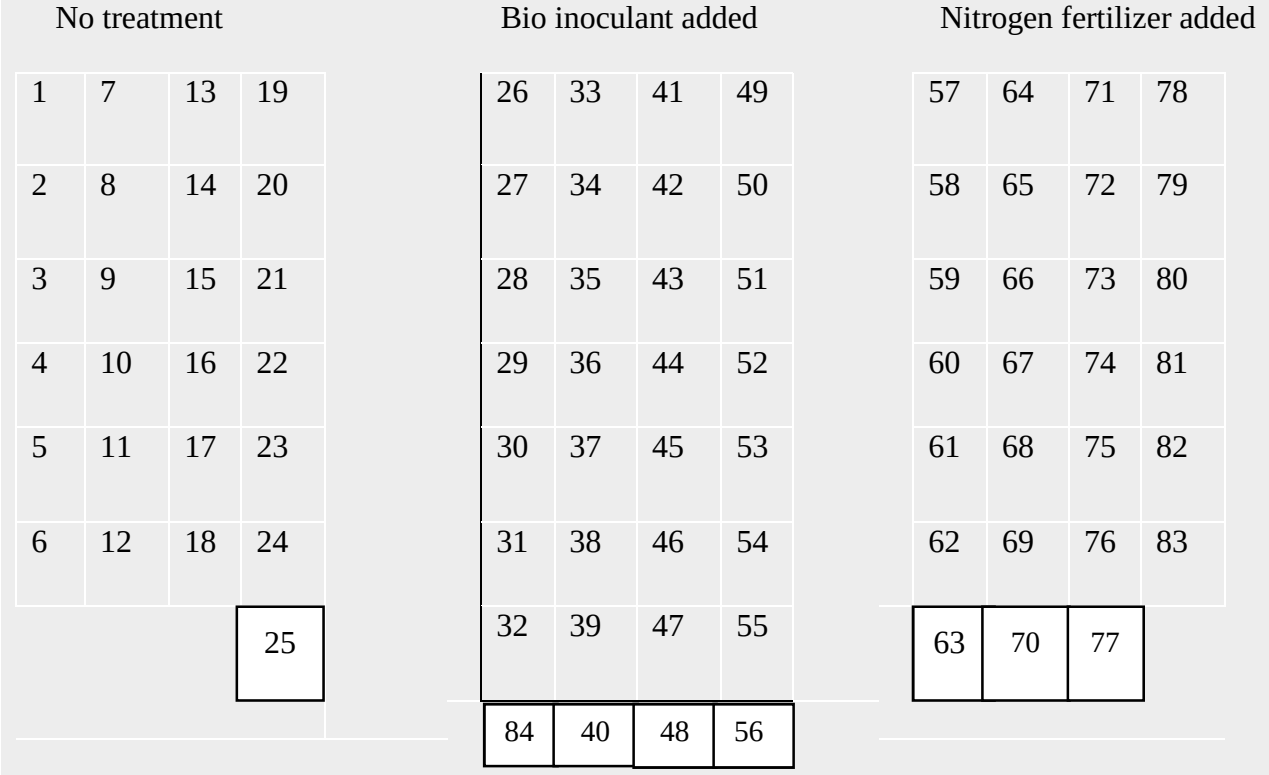


Figure 8. Plot layout of the study with numbers indicating different subplots.

3.3 Treatment Preparation and Planting

The inoculant treatment was performed by getting a substantial amount of bacterial inoculant strains called (1-7) and (14-3). The strains were grown in fresh Modified Arabinose Gluconate medium, with peat as a carrier, then packed in Whirl-Pack® sample bags. The bags were stored at room temperature avoiding their exposure to direct sunlight for long hours.

Just before planting the bio inoculant treated seeds, a small amount of Polyvinylpyrrolidone (PVP-40) was poured into 25 ml of distilled water. The PVP-40 was used because it is sticky, hence it helped facilitate the sticking of the inoculant better to

the seeds. A Small can was used to mix the inoculant, one seed cultivar and the PVP-40, all the six different cultivars were mixed this way before planting. This mixture was used to plant on subplots 26-39, 41-47 and 49-55. Subplots 84 and 40 each had the six different cultivars planted on the subplots as the destruction plots.

The cowpeas were grown under rain fed conditions to simulate the natural environmental conditions of the area. The field was ploughed back and forth to homogenize the soil before planting. Thereafter, the field was divided into 3 sections. One section had a treatment of the 6 different cowpea cultivars plus nitrogen fertilizer, the other section had the cowpea cultivars plus bio inoculants while the third only had the cowpea cultivars minus any other treatment. This design is illustrated in the diagram in Figure 8.

According to Figure 8 each subplot was 300 cm × 600 cm in area and 40 cm space was left between the subplots. Subplots 1-24, had 6 different cowpea cultivars planted on them after digging holes of about one inch in depth. Subplot 25, which was named the 'destruction plot', had all the different cultivars planted on it. Each subplot had 120 slots for seeding, hence after the integrity of the seeds was checked, with the good-looking ones being selected; a subplot had approximately 126 plants, one seed per slot.

Urea was added on the soils where the nitrogen fertilizer treatment was to be performed. Subplot 57-62, 64-69, 71-76 and 78-83 each had a different cultivar planted on them. Subplot 77 had all the six different cultivars planted on it and it contained chemical fertilizer. The plots were weeded on a regular basis.

A simpler design was used for the pot experiments at the Namibian University of Science and Technology laboratories in Windhoek. Pots with soils from the Kavango had cowpea

planted; some had the seeds with the inoculant while others only had the seeds without the inoculant and the fertilizer treatment.

3.4 Samples

3.4.1 Harvesting of root nodules

After the plants had been grown for about 60 days, plants from the destruction plot of each treatment were carefully uprooted randomly without disturbing the roots. These roots were then washed under running water, and the nodules that had a pink/red coloration were carefully cut with a sterile blade and stored in a silica gel vial as performed in Grönemeyer et al. (2014) for transportation from the study area to the laboratory. The University Of Bremen laboratories in Germany were used to perform the molecular biology work on the obtained nodules. The harvested nodules were labeled by indicating the acronym of the cultivar coupled with the treatment performed or type of inoculant added. Figure 9 shows how the nodules were stored and transported to Bremen, Germany.

Figure 9. Picture of how the nodules were collected in properly labeled silica gel tubes for transportation.

3.4.2 Surface sterilization of nodules

Senescent looking nodules were discarded while those that showed a pink like color were chosen and rehydrated for an hour in a petri dish containing distilled water, with some being rehydrated overnight. The rehydrated nodules were surface sterilized by first placing them in 98% ethanol for 5 seconds; followed by immersion in a petri dish with bleach (2% sodium hypochlorite) for 3 minutes following the descriptions of Grönemeyer et al. (2013). This was followed by five successive washes in sterile distilled water. Lastly the nodules were placed on a petri dish containing 100 µl of distilled water, one at a time, and crushed with a sterilized rod to obtain a bacteroid milky suspension. The suspension was then poured on plates containing Modified Arabinose Gluconate Agar and incubated at 30 ^{o}C for 2 weeks. Gram straining was used to differentiate these isolates (Hamza & Alebejo, 2017) by smearing the resultant colonies on glass and heat fixing them then applying a crystal violet stain followed by the addition of iodide. Decolourization was carried out with acetone and finally counterstaining with safranin before being viewed under the light microscope (Hamza & Alebejo, 2017).

3.4.3 Bacterial Culturing and DNA extraction

MAG agar inoculated plates were grown for two weeks at 30 ^{o}C and pure cultures were obtained after sub culturing twice. A colony lysis buffer made of 0.1% (v/v) Tween 20 was used after which a single colony was picked from each plate. The picked colony was transferred into a separate sterile Eppendorf tubes containing 50µl of the Tween 20 lysis

buffer to make a solution. This solution of cells was incubated at 95 ºC for 15 minutes with the help of a thermomixer while in the Eppendorf tubes which were then taken out and allowed to cool. After cooling, spinning was performed at maximum speed for 5 minutes in a centrifuge. The supernatant was then collected and put in a separate tube and this saved as the DNA template for PCR reactions.

3.4.4 Amplification of ITS regions

PCR was used to amplify the ITS region of DNA using the primer pairs FGPS1490 and FGPS130 (Grönemeyer, et al., 2014). A 50 µl PCR mixture composed of 5 µl 50X Molzym Taq Buffer (1X), dNTP mix of 2µl , forward primer: FGPs 130 and reverse primer FGPS 1490 both with a volume of 2 µl each, 0.5 µl of Mol Taq, 36.5µl of nuclease free water and finally 2 µl of colony lysis template. The PCR reaction was then carried out in an Esco Swift™ MaxPro Thermal Cycler made in Germany. The following PCR profile was employed: initial denaturation at 95 °C for 4 mins, this was followed by 40 cycles at 94°C for 1min, 58 °C for 1 min and 72 °C for 2 mins, final extension at 72 °C for 10 min and held at 4 °C (Cooper & Rao, 2006). The obtained amplicons were cleaned using a Monarch® PCR & DNA Cleanup kit in Bremen, Germany as per manufacturer's instructions and viewed in an Image Master® VDS in Bremen after being run on 1.5% gel electrophoresis at a voltage of 120 for 45 minutes for confirmation of amplification. These amplicons were then sequenced at the University Of Bremen laboratories in Germany.

3.5 Yield Assessment

3.5.1 Harvest Data collection

The yield of the different cowpea cultivars under the three treatments namely: nitrogen fertilizer, bio inoculant and no treatment was assessed and compared. Ten cowpea plants were selected from the middle rows of a subplot avoiding the border plants. They were used to calculate the root dry matter, grain and plant dry matter and converted into yield per hectare. All the subplots minus the destruction plots were harvested for yield assessment. The samples consisted of roots, shoots and pods. They had to be weighed in grams immediately after harvesting to get the root and shoot wet weights, with a beam balance and a hanging balance respectively.

Spades were used to dig up the 10 plants from the middle section of each subplot during the flowering phase. This was done carefully to try to excavate as many roots as possible attached to that plant. The non-inoculated plots were harvested first, these being subplots 1-24 according to Figure 8. For those subplots that had a few plants growing on them due to some having been dried or not germinated, less than 10 middle plants were harvested. This was followed by the harvesting of the bio inoculant treatment plots 26-55 without 40 and 48. The roots from these plants were passed through running tap water and soils attached to them removed.

3.5.2 Shoot and root biomass

The harvested 10 middle plants had their shoots separated from their roots. These shoots were then weighed and dry mass recorded immediately with a Highland™ portable balance. They were then dried in an open space in sunlight for 4 days and their dry weight was recorded. These measurements were used to extrapolate the shoot dry matter yield per subplot and were carried out on all the plots.

The roots that had been separated from the shoots were used to extrapolate the root dry matter yield per subplot of each plot. The wet roots were immediately weighed after being separated from the shoots. This was recorded as the root wet weight; these roots were then dried for 4 days in the open and the root dry matter yield recorded after these 4 days. This was recorded as the root dry matter yield and was used to extrapolate the root dry matter yield of the subplot were those roots were harvest. This was done for all plots.

3.5.3 Grain yield

For the grain yield data collection, 10 randomly excavated plants had their pod numbers counted. This was used to give an average number of pods per subplot based on the number of plants that were on that subplot. This was carried out for all plots. From these pods, 40 were selected randomly and the number of seeds in these 40 pods counted to get the average seeds per pod. The seed weight per subplot was also recorded and the above procedure was done for all the subplots. This was done by threshing all the pods from a subplot and the seeds weighed. The plants that were part of the pot experiments all withered and underwent etiolement hence no data was obtained from them.

Chapter 4. Results

4.1 Cowpea nodule collection and bacteria isolation

Nodules came in different shapes and colours as on the diagram below.

Figure 10. One of the Cowpea roots and its nodules that were used to extract rhizobia during the study.

A total of 6 cowpea root nodules that did not show signs of senescence and had a red/pink colour were chosen as representatives for analysis. Bacterial isolates were extracted from the representatives of the nodules. These isolates were slow growers and had different colours after being grown on MAG agar.

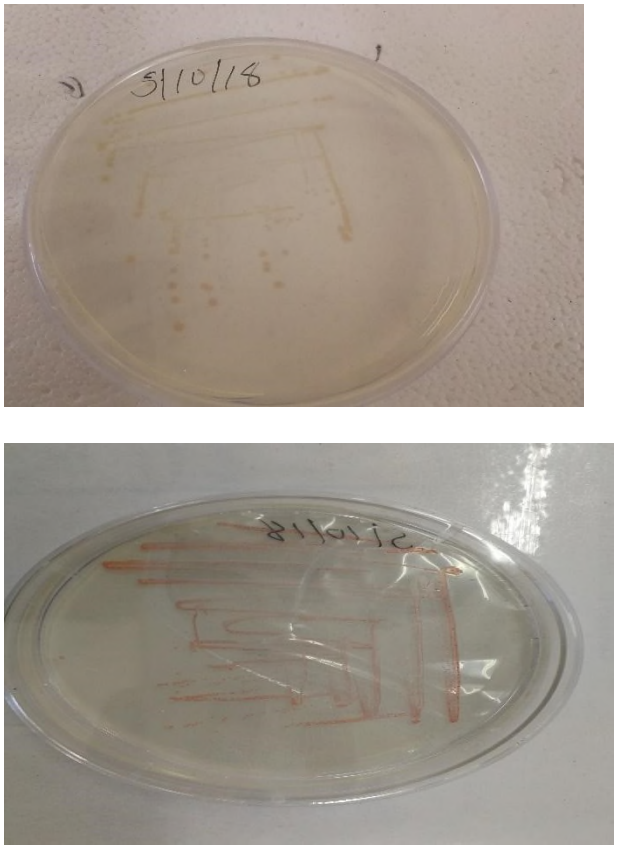


Figure 11. A representative of the colony appearance of the obtained isolates on MAG agar.

4.3 ITS region Amplification

The lysed pure cultures were amplified with an expected product range between 0.8 kb and 1 kb. The resulting amplicons are shown on Figure 12. The ITS sequencing results were then blasted in the NCBI data base from type material and were identified as *Bradyrhizobium vignae* strain 7-2 (KM378504), *Bradyrhizobium americanum* strain CMVU44 (LMG29514), *Bradyrhizobium ferriligni* strain CCBAU 51502 (KP411886) and *Bradyrhizobium kavangogense* strain 14-3 (KM378507). The sequence information of these strains is shown in the appendices and so are the blast outputs. A phylogenetic

tree was constructed from MEGA version 10.0.5 and the output tree is shown in Figure 13.

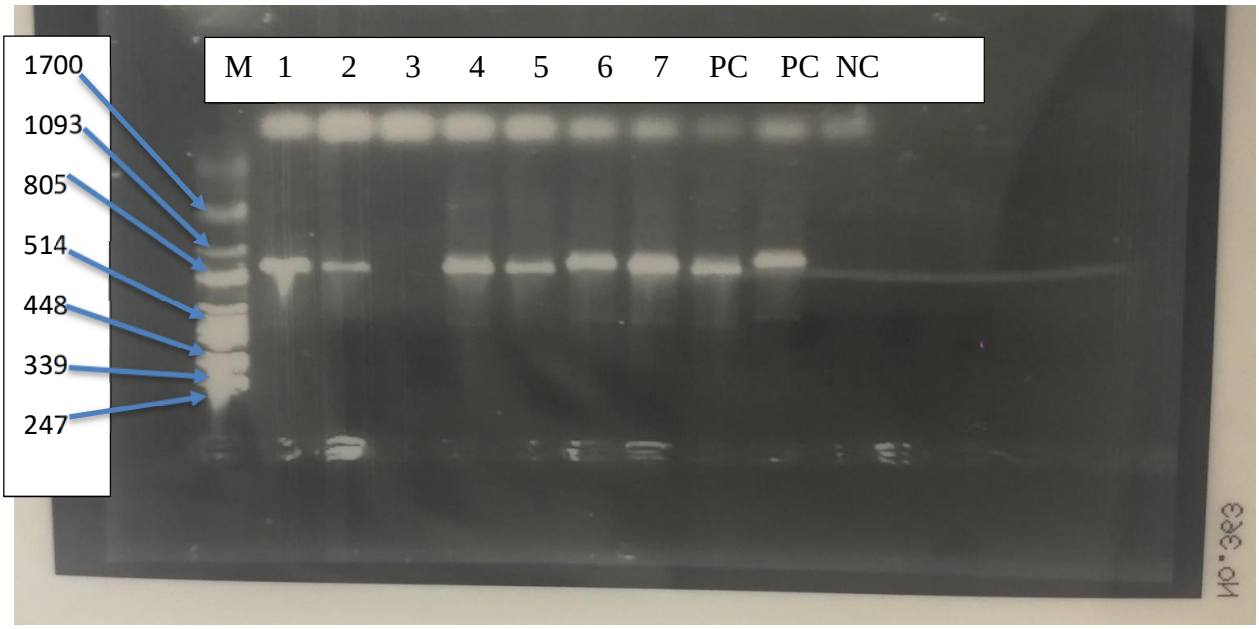


Figure 12: A 1.5% agarose gel consisting of the 7 (representatives) amplified isolates (1-7). PC were the positive controls of one genomic DNA and one nodule isolate of *Bradyrhizobium namibiense* while NC was the negative control. M the ladder in kbs.

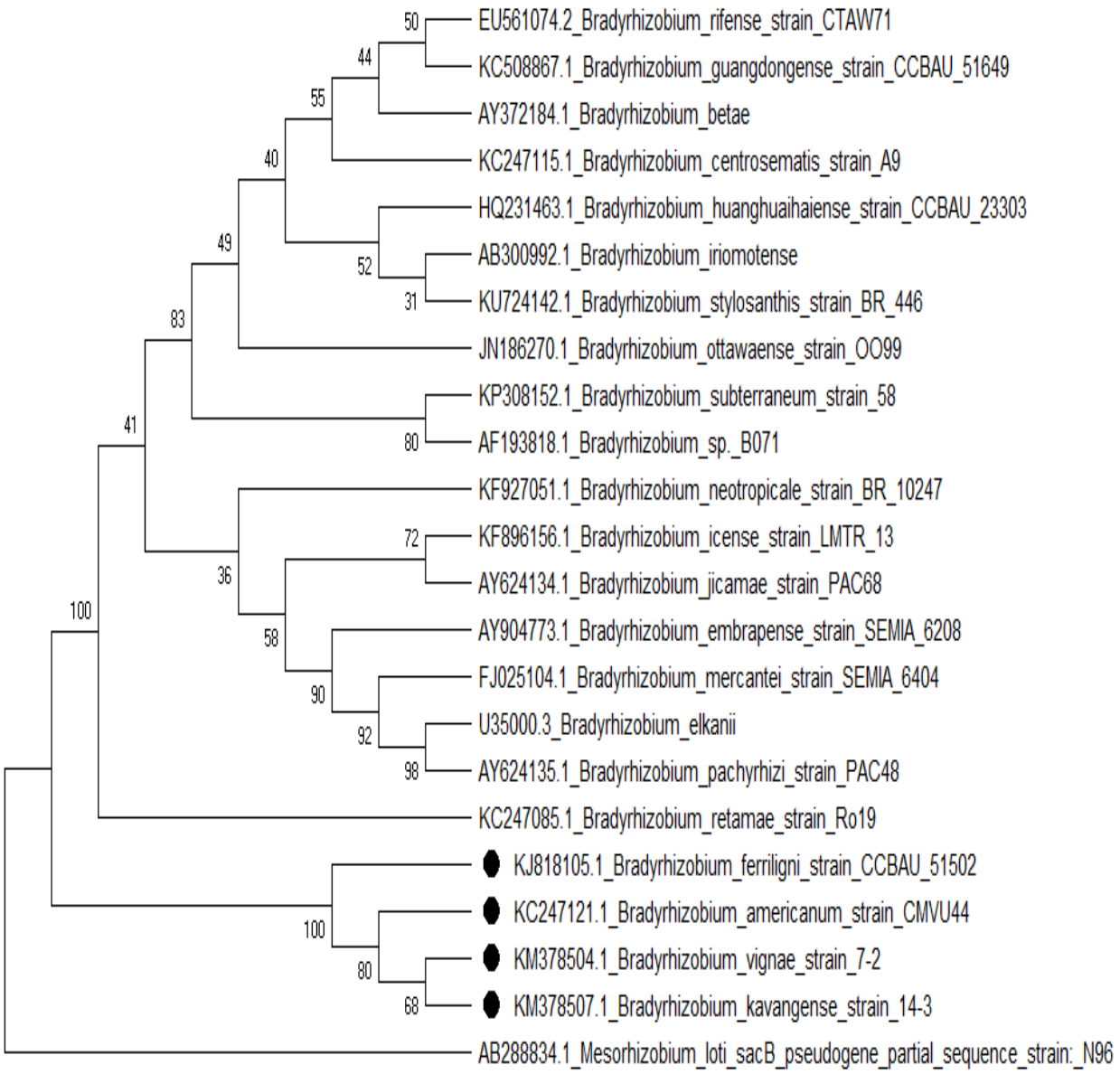


Figure 13: The phylogenetic relationship of the obtained isolates from *Vigna unguiculata* from the Kavango region inferred using the Neighbor-Joining method. *Mesorhizobium loti* was used as an outgroup to root the tree and its sequence was obtained from the NCBI database. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches.

4.6 Shoot, Root and Grain yield results.

Though each subplot was expected to have an estimated 120 plants, some subplots recorded only about half the amount or less during harvesting. This was due to some plants not being adapted to the local environment or some having been dried up by the time the harvesting took place. The figure that follows shows the field before harvesting.

Figure 14. Picture depicting the Cowpea study field at Mashare just before harvesting.

4.6.1 Harvesting

During harvesting time Nakare and Shindimba cultivars had a few cowpea plants growing on the designated subplots during harvesting hence only 5 middle plants of the bio inoculant treatment were dug up. The nitrogen fertilizer treatment plant was the last to be harvested, Subplots 57-83 minus subplots 63, 70 and 77, with 5 plants of the Nigerian

cultivar receiving nitrogen fertilizer being harvested. The tables that follow show the harvesting data.

Table 1: Average yield data of cowpea seeds at the physiological maturity stage. The following measurements are for the non-inoculant treatment, which is indicated by (-) after the cultivar name.

Cultivar Name	Shoot Wet weight(g)	Shoot Dry weight(g)	Plant Dry matter yield(kg/ha)	Root wet weight(g)	Root Dry Weight(g)	Root Dry Matter yield(kg/ha)	Grain yield (g)	Grain yield(kg/ha)
NAKARE - Average Yield	383.33	225.21	15013.89	138.73	70.17	4678.22	2273.23	1262.90
SILWANA - Average Yield	1125	225	15000.00	109.055	33.7525	2250.17	2401.86	1334.37
SHINDIMBA - Average Yield	300	360	24000.00	339.24	80.31	5354.22	273.18	151.77
LUTEMBWE - Average Yield	2500.00	1264.71	84313.90	105.30	27.65	1843.33	3560.66	1978.14
BIRA - Average Yield	1800.00	990.00	66000.00	158.38	35.50	2366.33	4270.64	2372.58

Table 2. Average yield data of cowpea at the physiological maturity stage. The following measurements are for the two bio inoculant treatments, which is indicated by (+*Bradyrhizobium* strain 1-7 and 14-3) after the cultivar name.

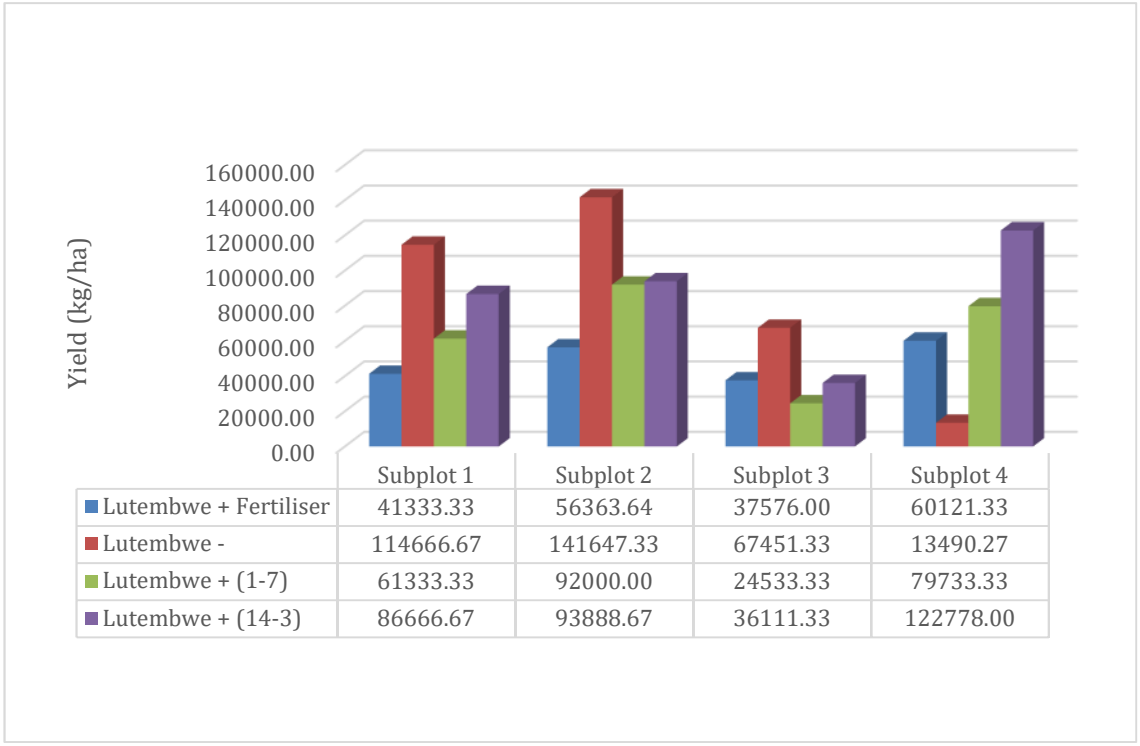
Cultivar name	Shoot Wet weight(g)	Shoot Dry weight(g)	Plant Dry matter yield(kg/ha)	Root wet weight(g)	Root Dry Weight(g)	Root Dry Matter yield(kg/ha)	Grain yield (g)	Grain yield(kg/ha)
LUTEMBWE + *Bradyrhizobium* strain 1-7 Average Yield	2100	966	64400	146.62	40.4	2693.5	4901.76	2723.19
LUTEMBWE + *Bradyrhizobium* strain 14-3 Average Yield	2350	1272.92	84861.17	135.73	43.78	2918.33	4922.36	2734.64
NAKARE + *Bradyrhizobium* strain 1-7 Average Yield	300	427.5	28500	130.26	62.465	4164.33	4918.5	2732.5
NAKARE + *Bradyrhizobium* strain 14-3 Average Yield	850	578	38533.33	209.73	99.46	6630.67	8118	4510
SHINDIMBA + *Bradyrhizobium* stain 1-7Average Yield	175	159.25	10616.67	188.8	53.32	3554.67	2921.4	1623
SILWANA + *Bradyrhizobium* strain 14-3Average Yield	2300	1086.11	72407.33	168.43	41.99	2799	5844.51	3246.95
BIRA + *Bradyrhizobium* stain 1-7Average Yield	550	403.335	26889	129.82	31.75	2116.67	3324.88	1847.16
BIRA + *Bradyrhizobium* strain 14-3Average Yield	1100	407	27133.33	142.76	35.905	2393.67	2664.01	1480.01

Table 3: Fertilizer treatment harvest data at the physiological maturity stage. Illustrated by a (+) fertilizer after the cultivar name.

Cultivar Name	Shoot Wet weight(g)	Shoot Dry Wet(g)	Plant dry matter yield(kg/ha)	Root wet weight(g)	Root Dry weight(g)	Root dry matter yield(kg/ha)	Grain Yield (g)	Grain yield(kg/ ha)
Lutembwe. Fert Average Yield	2600	732.73	48848.58	118.91	32.53	2168.5	2264.9	1258.21
ShindimbaFert Average Yield	575	517.5	34500	147.3	33.39	2226.00	463.97	257.76
Nakare.Fert Average Yield	966.67	172.07	11471.11	236.5	40.07	2671.33	1873.4	1040.79
Silwana.Fert Average Yield	2000	600	40000	110.28	29.13	1942.16	3703.4	2057.43
Bira.Fert Average Yield	2550	493	32866.67	104.38	39.7	2646.65	2460.9	1367.21
Nigerian Cultivar.Fert Average Yield	350	105	7000	54.47	13.13	875.58	1652.9	918.31

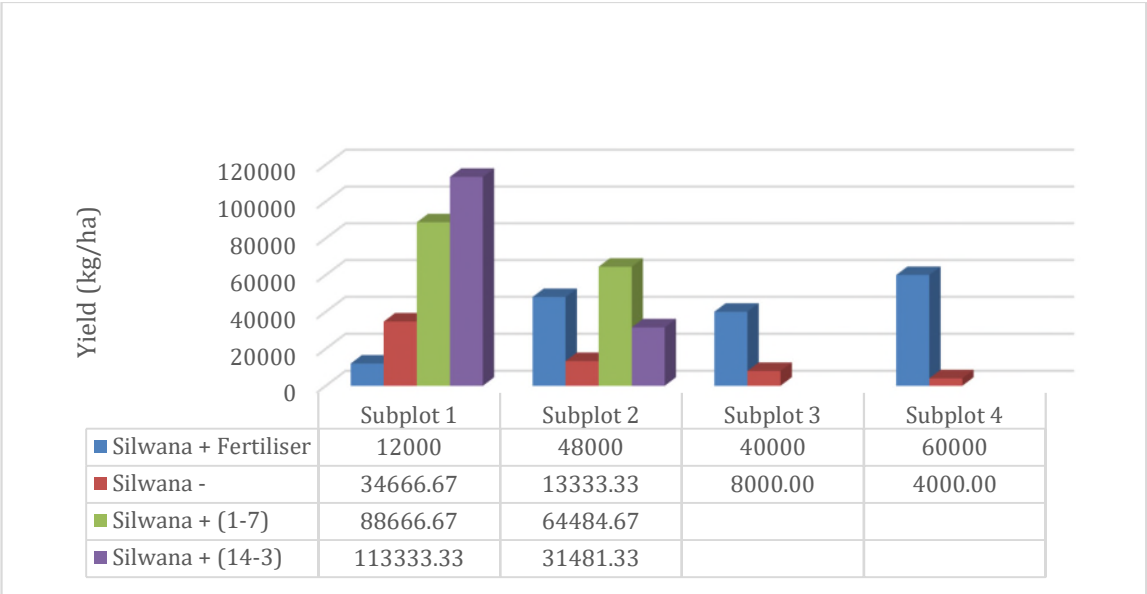
4.6.2 Shoot Biomass

The dry matter yield of the shoots was compared amongst the three treatments of fertilizer, bio-inoculant and a negative control of no treatment to figure out which was more effective by measuring the yield. 10 plants were chosen as a standard per cultivar in a treatment except for those plots that had a few plants due to reasons mentioned above. The graphs that follow are the results of these comparisons in terms of the shoots, roots and the grain yield of each cultivar planted.



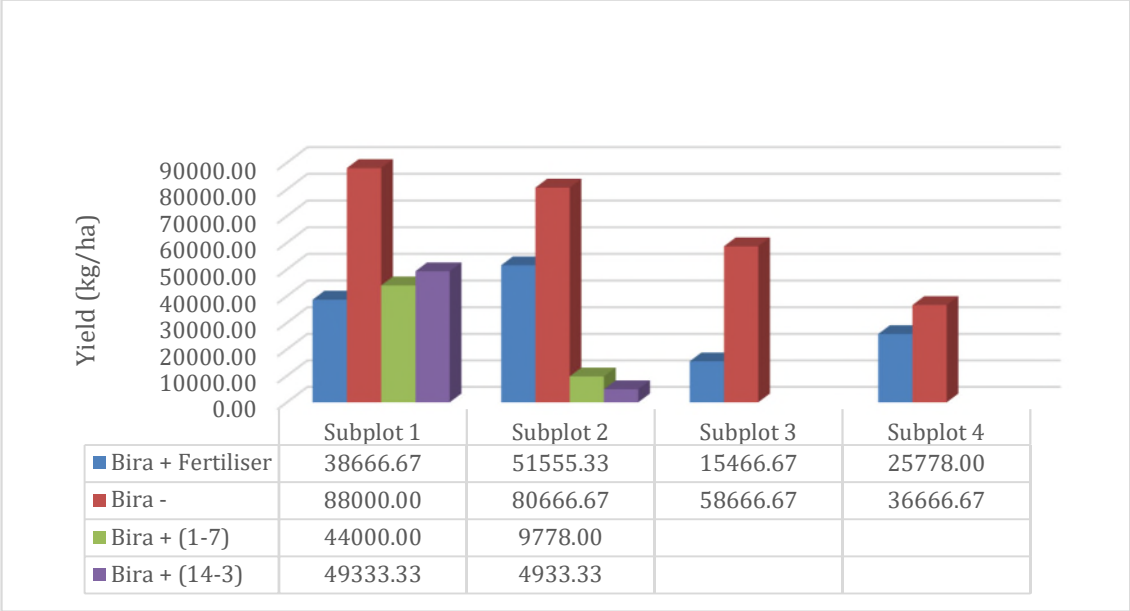
	Subplot 1	Subplot 2	Subplot 3	Subplot 4
Lutembwe + Fertiliser	41333.33	56363.64	37576.00	60121.33
Lutembwe -	114666.67	141647.33	67451.33	13490.27
Lutembwe + (1-7)	61333.33	92000.00	24533.33	79733.33
Lutembwe + (14-3)	86666.67	93888.67	36111.33	122778.00

Figure 15a. A comparison of the plant dry matter yield of the Lutembwe cultivar across the three treatments.



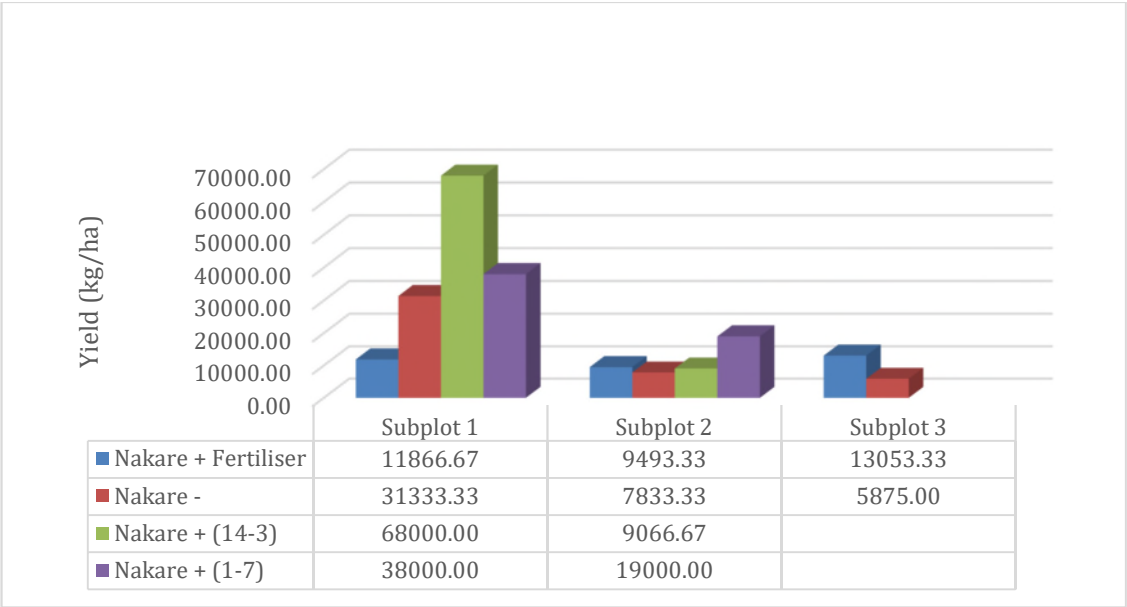
	Subplot 1	Subplot 2	Subplot 3	Subplot 4
Silwana + Fertiliser	12000	48000	40000	60000
Silwana -	34666.67	13333.33	8000.00	4000.00
Silwana + (1-7)	88666.67	64484.67		
Silwana + (14-3)	113333.33	31481.33		

Figure 15b. Showing a comparison of the plant dry matter yield of the Silwana cultivar across the three treatments. Subplots 3 and 4 did not have the bio-inoculant treatments.



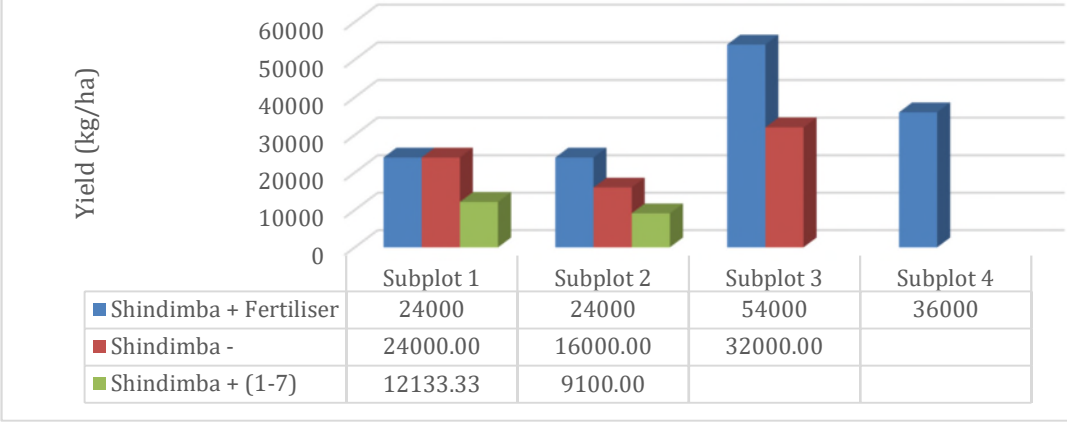
	Subplot 1	Subplot 2	Subplot 3	Subplot 4
Bira + Fertiliser	38666.67	51555.33	15466.67	25778.00
Bira -	88000.00	80666.67	58666.67	36666.67
Bira + (1-7)	44000.00	9778.00		
Bira + (14-3)	49333.33	4933.33		

Figure 15c. Showing a comparison of the plant dry matter yield of the Bira cultivar across the three treatments. Subplot 3 and subplot 4 did not have the bio-inoculant treatments.



	Subplot 1	Subplot 2	Subplot 3
Nakare + Fertiliser	11866.67	9493.33	13053.33
Nakare -	31333.33	7833.33	5875.00
Nakare + (14-3)	68000.00	9066.67	
Nakare + (1-7)	38000.00	19000.00	

Figure 15d. Showing a comparison of the plant dry matter yield of the Nakare cultivar across the three treatments. Only 3 subplots were harvested and subplot 3 lacked the bio-inoculants.

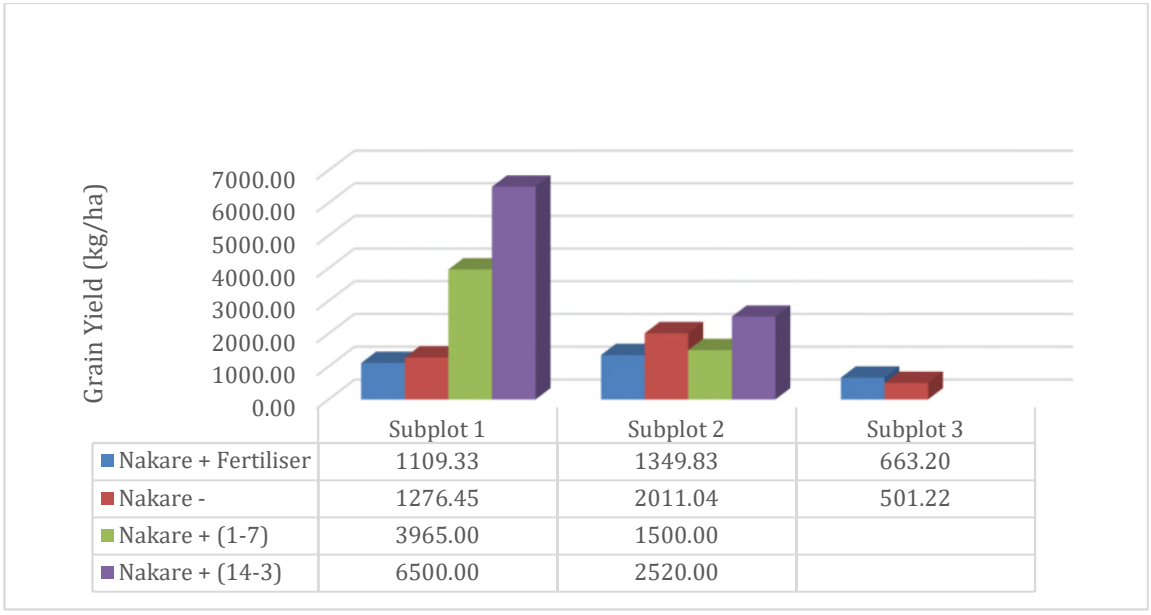


	Subplot 1	Subplot 2	Subplot 3	Subplot 4
Shindimba + Fertiliser	24000	24000	54000	36000
Shindimba -	24000.00	16000.00	32000.00	
Shindimba + (1-7)	12133.33	9100.00		

Figure 15e. Showing a comparison of the plant dry matter yield of the Shindimba cultivar across the three treatments. Only one bio-inoculant (1-7) was applied but not on subplots 3 and 4.

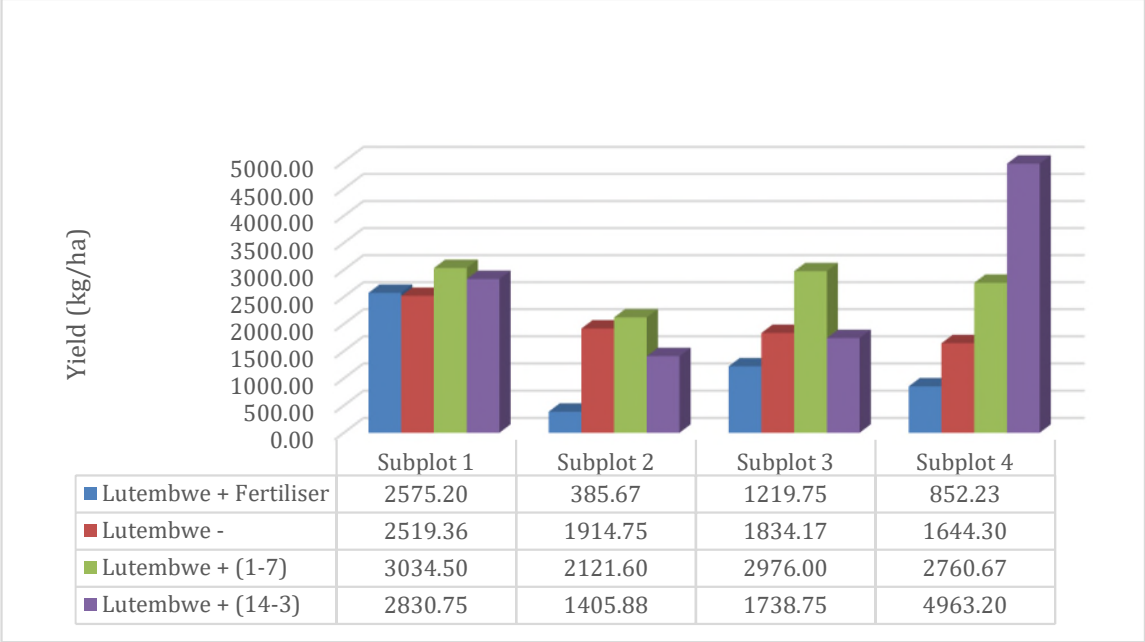
4.6.3 Grain and Root yield

The graphs that follow show the grain yield per hectare of each subplot across all the studied treatments, some treatments had less than 4 subplots of grains harvested due to some subplots drying up and hence nothing remained to be harvested.



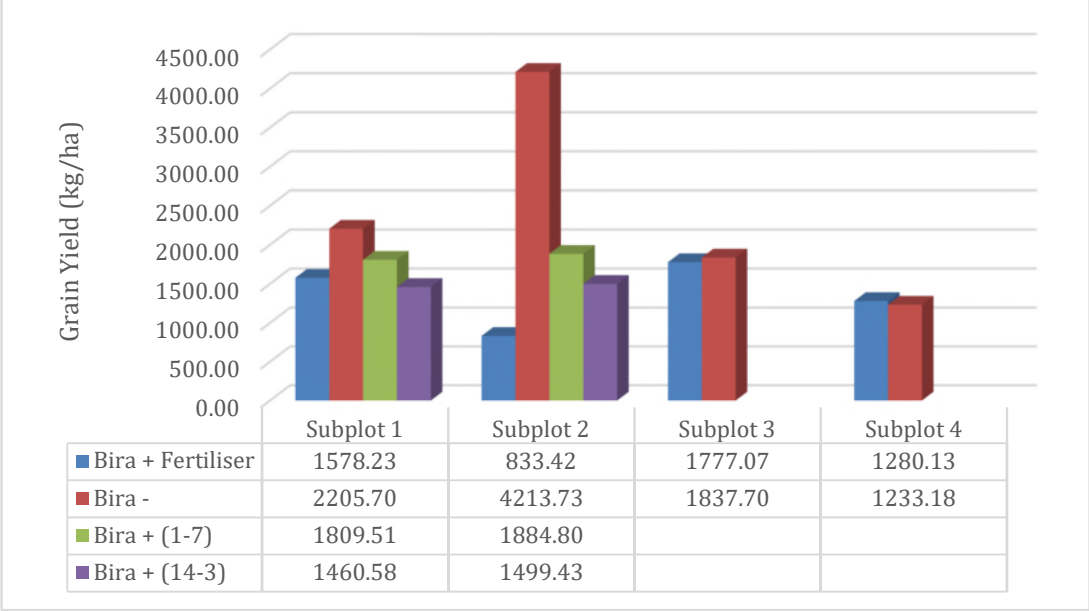
	Subplot 1	Subplot 2	Subplot 3
Nakare + Fertiliser	1109.33	1349.83	663.20
Nakare -	1276.45	2011.04	501.22
Nakare + (1-7)	3965.00	1500.00	
Nakare + (14-3)	6500.00	2520.00	

Figure 16a. Graphical representation of the Grain Yield of the Nakare cultivar across all the three treatments. Only 3 subplots were harvested and subplot 3 lacked the bio-inoculant treatment.



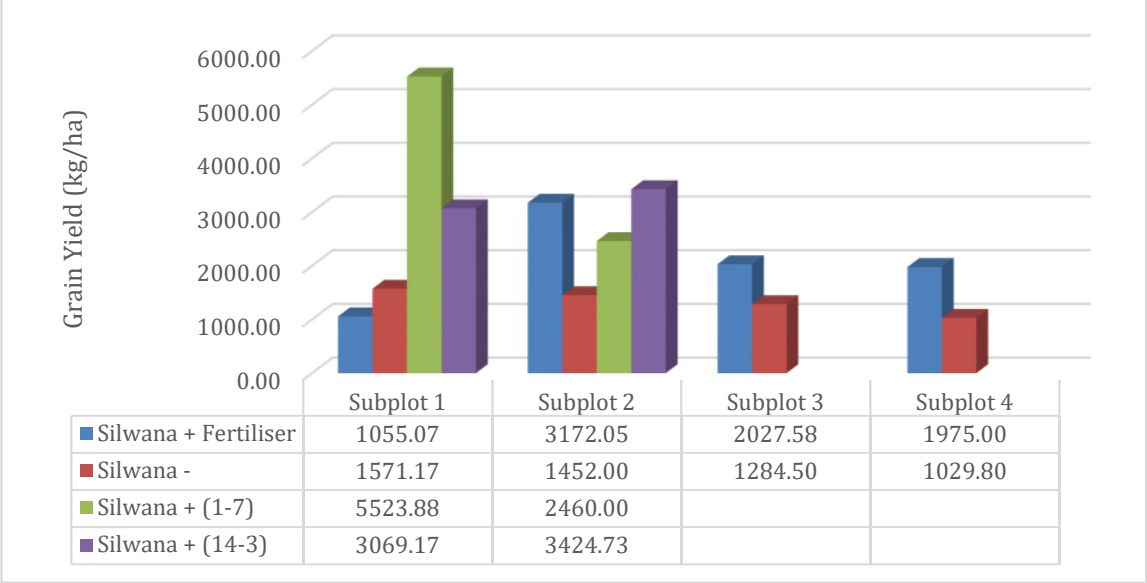
	Subplot 1	Subplot 2	Subplot 3	Subplot 4
Lutembwe + Fertiliser	2575.20	385.67	1219.75	852.23
Lutembwe -	2519.36	1914.75	1834.17	1644.30
Lutembwe + (1-7)	3034.50	2121.60	2976.00	2760.67
Lutembwe + (14-3)	2830.75	1405.88	1738.75	4963.20

Figure 16b. Graphical representation of the Grain Yield of the Lutembwe cultivar across all the three treatments.



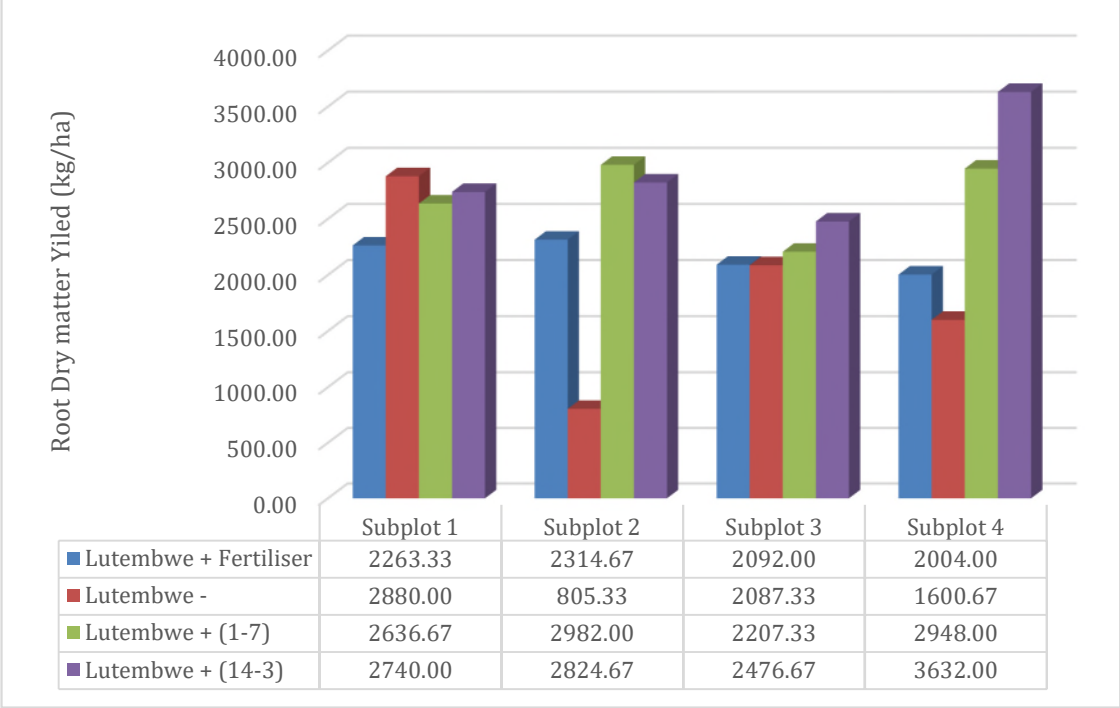
	Subplot 1	Subplot 2	Subplot 3	Subplot 4
Bira + Fertiliser	1578.23	833.42	1777.07	1280.13
Bira -	2205.70	4213.73	1837.70	1233.18
Bira + (1-7)	1809.51	1884.80		
Bira + (14-3)	1460.58	1499.43		

Figure 16c. Graphical representation of the Grain yield of Bira cultivar across all the three treatments. Subplots 3 and 4 lacked the bio-inoculant treatment.



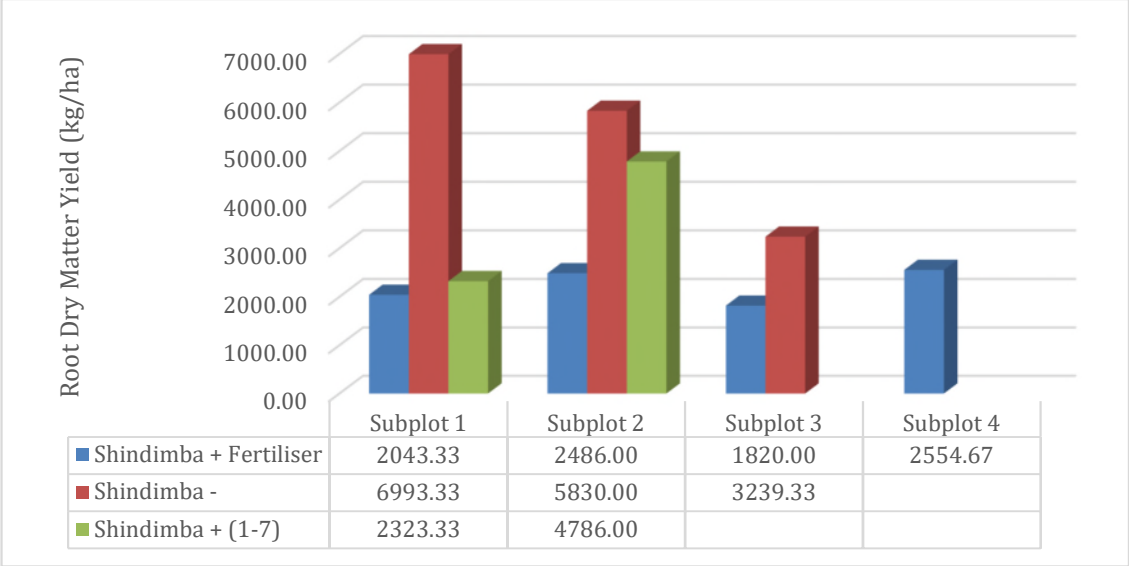
	Subplot 1	Subplot 2	Subplot 3	Subplot 4
Silwana + Fertiliser	1055.07	3172.05	2027.58	1975.00
Silwana -	1571.17	1452.00	1284.50	1029.80
Silwana + (1-7)	5523.88	2460.00		
Silwana + (14-3)	3069.17	3424.73		

Figure 16d. Graphical representation of the Grain yield of the Silwana cultivar across all the three treatments. Subplots 3 and 4 lacked the bio-inoculant treatment.



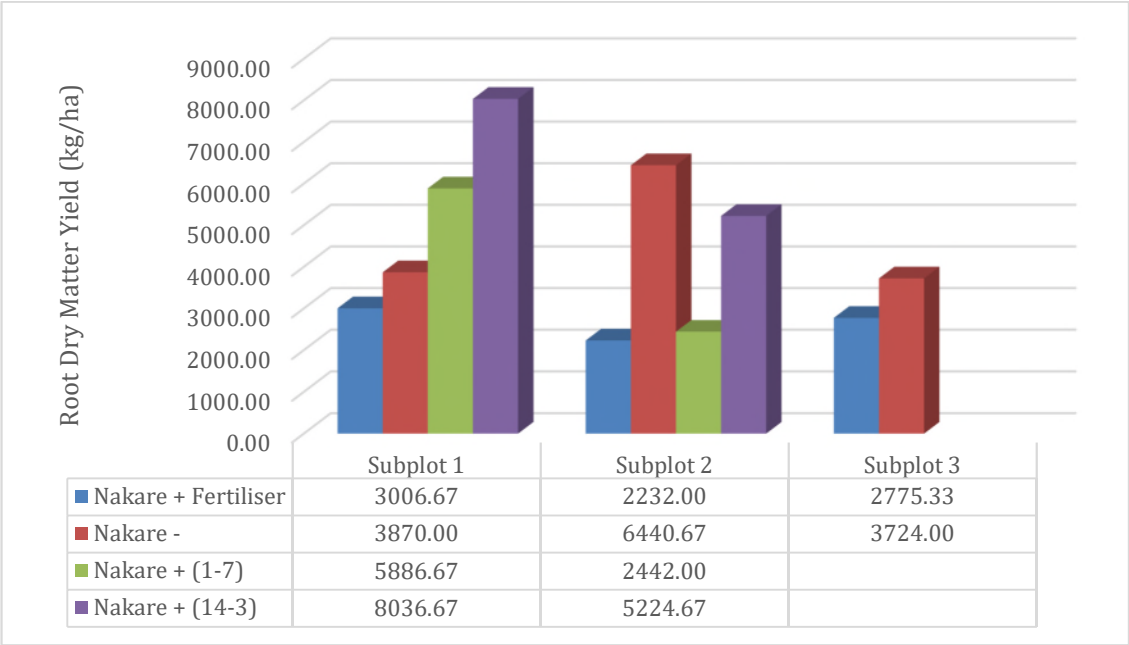
	Subplot 1	Subplot 2	Subplot 3	Subplot 4
Lutembwe + Fertiliser	2263.33	2314.67	2092.00	2004.00
Lutembwe -	2880.00	805.33	2087.33	1600.67
Lutembwe + (1-7)	2636.67	2982.00	2207.33	2948.00
Lutembwe + (14-3)	2740.00	2824.67	2476.67	3632.00

Figure 17a. A graphical representation of Lutembwe's Root yield across all the three treatments.



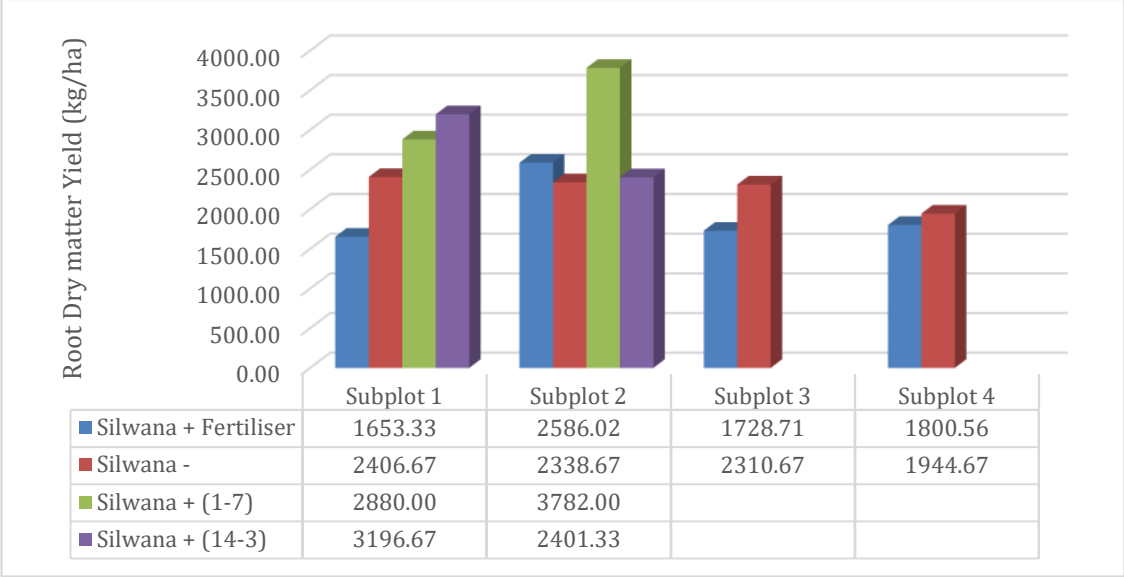
	Subplot 1	Subplot 2	Subplot 3	Subplot 4
Shindimba + Fertiliser	2043.33	2486.00	1820.00	2554.67
Shindimba -	6993.33	5830.00	3239.33	
Shindimba + (1-7)	2323.33	4786.00		

Figure 17b. Shindimba's root yield across all the three treatments with subplots 3 lacking the bio-inoculant treatment and Subplot 4 only having the fertilizer treatment.



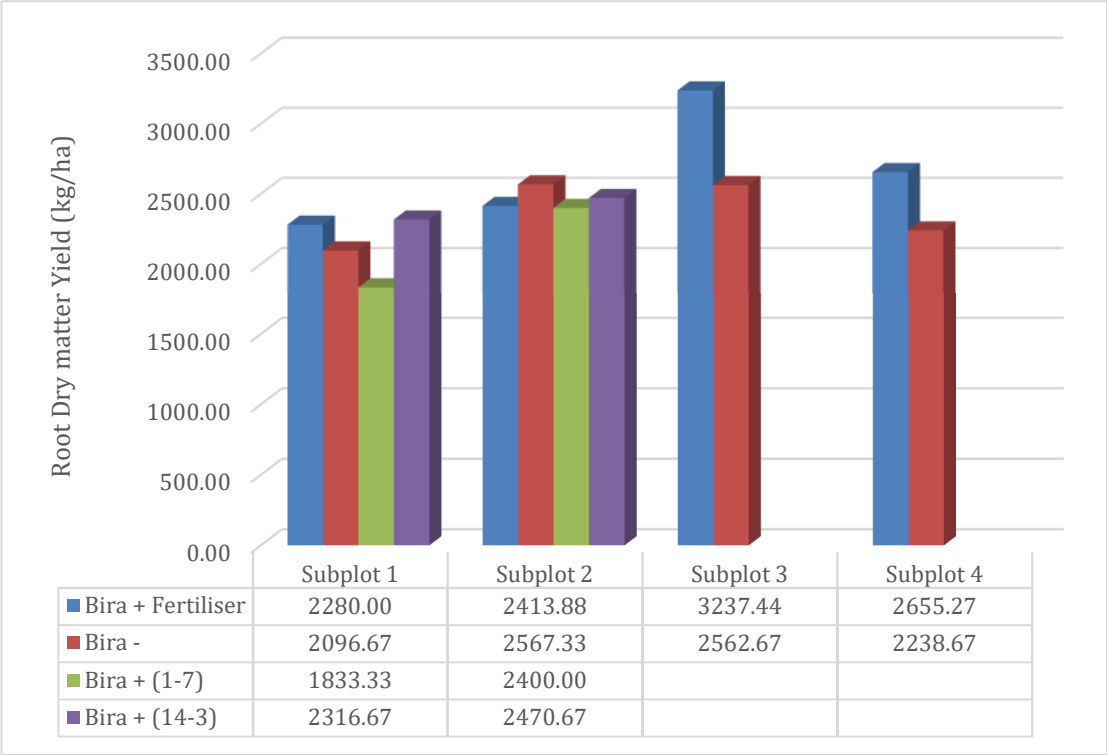
	Subplot 1	Subplot 2	Subplot 3
Nakare + Fertiliser	3006.67	2232.00	2775.33
Nakare -	3870.00	6440.67	3724.00
Nakare + (1-7)	5886.67	2442.00	
Nakare + (14-3)	8036.67	5224.67	

Figure 17c. Graphical representation of the Nakare cultivars' root dry matter yield, with three subplots of the cultivar being harvested.



	Subplot 1	Subplot 2	Subplot 3	Subplot 4
Silwana + Fertiliser	1653.33	2586.02	1728.71	1800.56
Silwana -	2406.67	2338.67	2310.67	1944.67
Silwana + (1-7)	2880.00	3782.00		
Silwana + (14-3)	3196.67	2401.33		

Figure 17d. Graphical representation of the Silwana cultivars' root dry matter yield, with subplots 3 and 4 not having the inoculant treatment.



	Subplot 1	Subplot 2	Subplot 3	Subplot 4
Bira + Fertiliser	2280.00	2413.88	3237.44	2655.27
Bira -	2096.67	2567.33	2562.67	2238.67
Bira + (1-7)	1833.33	2400.00		
Bira + (14-3)	2316.67	2470.67		

Figure 17e. Graphical representation of the Bira cultivars' root dry matter yield, with Subplots 3 and 4 only having the fertilizer and non-inoculant treatments harvested but no inoculant.

4.7 Data analysis of Cowpea Yield parameters

A 2-Way ANOVA was carried out on the above datasets of namely: Shoot Dry Matter Yield (kg/ha), Root Dry Matter Yield (kg/ha) and Grain yield (kg/ha) after the ANOVA assumptions were met i.e. data being normally distributed, independence of samples and equal variances between the three treatments. This Analysis of variance tested the hypotheses below.

1. H_0: There is no significance difference in the Shoot, Root and Grain yields across the 3 different treatments of the different cultivars.
2. H_0: There is no significant difference in the Shoot, Root and Grain yields across the different cultivars.

4.7.1 Plant Biomass Yield

Figure 18: Lutembwe shoot immediately after harvesting.

The p-value of treatment to plant dry matter yield was 0.434, indicating a lack of significant difference between the treatments and shoot biomass yield at the 0.05 level of

significance. On the other hand there was a statistical difference between cultivar name and plant dry matter yield and the interaction between Treatment and cultivar name at the 0.05 level of significance with p values of 0.00 and 0.016 respectively. The pairwise comparisons of the cultivars is shown in the Appendix 2.

Therefore there was insufficient evidence to reject the first null hypothesis and enough evidence to reject the second null hypothesis. Indicating a significant difference in plant dry matter yield across the six different cultivars.

4.7.2 Root biomass Yield

Some roots had massive tumor like growth when excavated such as the root of the cultivar shown below

Figure 19. The extensive root yield of the Shindimba cultivar without inoculant.

From the analysis of variance of the root dry matter yield, there was a statistical difference in the means of the yield across the treatments and cultivar names with p-values of 0.001 and 0.000 respectively. Also, the interaction of Treatment and Cultivar name with Root

Dry matter yield was of statistical significance at an alpha level of 0.05. The pairwise comparisons and other supporting data are in Appendix 2. The root yield was important in our study because a large root weight provides for a larger surface area for microbes to colonize the legume.

4.7.3 Grain Yield

For Treatment and Cultivar name statistics, the null hypotheses state:

1. H_0: There is no significance difference in the Grain yields across the 3 different treatments.
2. H_0: There is no significant difference in the Grain yields across the different cultivars.

These hypotheses are rejected at the 0.05 level of significance. For the grain yield statistical analysis, there was a statistical difference across the means of the different treatments and cultivar names at the 0.05 level of significance with p-values of 0.00 and 0.009 respectively. On the contrary there was no statistical difference in means of the interaction of Treatment and Cultivar name with grain yield with a p-value of 0.059.

Chapter 5. Discussion

Bradyrhizobium spp. have been reported to be the dominant rhizobia colonizing most legumes in the Kavango region (Grönemeyer & Reinhold-Hurek, 2018) . These are slow growers that have been reported to induce nodulation in both legumes and non-legume plants (Jaiswal et al. 2017). This concurs with the study done by Ndugu et al. (2018) who reported that the genus *Bradyrhizobium* was the most dominant cowpea root nodule symbiont based on an isolation-based approach.

From the obtained isolates, 4 bacterial species were identified using sequence analysis from cowpea nodules from the Kavango region. These were characterized as nitrogen fixers in previous studies (Grönemeyer et al. 2015: Grönemeyer et al. 2016: Ramirez-Bahena et al. 2016: Yao et al. 2015) and were observed to be slow growers during culturing. Cowpea is known to be promiscuous in nature by accepting microbes from different genera (Guimaraes, et al., 2012). The isolated *Bradyrhizobium* spp. from this study are so diverse and possess properties that are crucial as bio-fertilizers and have been reported to have plant growth promoting characteristics (Suyal, et al., 2016).

Bradyrhizobium vignae type strain 7-2 was first isolated and characterized in the study area (Mashare) by Grönemeyer et al. (2016). This strain is a member of the larger *Bradyrhizobium vignae* group. The group comprises 21 strains and the original crop of isolation of *Bradyrhizobium vignae* 7-2 was *Vigna unguiculata* (Grönemeyer et al. 2016). Grönemeyer et al. (2016) reported a DNA G+C content of the 7-2 strain of 65.4mol% and there is no other mention of this *Bradyrhizobium vignae* 7-2 strain apart from that of

Grönemeyer et al. (2014) and Grönemeyer et al. (2016). *Bradyrhizobium vignae* was also isolated from *Vigna unguiculata* in Spain by Bejrarano et al. (2014).

In this study, *Bradyrhizobium americanum* strain CMVU44 was isolated after being identified using sequence analysis. This strain has never been reported in this region before. This was characterized as a novel species after it was isolated from *Centrosema macrocarpum* root nodules in Venezuela (Ramirez-Bahena et al. 2016). According to Ramirez-Bahena et al. (2016), they had a 62.7% G+C content which gives an optimal melting point and enables an easy design for the primers. This case illustrates the reported promiscuity of *Vignia unguiculata* as it is known to trap a wide host of rhizobia. *Bradyrhizobium americanum* had not been reported in cowpea prior to this study.

Bradyrhizobium kavangense strain 14-3 is one of the strains that was used as a bio-inoculant in this study. A bio-inoculant has to be native in an area for it to successfully establish itself. The strain 14-3 was chosen as it had been isolated from the Kavango region as a novel species by Grönemeyer et al. (2015). To the best of my knowledge, this strain has only been reported by (Grönemeyer et al. 2015).

Bradyrhizobium ferriligni strain CCBAU 51502 was also isolated in this study. This was the second time it has been reported. This strain was first isolated from *Erythrophloeum fordii* (Rosewood) which is dominant in Asia (Yao et al. 2015). It has a DNA G+C content of 61.95 mol % (Yao et al. 2015).

To my knowledge there is no information on how these isolates affect biomass or their effect on yield. They have not been widely reported and some had such as *Bradyrhizobium kavangense* and *Bradyrhizobium vignae* were first isolated as novel species from the same

region in 2014 and 2016 respectively by Grönemeyer et al. (2015) and Grönemeyer et al. (2016).

In addition to the importance of isolates it is also of utmost importance to assess the yield of the different cowpea cultivars in relation to their subjected treatments and how these yield components respond to reach a conclusion as to which treatment would best suit a farmer's needs. From the yield assessment of the different cowpea cultivars in terms of plant dry matter yield, there was no statistical difference in the obtained plant dry matter measurements across the three different treatments ($p > 0.05$). This is despite there being an observed difference in the obtained mean values of the treatments. This correlates to a study done by Andrade et al. (2013), who reported that the use of mineral fertilizer did not have any significant effect on the shoot dry biomass.

My results indicate that the use of nitrogen fertilizer to enhance shoot biomass of the bean has not been significant with a p value of 0.434 in comparison to the use of bio-inoculant treatment. This was in line with Hungria et al. (2006) who stated that soybean that was treated with nitrogen fertilizer, did not indicate any benefits as compared to the application of bio-inoculants on the soybean grown in Brazilian soils.

With respect to the different cultivars' relation to plant biomass yield, it was observed that the obtained yield measurements differed significantly depending by the type of cultivar used with a p-value less than 0.05. For instance, when subjected to all the three different treatments, the cultivar Lutembwe had the largest plant dry yield (Table 1, 2 and 3) as compared to the other cultivars. To my best of knowledge there is no study that has been carried out elsewhere on yield comparisons of these cultivars. Hence for farmers that would like to grow cowpea for forage use, Lutembwe would be the recommended cultivar.

Lutembwe's plant dry matter yield is seconded by Silwana followed by Bira, with the Nigerian cultivar not faring well in terms of shoot yield as compared to the other cultivars. This poor performance of the cultivar could be attributed to it not natively grown in the Southern African soils hence the poor yield.

The cultivars Lutembwe and Nakare had a poor shoot dry matter yield with the fertilizer treatments with Bira and Shindimba reporting the lowest shoot dry matter yield with the bio-inoculant treatment while Silwana and Nakare reported the highest yield with bio-inoculant. The Nigerian cultivar was only subjected to the fertilizer treatment due to an insufficient amount of seeds from Nigeria. Therefore with regards to the shoot biomass, with fertilizer treatment maximum yield was achieved by Lutembwe, while with bio-inoculant maximum yields are achieved by the cultivar Silwana. Such information is handy when cowpea is cultivated for its shoot biomass with an indication that the use of fertilizer when cultivating cowpea for this purpose is non profitable.

There is a need to measure the root dry weight in crops because in most cases it is a relative indicator that predicts shoot dry matter yield as it tends to give a relationship between the two (Fageria, 2005). Based on our analysis, there was a significant interaction between the means of the treatment, cultivar name, interaction of treatment and cultivar name and root dry matter yield at the 0.05 level of significance.

These results illustrate that if the sole purpose of growing cowpea is to enhance the root dry matter yield then the bio inoculant treatment should be prioritized over the usage of mineral fertilizers. The post hoc tests revealed a significant mean difference of 1,229.13kg/ha of the yield between the fertilizer treatment and the bio-inoculant treatments. There was no significant difference between the bio-inoculant root yield and

the negative control, in addition to this, there was a significant mean difference of 1,044.57 between the fertilizer treatment and the negative control at the 0.05 level of significance.

The Nakare and Shindimba cultivars had the largest estimated means of root dry matter yield. Surprisingly, these cultivars had the smallest shoot biomass yield, hence it can be hypothesized that the root dry matter yield does not correlate to the shoot dry matter yield based on this study's findings. Those with a large root dry matter mean had gigantic roots that appeared to have tumor like growths. An increased root yield leads to an increased root surface area resulting in an increase in uptake of water and minerals for the crop (Matiru & Dakora, 2004).

Cowpea grain yield is considered one of the most important parameters for farmers in terms of assessing how different treatments affect yield (Horn et al. 2017). This is due to the grains having a high protein content for consumption (Hungria et al. 2006). In addition, the more the grain yield the more profits the farmers expect from the legumes and also this entails the farmer has a surplus to plant for the next season.

There was a significant difference in the means of the different treatments and cultivars with relation to the grain yields. The bio-inoculant recorded a grain yield of 2,651.24kg/ha as compared to the bio-inoculant and the negative control. This figure is substantially high as compared to the grain yield of 1,441kg/ha reported by (Dekhane et al. 2011). A 10% increase in grain yield between a treatment and no treatment is a substantial indication that a treatment is working according to Ronner et al. (2016) henceforth this study's results indicate that the bio-inoculant treatment worked by producing a grain yield greater than 10% as compared to the negative control. These findings are close to the 30% increase in grain yield by bio-inoculant application reported by (Martins et al. 2003).

If a farmer's aim is to increase the grain yield of their cowpea then it's recommended to use bio-inoculant as opposed to mineral fertilizer because not only is it eco-friendly but also is a cheaper alternative, this is supported by our findings. In addition to this, the cultivars Silwana, Nakare and Lutembwe gave the largest grain yield with the bio-inoculant treatment with yields of 3,619.45, 3,621.25 and 2,728.92 kg/ha respectively and Shindimba had the lowest grain yield.

Chapter 6: Conclusion and Recommendations

To sum up, as per objectives, cowpea nodule symbionts were isolated and identified as related to the genus *Bradyrhizobium*. These are good candidates for the development of bio-fertilizers as they possess the characteristics of a good bio-inoculant and have proved to have plant growth promoting capabilities. Further, cowpea yield was substantially increased in the bio-inoculant treatment as compared to the fertilizer treatment and the negative control under rain fed conditions. The results of this study were significant in providing the subsistence farmers with bio-inoculants that can effectively fix biological nitrogen when in symbiosis with cowpea since the inoculants ecological origin (Kavango soils) and their climatic adaptation are known. This will in turn increase profits and crop productivity at large as less money will be spent on chemicals in trying to enrich the poor soils in the regions and Namibia at large.

Therefore based on the results obtained in this study, it is recommended that another similar study be carried as this was a pilot study. More resources should be channeled into this effort as the development of a bio-inoculant technology will result in an economical gain for the farmers. Hence other studies under rain fed conditions to support our study are necessary. Other indigenous legumes such as *Cicer arietinum* (Chickpea) or *Vigna subterranea* (Bambara groundnut) could be used as trapping plant so a wide range of microbes with plant growth promoting characteristics can be obtained.

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Appendices

Appendix 1

Table 1. Raw harvest data for the non-inoculant plots (-)

Cultivar Name & Nu	Shoot Wet weight(g	Shoot Dry weight(g)	Plant Dry matter yiel	Root wet weight(g)	Root Dry Weight(g)	Root Dry Matter yiel	Grain yield (g)	Grain yield(kg/ha)
NAKARE -								
7								
Subplot 1	800	470	31333.33	114.76	58.05	3870.00	2297.61	1276.45
Subplot 2	200	117.5	7833.33	191	96.61	6440.67	3619.88	2011.04
Subplot 3	150	88.125	5875.00	110.44	55.86	3724.00	902.19	501.22
SILWANA -								
10								
Subplot 1	2600	520	34666.67	116.64	36.1	2406.67	2828.10	1571.17
Subplot 2	1000	200	13333.33	113.34	35.08	2338.67	2613.60	1452.00
Subplot 3	600	120	8000.00	112	34.66	2310.67	2312.10	1284.50
Subplot 4	300	60	4000.00	94.24	29.17	1944.67	1853.64	1029.80
SHINDIMBA -								
10								
Subplot 1	300	360	24000.00	409.12	104.9	6993.33	294.15	163.42
Subplot 2	200	240	16000.00	341.08	87.45	5830.00	217.15	120.64
Subplot 3	400	480	32000.00	267.52	48.59	3239.33	308.25	171.25
LUTEMBWE -								
10								
Subplot 1	3400	1720	114666.67	164.52	43.2	2880.00	4534.85	2519.36
Subplot 2	4200	2124.71	141647.33	46	12.08	805.33	3446.55	1914.75
Subplot 3	2000	1011.77	67451.33	119.24	31.31	2087.33	3301.50	1834.17
Subplot 4	400	202.354	13490.27	91.44	24.01	1600.67	2959.74	1644.30
BIRA -								
10								
Subplot 1	2400	1320	88000.00	150.96	31.45	2096.67	3970.26	2205.70
Subplot 2	2200	1210	80666.67	181.84	38.51	2567.33	7584.72	4213.73
Subplot 3	1600	880	58666.67	184.52	38.44	2562.67	3307.86	1837.70
Subplot 4	1000	550	36666.67	116.2	33.58	2238.67	2219.72	1233.18

Table 2: Raw data for each subplot of the bio-inoculant plots

Cultivar Name & Num	Shoot Wet weight(g)	Shoot Dry weight(g)	Plant Dry matter yiel	Root wet weight(g)	Root Dry Weight(g)	Root Dry Matter yield	Grain yield (g)	Grain yield(kg/ha)
LUTEMBWE + Bradyrhizobium stain 1-7								
10								
Subplot 1	2000	920	61333.33	143.52	39.55	2636.67	5462.10	3034.50
Subplot 2	3000	1380	92000.00	162.32	44.73	2982.00	3818.88	2121.60
Subplot 3	800	368	24533.33	120.14	33.11	2207.33	5356.80	2976.00
Subplot 4	2600	1196	79733.33	160.48	44.22	2948.00	4969.20	2760.67
LUTEMBWE + Bradyrhizobium strain 14-3								
10								
Subplot 1	2400	1300	86666.67	128.9	41.1	2740.00	5095.35	2830.75
Subplot 2	2600	1408.33	93888.67	132.88	42.37	2824.67	2530.58	1405.88
Subplot 3	1000	541.67	36111.33	116.52	37.15	2476.67	3129.75	1738.75
Subplot 4	3400	1841.67	122778.00	164.6	54.48	3632.00	8933.76	4963.20
NAKARE + Bradyrhizobium strain 14-3								
10								
Subplot 1	1500	1020	68000.00	254.2	120.55	8036.67	11700.00	6500.00
Subplot 2	200	136	9066.67	165.26	78.37	5224.67	4536.00	2520.00
NAKARE + Bradyrhizobium stain 1-7								
10								
Subplot 1	400	570	38000.00	184.25	88.3	5886.67	7137.00	3965.00
5								
Subplot 2	200	285	19000.00	76.2	36.63	2442.00	2700.00	1500.00
SHINDIMBA + Bradyrhizobium stain 1-7								
5								
Subplot 1	200	182	12133.33	123.4	34.85	2323.33	2550.00	1416.67
Subplot 2	150	136.5	9100.00	254.2	71.79	4786.00	3292.80	1829.33
SHINDIMBA + Bradyrhizobium strain 14-3								
0								
SILWANA + Bradyrhizobium stain 1-7								
10								
Subplot 1	2200	1330	88666.67	112.35	43.2	2880.00	9942.98	5523.88
Subplot 2	1600	967.27	64484.67	147.55	56.73	3782.00	4428.00	2460.00
SILWANA + Bradyrhizobium strain 14-3								
10								
Subplot 1	3600	1700	113333.33	192.35	47.95	3196.67	5524.50	3069.17
Subplot 2	1000	472.22	31481.33	144.5	36.02	2401.33	6164.52	3424.73
BIRA + Bradyrhizobium stain 1-7								
10								
Subplot 1	900	660	44000.00	112.44	27.5	1833.33	3257.12	1809.51
Subplot 2	200	146.67	9778.00	147.2	36	2400.00	3392.64	1884.80
BIRA + Bradyrhizobium strain 14-3								
10								
Subplot 1	2000	740	49333.33	138.16	34.75	2316.67	2629.05	1460.58
Subplot 2	200	74	4933.33	147.36	37.06	2470.67	2698.97	1499.43

Table 3: Raw data for the fertilizer treatment of all subplots

Cultivar Name	Shoot Wet wei	Shoot Dry Wet(g)	Plant dry matter yi	Root wet weight(g	Root Dry weight(g)	Root dry matter yie	Grain Yield (g)	Grain yield(kg/ha)
LUTEMBWE.Fertiliser								
10								
Subplot 1	2200	620	41333.33	124.1	33.95	2263.33	4635.36	2575.20
Subplot 2	3000	845.45	56363.64	126.92	34.72	2314.67	694.20	385.67
Subplot 3	2000	563.64	37576.00	114.72	31.38	2092.00	2195.55	1219.75
Subplot 4	3200	901.82	60121.33	109.88	30.06	2004.00	1534.02	852.23
SHINDIMBA.Fertiliser								
10								
Subplot 1	400	360	24000	135.21	30.65	2043.33	411.97	228.87
Subplot 2	400	360	24000	164.51	37.29	2486.00	430.41	239.12
Subplot 3	900	810	54000	120.44	27.3	1820.00	637.28	354.04
Subplot 4	600	540	36000	169.04	38.32	2554.67	376.20	209.00
Nakare.Fertiliser								
10								
Subplot 1	1000	178	11866.67	266.2	45.1	3006.67	1996.80	1109.33
Subplot 2	800	142.4	9493.33	197.6	33.48	2232.00	2429.70	1349.83
Subplot 3	1100	195.8	13053.33	245.7	41.63	2775.33	1193.76	663.20
SILWANA.Fertiliser								
10								
Subplot 1	600	180	12000	93.88	24.8	1653.33	1899.12	1055.07
Subplot 2	2400	720	48000	146.84	38.79	2586.02	5709.69	3172.05
Subplot 3	2000	600	40000	98.16	25.93	1728.71	3649.65	2027.58
Subplot 4	3000	900	60000	102.24	27.01	1800.56	3555.00	1975.00
BIRA.Fertiliser								
10								
Subplot 1	3000	580	38666.67	89.92	34.2	2280.00	2840.82	1578.23
Subplot 2	4000	773.33	51555.33	95.2	36.21	2413.88	1500.15	833.42
Subplot 3	1200	232	15466.67	127.68	48.56	3237.44	3198.72	1777.07
Subplot 4	2000	386.67	25778.00	104.72	39.83	2655.27	2304.23	1280.13
NIGERIAN Cultivar.Fertiliser								
5								
Subplot 1	600	180	12000	80.04	19.3	1286.67	2116.80	1176.00
Subplot 2	200	60	4000	41.44	9.99	666.16	1670.69	928.16
Subplot 3	200	60	4000	38.03	9.17	611.34	1499.46	833.03
Subplot 4	400	120	8000	58.36	14.07	938.15	1324.86	736.03

Appendix 2

Data Analysis

Normality Tests

Descriptives

	Treatment			Statistic	Std. Error
Plant_Dry_Matter_Yield	Fertiliser	Mean		29881.4913	3997.32379
		95% Confidence Interval for Mean	Lower Bound	21591.5491	
			Upper Bound	38171.4335	
		5% Trimmed Mean		29640.2401	
		Median		25778.0000	
		Variance		367507742.8	
		Std. Deviation		19170.49146	
		Minimum		4000.00	
		Maximum		60121.33	
		Range		56121.33	
		Interquartile Range		36000.00	
		Skewness		.201	.481
		Kurtosis		-1.426	.935

Based on the Descriptive statistics of the plant dry matter yield in terms of yield per hectare for the fertilizer treatment, the Z values when the measure is divided by its stand error lies between -1.96 and +1.96. For example the skewness Z value is (0.201/0.481) = 0.418 and the Kurtosis Z value is (-1.426/0.935)= -1.525. Both values indicate a little skewness and kurtosis but lie within the range of -1.96 and +1.96. Hence based on these 2 it can be said that the plant dry matter yield values are approximately normally distributed. This is supported by the Normal Q-Q Plot below which shows that the data are approximately normally distributed along the line and also by a visual inspection of the histograms.

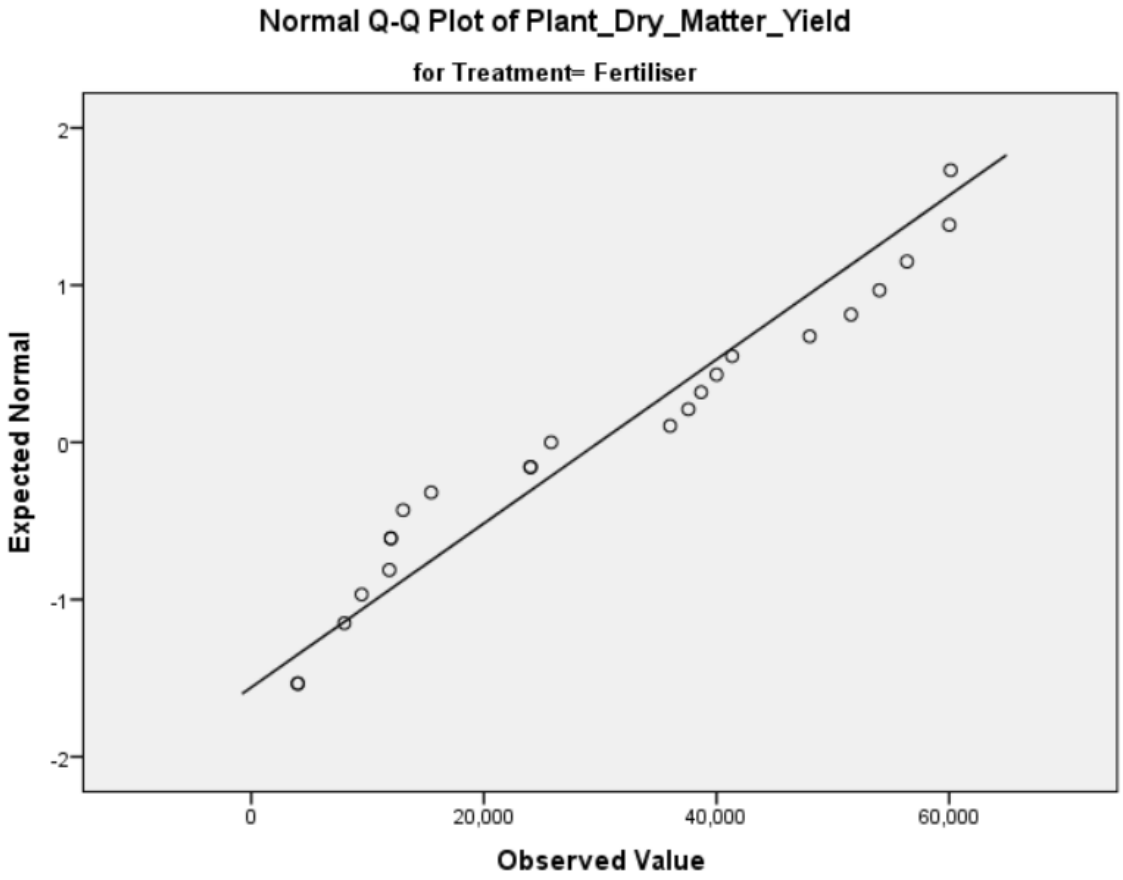
SPSS output of the plant dry matter yield tests between-subjects effects

Tests of Between-Subjects Effects

Dependent Variable: Plant_Dry_Matter_Yield

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	39886390060.000[a]	15	2659092671.000	4.194	.000	.572
Intercept	73585686100.000	1	73585686100.000	116.055	.000	.712
Treatment	1076062735.000	2	538031367.300	.849	.434	.035
Cultivar_Name	20984435470.000	5	4196887093.000	6.619	.000	.413
Treatment * Cultivar_Name	13659285540.000	8	1707410693.000	2.693	.016	.314
Error	29800844500.000	47	634060521.300			
Total	178972827000.000	63				
Corrected Total	69687234570.000	62				

a. R Squared = .572 (Adjusted R Squared = .436)



Descriptives

	Treatment			Statistic	Std. Error
Plant_Dry_Matter_Yield	Negative control	Mean		48883.7073	10790.80697
		95% Confidence Interval for Mean	Lower Bound	25739.7282	
			Upper Bound	72027.6865	
		5% Trimmed Mean		46223.7120	
		Median		34666.6700	
		Variance		1746622727	
		Std. Deviation		41792.61570	
		Minimum		4000.00	
		Maximum		141647.33	
		Range		137647.33	
		Interquartile Range		67176.40	
		Skewness		.986	.580
		Kurtosis		.120	1.121

Tests of Normality

	Treatment	Kolmogorov-Smirnov[a] Statistic	df	Sig.	Shapiro-Wilk Statistic	df	Sig.
Plant_Dry_Matter_Yield	Negative control	.215	15	.060	.891	15	.070

a. Lilliefors Significance Correction

The plant dry matter yield per hectare measurements of the negative control with no inoculant are normally distributed as can be seen by the p-value obtained of 0.07 which is greater than 0.05. This is supported by Z values that lie between -1.96 and +1.96 by the skewness Z value being (0.986/0.580) = 1.7 and the Kurtosis Z value is (0.120/1.121)= 0.107 based on the Descriptive statistics table above. The Normal Q-Q Plot in the Annex, visual inspection of the histogram and box plots support this normality claim.

Descriptives

	Treatment			Statistic	Std. Error
Plant_Dry_Matter_Yield	Bio-inoculant	Mean		48917.4271	9451.89704
		95% Confidence Interval for Mean	Lower Bound	28497.8450	
			Upper Bound	69337.0093	
		5% Trimmed Mean		47782.3268	
		Median		46666.6650	
		Variance		1250737008	
		Std. Deviation		35365.76039	
		Minimum		4933.33	
		Maximum		113333.33	
		Range		108400.00	
		Interquartile Range		70422.17	
		Skewness		.339	.597
		Kurtosis		-1.101	1.154

Tests of Normality

	Treatment	Kolmogorov-Smirnov[a] Statistic	df	Sig.	Shapiro-Wilk Statistic	df	Sig.
Plant_Dry_Matter_Yield	Bio-inoculant	.137	14	.200*	.934	14	.352

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

The p-value obtained above from the bio-inoculant Plant dry matter yield per hectare measurements is 0.352, indicating a normal distribution of the data. Also a visual inspection of the Normal Q-Q plots and the Box plots which are as symmetrical as possible support this claim.

Tests of Between-Subjects Effects

Dependent Variable: Root_Dry_Matter_Yield

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	86181826.680[a]	15	5745455.112	7.059	.000	.693
Intercept	447682550.000	1	447682550.000	550.014	.000	.921
Treatment	13105288.290	2	6552644.144	8.050	.001	.255
Cultivar_Name	40806981.430	5	8161396.286	10.027	.000	.516
Treatment * Cultivar_Name	22891009.070	8	2861376.133	3.515	.003	.374
Error	38255541.520	47	813947.692			
Total	615078763.100	63				
Corrected Total	124437368.200	62				

a. R Squared = .693 (Adjusted R Squared = .594)

Descriptives

	Treatment			Statistic	Std. Error
Root_Dry_Matter_Yield	Fertiliser	Mean		2063.0230	143.79898
		95% Confidence Interval for Mean	Lower Bound	1764.8022	
			Upper Bound	2361.2439	
		5% Trimmed Mean		2079.7017	
		Median		2232.0000	
		Variance		475597.347	
		Std. Deviation		689.63566	
		Minimum		611.34	
		Maximum		3237.44	
		Range		2626.10	
		Interquartile Range		825.96	
		Skewness		-.663	.481
		Kurtosis		.115	.935

Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Treatment	Statistic	df	Sig.	Statistic	df	Sig.
Root_Dry_Matter_Yield	Fertiliser	.119	23	.200*	.950	23	.290

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

The obtained measurements for the root dry matter yield in kilograms per hectare are normally distributed as can be seen in the Descriptive table and Shapiro-Wilk tests above and in the Annex. All three treatment measurements have a p-value greater than 0.05 and their histograms and Normal Q-Q plots, also in the Annex, indicate normally distributed data.

Tests of Between-Subjects Effects

Dependent Variable: Grain_Yield

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	61400644.780[a]	15	4093376.319	4.758	.000	.603
Intercept	163213020.900	1	163213020.900	189.721	.000	.801
Treatment	22438450.780	2	11219225.390	13.041	.000	.357
Cultivar_Name	14938031.290	5	2987606.259	3.473	.009	.270
Treatment * Cultivar_Name	14217142.250	8	1777142.782	2.066	.059	.260
Error	40433110.910	47	860278.956			
Total	308977495.600	63				
Corrected Total	101833755.700	62				

a. R Squared = .603 (Adjusted R Squared = .476)

Descriptives

	Treatment			Statistic	Std. Error
Grain_Yield	Fertiliser	Mean		1154.6961	158.72610
		95% Confidence Interval for Mean	Lower Bound	825.5183	
			Upper Bound	1483.8739	
		5% Trimmed Mean		1099.3406	
		Median		1055.0700	
		Variance		579461.401	
		Std. Deviation		761.22362	
		Minimum		209.00	
		Maximum		3172.05	
		Range		2963.05	
		Interquartile Range		915.03	
		Skewness		1.010	.481
		Kurtosis		.986	.935

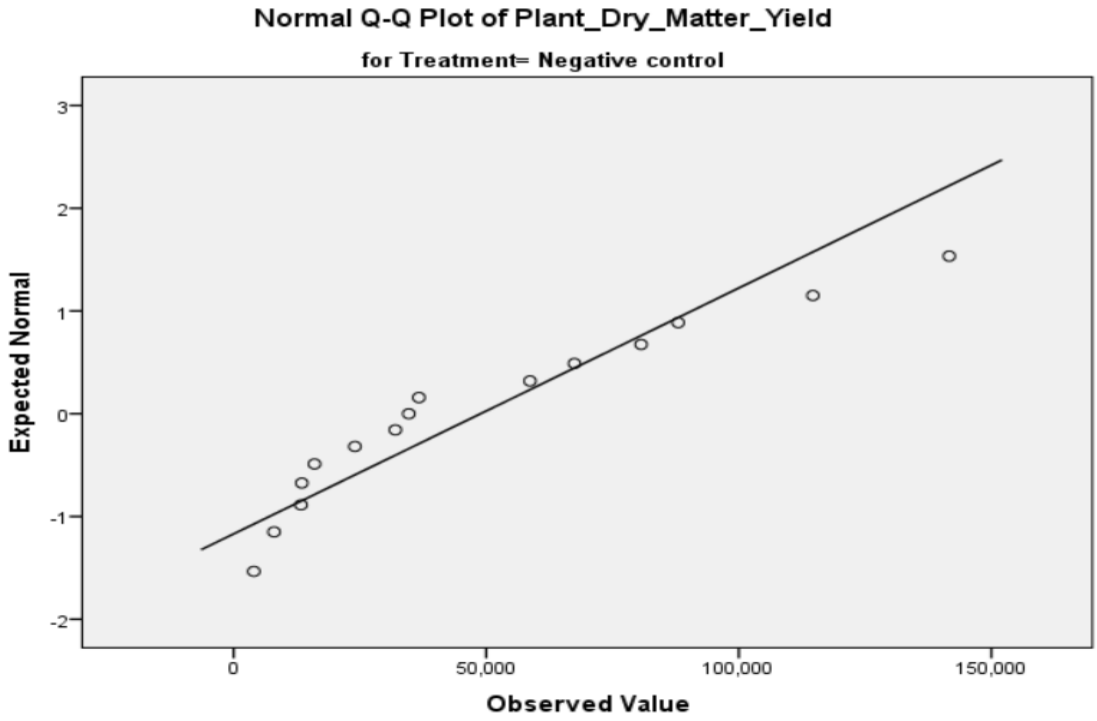
Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Treatment	Statistic	df	Sig.	Statistic	df	Sig.
Grain_Yield	Fertiliser	.138	23	.200*	.923	23	.078

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

The normality tests for the grain yield data indicate a normally distributed dataset across all three treatments of fertilizer, negative control and bio-inoculant. This is evident from the Shapiro-Wilk p-values of all treatments, with the Negative control and bio-inoculant normality tests results in the Appendices.


Normal Q-Q Plot of Plant_Dry_Matter_Yield
for Treatment= Negative control
Expected Normal
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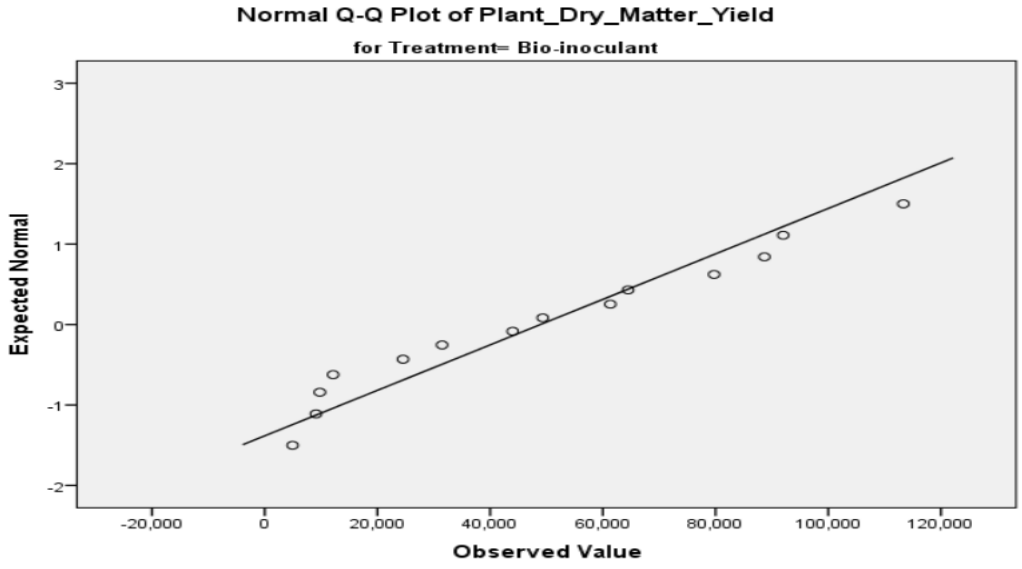
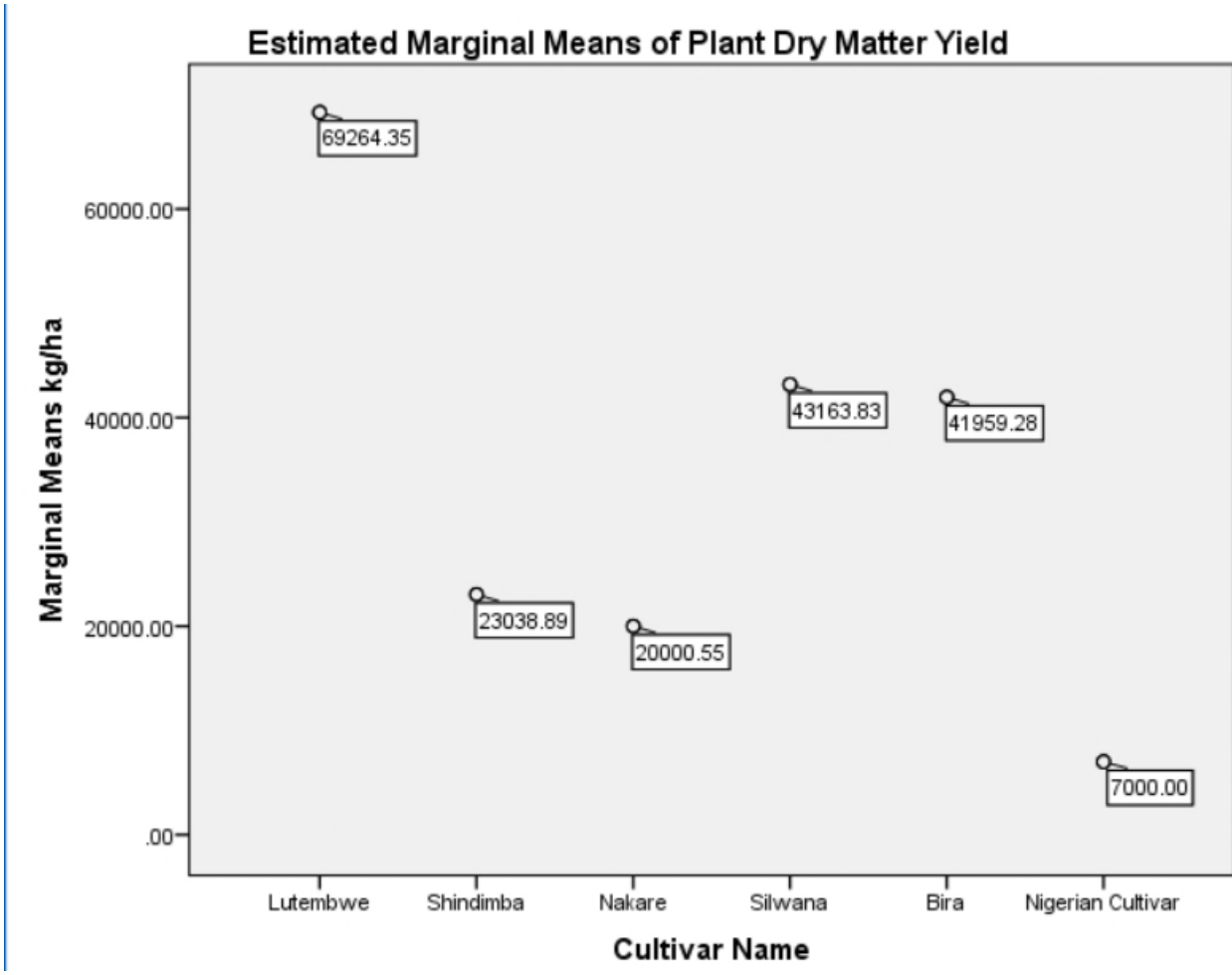
2
1
0
-1
-2
0
50,000
100,000
150,000
Observed Value


Normal Q-Q Plot of Plant_Dry_Matter_Yield
for Treatment= Bio-inoculant
Expected Normal
3
2
1
0
-1
-2
-20,000
0
20,000
40,000
60,000
80,000
100,000
120,000
Observed Value


Estimated Marginal Means of Plant Dry Matter Yield
Marginal Means kg/ha
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40000.00
20000.00
.00
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23038.89
20000.55
43163.83
41959.28
7000.00
Lutembwe
Shindimba
Nakare
Silwana
Bira
Nigerian Cultivar
Cultivar Name

Pairwise Comparisons

Dependent Variable: Plant_Dry_Matter_Yield

(I) Cultivar Name	(J) Cultivar Name	Mean Difference (I-J)	Std. Error	Sig.[d]	95% Confidence Interval for Difference[d]	
					Lower Bound	Upper Bou
Lutembwe	Shindimba	46225.464[*]	10970.601	.002	12297.014	80153
	Nakare	49263.798[*]	10421.719	.000	17032.859	81494
	Silwana	26100.519	9842.274	.163	-4338.388	56539
	Bira	27305.074	9842.274	.119	-3133.833	57743
	Nigerian Cultivar	62264.352[*,b]	14231.905	.001	18249.765	106278
Shindimba	Lutembwe	-46225.464[*]	10970.601	.002	-80153.914	-12297
	Nakare	3038.334	11870.229	1.000	-33672.370	39749
	Silwana	-20124.945	11364.879	1.000	-55272.768	15022
	Bira	-18920.390	11364.879	1.000	-54068.213	16227
	Nigerian Cultivar	16038.888[b]	15324.400	1.000	-31354.426	63432
Nakare	Lutembwe	-49263.798[*]	10421.719	.000	-81494.736	-17032
	Shindimba	-3038.334	11870.229	1.000	-39749.037	33672
	Silwana	-23163.279	10835.987	.567	-56675.413	10348
	Bira	-21958.724	10835.987	.726	-55470.858	11553
	Nigerian Cultivar	13000.555[b]	14936.377	1.000	-33192.734	59193
Silwana	Lutembwe	-26100.519	9842.274	.163	-56539.426	4338
	Shindimba	20124.945	11364.879	1.000	-15022.878	55272
	Nakare	23163.279	10835.987	.567	-10348.855	56675
	Bira	1204.555	10279.920	1.000	-30587.847	32996
	Nigerian Cultivar	36163.833[b]	14538.002	.247	-8797.412	81125
Bira	Lutembwe	-27305.074	9842.274	.119	-57743.981	3133
	Shindimba	18920.390	11364.879	1.000	-16227.433	54068
	Nakare	21958.724	10835.987	.726	-11553.410	55470
	Silwana	-1204.555	10279.920	1.000	-32996.957	30587
	Nigerian Cultivar	34959.278[b]	14538.002	.303	-10001.967	79920
Nigerian Cultivar	Lutembwe	-62264.352[*,c]	14231.905	.001	-106278.940	-18249
	Shindimba	-16038.888[c]	15324.400	1.000	-63432.203	31354
	Nakare	-13000.555[c]	14936.377	1.000	-59193.844	33192
	Silwana	-36163.833[c]	14538.002	.247	-81125.079	8797
	Bira	-34959.278[c]	14538.002	.303	-79920.524	10001

The mean difference is significant at the .05 level.

Descriptives

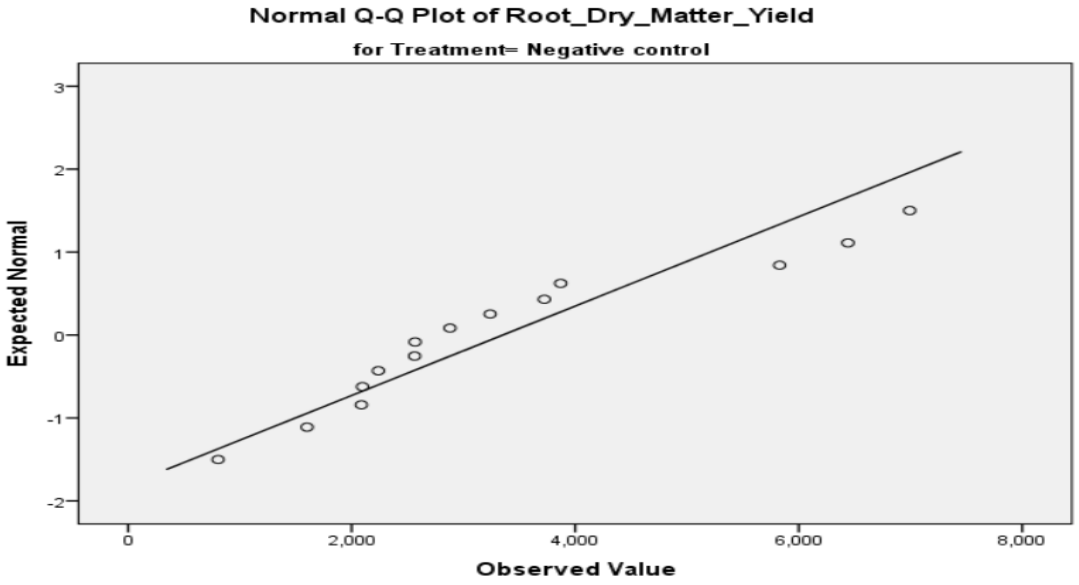
	Treatment			Statistic	Std. Error
Root_Dry_Matter_Yield	Negative control	Mean		3352.5714	496.07256
		95% Confidence Interval for Mean	Lower Bound	2280.8718	
			Upper Bound	4424.2710	
		5% Trimmed Mean		3291.8205	
		Median		2723.6650	
		Variance		3445231.809	
		Std. Deviation		1856.13356	
		Minimum		805.33	
		Maximum		6993.33	
		Range		6188.00	
		Interquartile Range		2265.67	
		Skewness		.908	.597
		Kurtosis		-.095	1.154

Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Treatment	Statistic	df	Sig.	Statistic	df	Sig.
Root_Dry_Matter_Yield	Negative control	.176	14	.200*	.896	14	.100

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction



Pairwise Comparisons

Dependent Variable: Root_Dry_Matter_Yield

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.[d]	95% Confidence Interval for Difference[d]	
					Lower Bound	Upper Bound
Fertilizer	Negative Control	-1210.087[*,b]	286.221	.000	-1920.686	-499.488
	Bio-inoculant	-1327.281[*,b]	283.841	.000	-2031.972	-622.591
Negative Control	Fertiliser	1210.087[*,c]	286.221	.000	499.488	1920.686
	Bio-inoculant	-117.194[b,c]	301.481	1.000	-865.680	631.291
Bio-inoculant	Fertiliser	1327.281[*,c]	283.841	.000	622.591	2031.972
	Negative Control	117.194[b,c]	301.481	1.000	-631.291	865.680

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. An estimate of the modified population marginal mean (J).

c. An estimate of the modified population marginal mean (I).

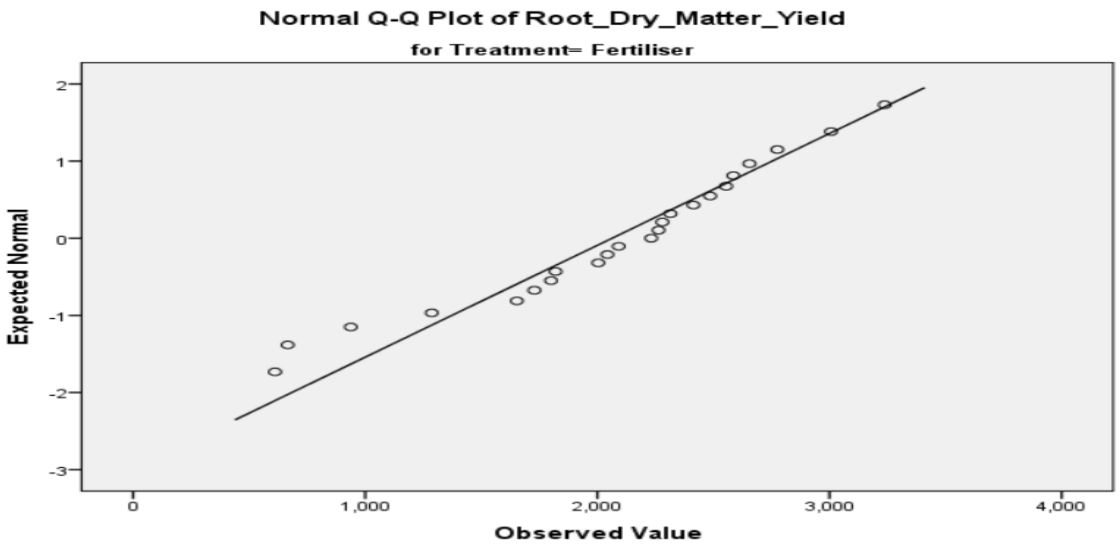
d. Adjustment for multiple comparisons: Bonferroni.

Estimates

Dependent Variable: Root_Dry_Matter_Yield

Cultivar_Name	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Lutembwe	2272.583	237.748	1794.296	2750.871
Shindimba	3711.628	313.010	3081.934	4341.323
Nakare	4249.020	287.927	3669.785	4828.255
Silwana	2419.108	260.440	1895.171	2943.046
Bira	2422.717	260.440	1898.779	2946.654
Nigerian Cultivar	875.580[a]	451.095	-31.907	1783.067

a. Based on modified population marginal mean.


Normal Q-Q Plot of Root_Dry_Matter_Yield
for Treatment= Fertiliser
Expected Normal
Observed Value
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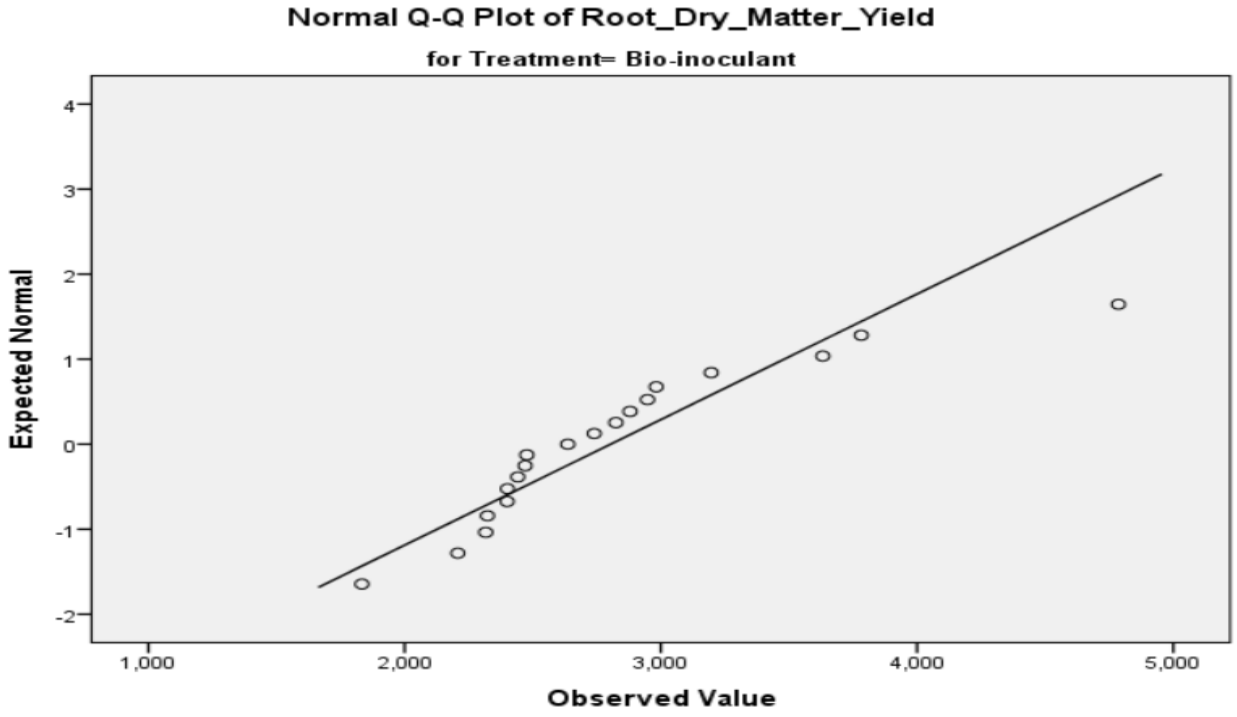
1
0
-1
-2
-3
0
1,000
2,000
3,000
4,000


Normal Q-Q Plot of Root_Dry_Matter_Yield
for Treatment= Bio-inoculant
Expected Normal
Observed Value
4
3
2
1
0
-1
-2
1,000
2,000
3,000
4,000
5,000

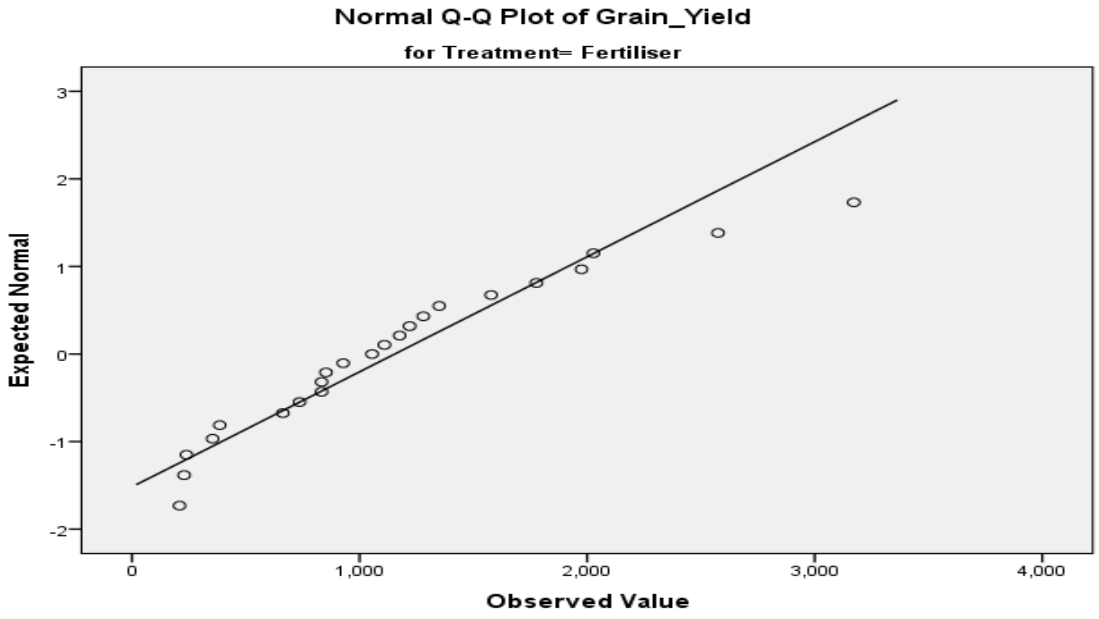
Descriptives

	Treatment			Statistic	Std. Error
Root_Dry_Matter_Yield	Bio-inoculant	Mean		2804.1758	155.37830
		95% Confidence Interval for Mean	Lower Bound	2477.7381	
			Upper Bound	3130.6135	
		5% Trimmed Mean		2748.0103	
		Median		2636.6700	
		Variance		458705.899	
		Std. Deviation		677.27830	
		Minimum		1833.33	
		Maximum		4786.00	
		Range		2952.67	
		Interquartile Range		582.00	
		Skewness		1.548	.524
		Kurtosis		3.087	1.014

Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Treatment	Statistic	df	Sig.	Statistic	df	Sig.
Root_Dry_Matter_Yield	Bio-inoculant	.186	19	.083	.870	19	.015

a. Lilliefors Significance Correction



Pairwise Comparisons

Dependent Variable: Grain_Yield

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.[d]	95% Confidence Interval for Difference[d]	
					Lower Bound	Upper Bound
Fertiliser	Negative Control	-270.003[a]	294.254	1.000	-1000.546	460.540
	Bio-inoculant	-1501.289[a,*]	291.808	.000	-2225.758	-776.820
Negative Control	Fertiliser	270.003[c]	294.254	1.000	-460.540	1000.546
	Bio-inoculant	-1231.286[a,*,c]	309.943	.001	-2000.779	-461.793
Bio-inoculant	Fertiliser	1501.289[*,c]	291.808	.000	776.820	2225.758
	Negative Control	1231.286[a,*,c]	309.943	.001	461.793	2000.779

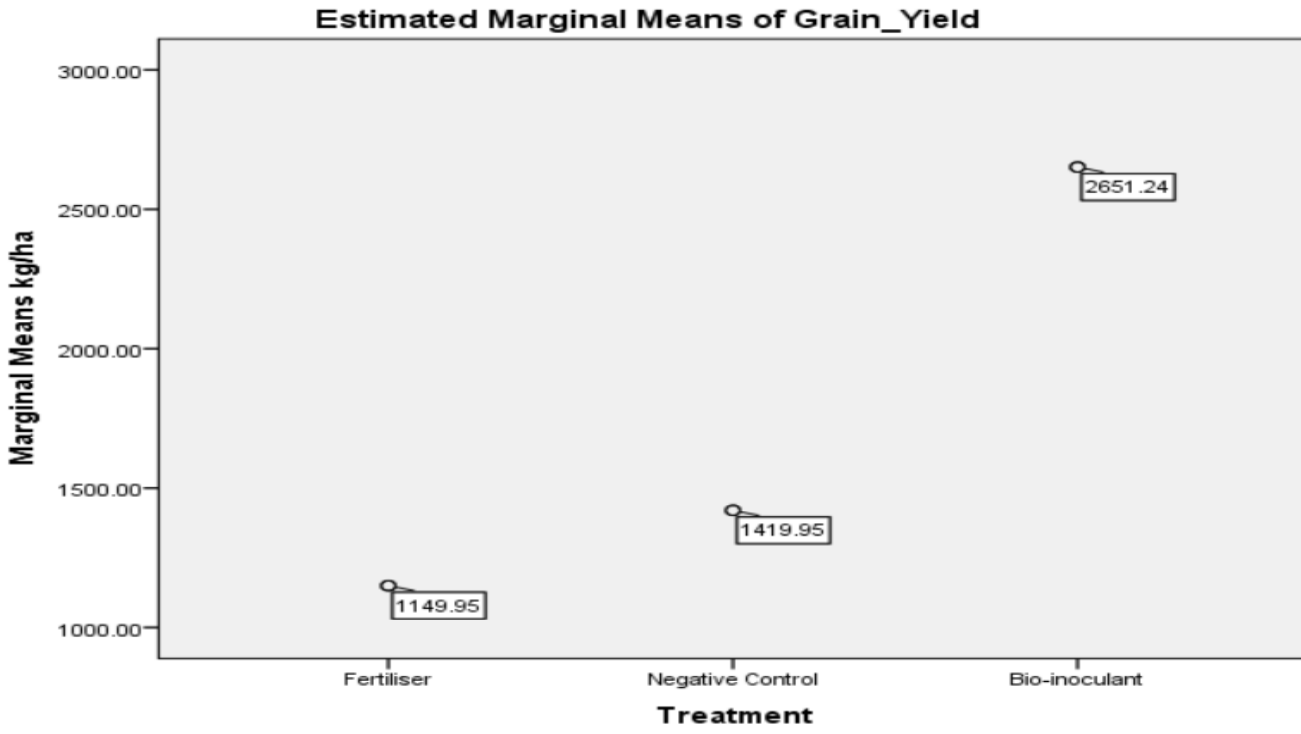
Based on estimated marginal means

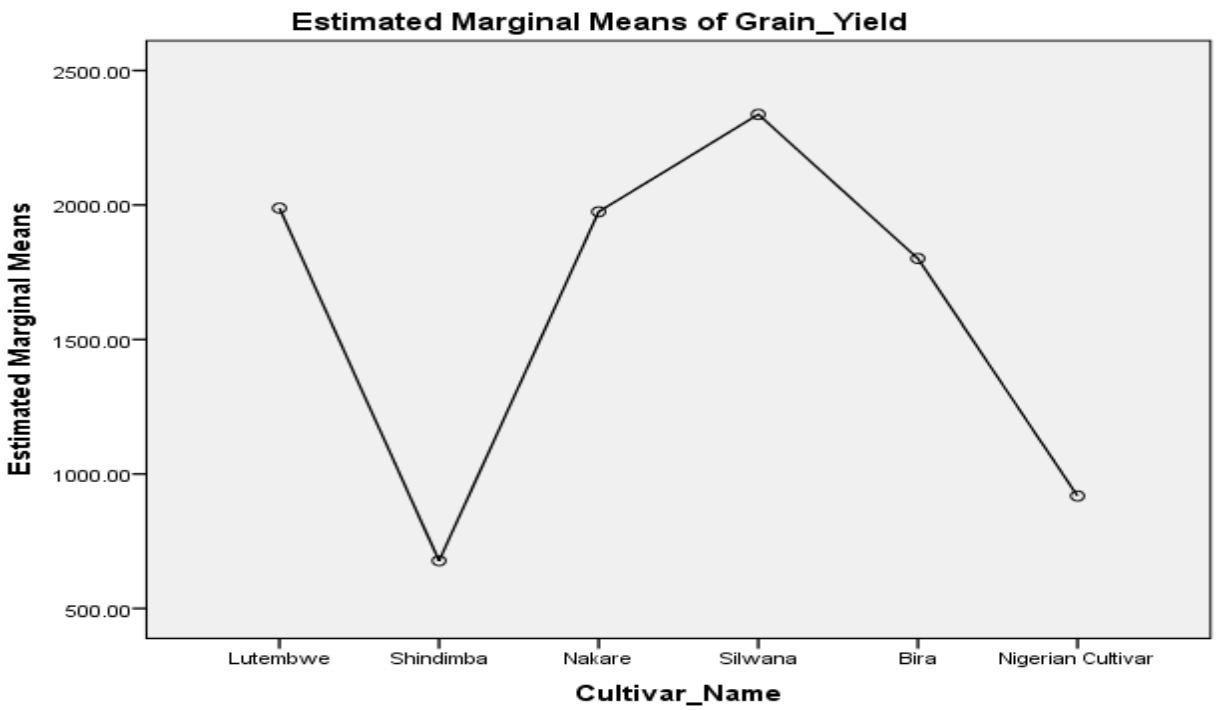
*. The mean difference is significant at the .05 level.

a. An estimate of the modified population marginal mean (J).

c. An estimate of the modified population marginal mean (I).

d. Adjustment for multiple comparisons: Bonferroni.




Estimated Marginal Means of Grain_Yield
Estimated Marginal Means
2500.00
2000.00
1500.00
1000.00
500.00
Lutembwe
Shindimba
Nakare
Silwana
Bira
Nigerian Cultivar
Cultivar_Name

Descriptives

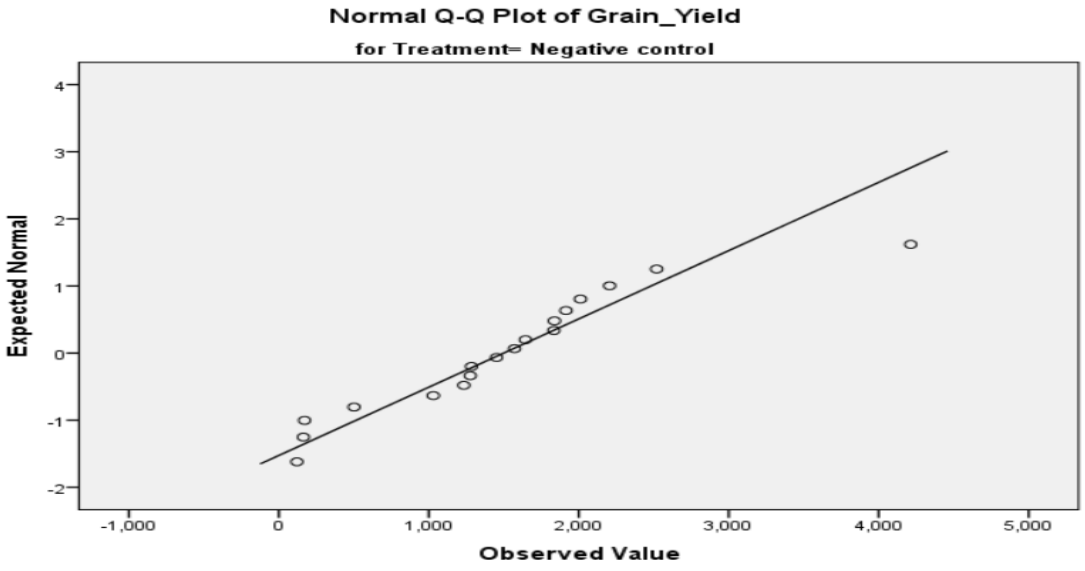
	Treatment			Statistic	Std. Error
Grain_Yield	Negative control	Mean		1499.1322	231.71300
		95% Confidence Interval for Mean	Lower Bound	1010.2605	
			Upper Bound	1988.0039	
		5% Trimmed Mean		1424.9041	
		Median		1511.5850	
		Variance		966436.482	
		Std. Deviation		983.07501	
		Minimum		120.64	
		Maximum		4213.73	
		Range		4093.09	
		Interquartile Range		1041.17	
		Skewness		.921	.536
		Kurtosis		2.375	1.038

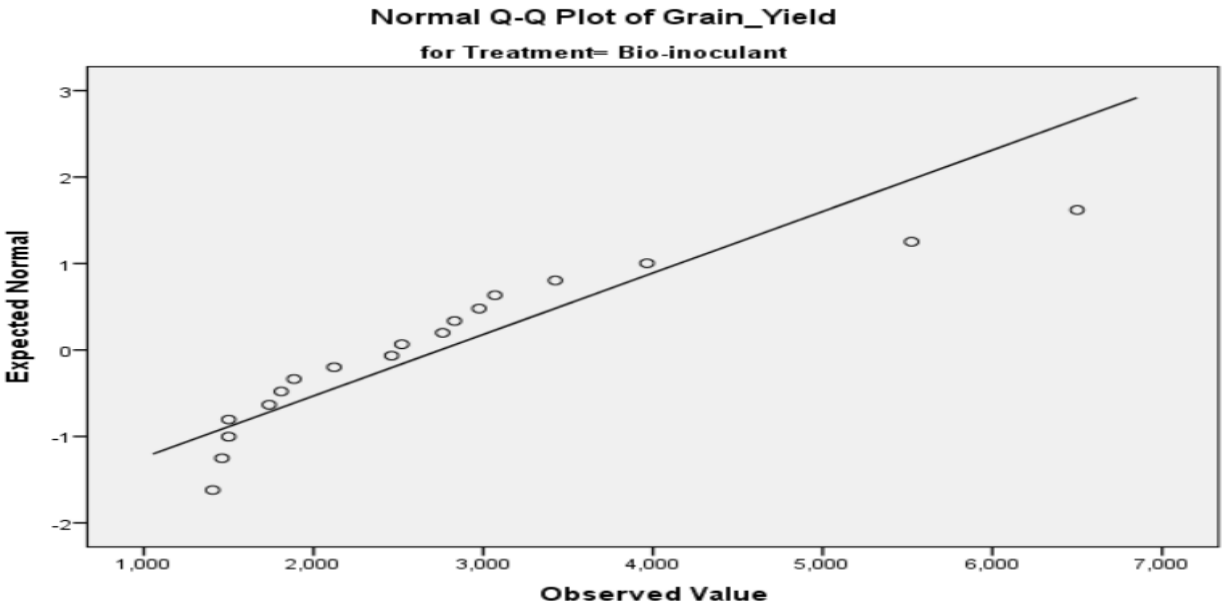
Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Treatment	Statistic	df	Sig.	Statistic	df	Sig.
Grain_Yield	Negative control	.135	18	.200*	.912	18	.093

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction





Descriptives

	Treatment			Statistic	Std. Error
Grain_Yield	Bio-inoculant	Mean		2747.2639	331.52024
		95% Confidence Interval for Mean	Lower Bound	2047.8173	
			Upper Bound	3446.7105	
		5% Trimmed Mean		2613.2999	
		Median		2490.0000	
		Variance		1978302.024	
		Std. Deviation		1406.52125	
		Minimum		1405.88	
		Maximum		6500.00	
		Range		5094.12	
		Interquartile Range		1479.00	
		Skewness		1.551	.536
		Kurtosis		2.246	1.038

Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Treatment	Statistic	df	Sig.	Statistic	df	Sig.
Grain_Yield	Bio-inoculant	.187	18	.095	.833	18	.005

a. Lilliefors Significance Correction

Correlations

Correlations

		Shoot_Yield	Root_Yield	Grain_Yield
Shoot_Yield	Pearson Correlation	1	-.010	.499**
	Sig. (2-tailed)		.936	.000
	N	63	63	63
Root_Yield	Pearson Correlation	-.010	1	.319*
	Sig. (2-tailed)	.936		.011
	N	63	63	63
Grain_Yield	Pearson Correlation	.499**	.319*	1
	Sig. (2-tailed)	.000	.011	
	N	63	63	63

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Sequence Data

Bradyrhizobium americanum strain CMVU44 16S-23S ribosomal RNA intergenic spacer and 23S ribosomal RNA gene, partial sequence

Sequence ID: KC247121.1Length: 800Number of Matches: 2
Related Information
Range 1: 286 to 786GenBankGraphics Next Match Previous Match
Alignment statistics for match #1

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Query  418
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Range 2: 59 to 278GenBankGraphics Next Match Previous Match First Match

Alignment statistics for match #2

Score	Expect	Identities	Gaps	Strand
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RNA intergenic spacer and 23S ribosomal RNA gene, partial sequence
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ATCAAGTGCCTAAGGGGTCGGGATGCTGCG
```

Bradyrhizobium vignae strain 7-2 16S-23S ribosomal RNA intergenic spacer, partial sequence

Sequence ID: KM378504.1Length: 814Number of Matches: 1

Related Information

Range 1: 402 to 750GenBankGraphics Next Match Previous Match

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
562 bits(304)	1e-157	335/350(96%)	2/350(0%)	Plus/Minus

```
Query  1
CCTCTCAGCGCCAAGGCATCCACCGAACACCCTTAAGGCACTTGATTGCTCTCATTATCA  60

||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  750
CCTCTCAGCGCCAAGGCATCCACCGAACACCCTTAAGGCACTTGATTGCTCTCATTATCA  691

Query  61
ATGTCCACACACTCGGCAGAATGTTGTCTGTACGATTGCCTTGCGGCGCGCACCCTCGAT  120
             |||||||||||||||||||||||||||| |  |||||||||||| |||
||||||||
```

```
Sbjct  690
ATGTCCACACACTCGGCAGAATGTTGTCTGCAGAATTGCCTTGCGGCACGCGCCCTCGAT  631

Query  121  GAATGCTA-
CGTACAGCCGGACATTGACTAGAAAGACCAGCTTGCTTCGTAAGATCGTTC  179
             ||| |||| |
||||||||||||||||||||||||||||||||||||||||||||
Sbjct  630  GAACGCTATCCG-
CAGCCGGACATTGACTAGAAAGACCAGCTTGCTTCGTAAGATCGTTC  572

Query  180
CGATAGCGAGGCGGTCAAGCTTCGCTAAAAGGATCATTTACAACCCGCATTCAACCTCAC  239
             ||||||||||||||||||||||||||||||||||||||||||||||||||||
|||||||||
Sbjct  571
CGATAGCGAGGCGGTCAAGCTTCGCTAAAAGGATCATTTACAACCCGCATCCAACCTCAC  512

Query  240
GGCTGAAAGCGAGCGCCGAAATGATCCGGATATAATGAAGGCCCGCGCAACTATATGCTG  299
             ||||| ||||||||||||||||||||||| |||||||||| ||||||||
|||||||
Sbjct  511
GGCTGGAAGCGAGCGCCGAAATGATCCGGAGATAATGAAGGCTCGCGCAACGATATGCTG  452

Query  300  CACGACCCAACTCGGATCGATCTCCTCTTTACGATGTCAGAAAACACGCA  349
             ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  451  CACGACCCAACTCGGATCGATCTCCTCTTTACGATGTCAGAAAACACGCA  402
```

GenBank: KM378504.1

GenBank Graphics PopSet

```
>KM378504.1 Bradyrhizobium vignae strain 7-2 16S-23S ribosomal RNA
intergenic spacer, partial sequence
TCAGTGGCCAACAGATCGCCAGATCGTTGAGCTGCATTGGCGGGATTTCGCCGTCTTCGTTTCTCTTTCT
TCGCGGACGAACACGCGCTGGGCCTGCAACGGCAGGATCCAGGGTCCTTAACGGGATGTGCGTTAGGGGC
TTGTAGCTCAGTTGGTTAGAGCGCGCGCTTGATAAGCGTGAGGTCGGAAGTTCAAGTCTTCCCAGGCCCA
CCACTTTCATCGAGTGAGCATTCGTCTTCTGGTTACGGGGCCATAGCTCAGCTGGGAGAGCGCGTGCTTT
GCAAGCATGAGGTCGTCGGTTCGATCCCGTCTGGCTCCACCAGATGGTTTGATCACCGAGATCTTGACCG
TCGTCCGCGAAACATCACTTCGCATCCTGCTAGTCTGGATAGACAGCAGTTTGCGTGTTTTCTGACATCG
TAAAGAGGAGATCGATCCGAGTTGGGTCGTGCAGCATATCGTTGCGCGAGCCTTCATTATCTCCGGATCA
TTTCGGCGCTCGCTTCCAGCCGTGAGGTTGGATGCGGGTTGTAAATGATCCTTTTAGCGAAGCTTGACCG
CCTCGCTATCGGAACGATCTTACGAAGCAAGCTGGTCTTTCTAGTCAATGTCCGGCTGCGGATAGCGTTC
ATCGAGGGCGCGTGCCGCAAGGCAATTCTGCAGACAACATTCTGCCGAGTGTGTGGACATTGATAATGAG
AGCAATCAAGTGCCTTAAGGGTGTTCGGTGGATGCCTTGGCGCTGAGAGGCGATGAAGGACGTGCTACGC
TGCGATAAGCCGTGGGGAGCTGCGAAGAAGCTTTGATCCGCGGA
```

Bradyrhizobium kavangense strain 14-3 16S-23S ribosomal RNA intergenic spacer, partial sequence
Sequence ID: KM378507.1Length: 899Number of Matches: 2
Related Information

Range 1: 135 to 870GenBankGraphics Next Match Previous Match

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
869 bits(470)	0.0	662/748(89%)	40/748(5%)	Plus/Minus

```
Query  1
CACGTCCTTCATCGCCTCTCAGCGCCAAGGCATCCACCGAACACCCTTAAGGCACTTGAT  60

||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  870
CACGTCCTTCATCGCCTCTCAGCGCCAAGGCATCCACCGAACACCCTTAAGGCACTTGAT  811

Query  61   TGCTCTCATTATCAATGTCCACACACTCGGCAGAATGTTGTCTGTACGATTGCC-----
-  114
            |||||||||||||||||||||||||||||||||||| || ||||||||||
Sbjct  810
TGCTCTCATTATCAATGTCCACACACTCGGCAGAATGTTGGCTCTACGATTGCCGGGAGA  751

Query  115  ------T--TGC-
GGCGCGCACCCTCGATGAATGCTACGTACAGCCGGACATTGACTAGA  165
                    |    |
||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  750
CCGAAATCCCCCAGGCGCGCACCCTCGATGAATGCTACGTACAGCCGGACATTGACTAGA  691

Query  166
AAGACCAGCTTGCTTCGTAAGATCGTTCCGATAGCGAGGCGGTCAAGCTTCGCTAAAAGG  225

||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  690
AAGACCAGCTTGCTTCGTAAGATCGTTCCGATAGCGAGGCGGTCAAGCTTCGCTAAAAGG  631

Query  226  ATCATTT-ACAACCCGCATTCAACCTCACGGCTGAAAGCGAGCGCCGAAA-
TGATCCGGA  283
            ||||||| ||||| ||||| |||||||||||| | ||||||||||||||
|||||||||
Sbjct  630
ATCATTTTACAACTCGCATCCAACCTCACGGCCGGAAGCGAGCGCCGAAAATGATCCGGA  571

Query  284  GATAATGAAGG-
CCCGCGCAACGATATGCTGCACGACCCAACTCGGATCGATCTCCTCTT  342
            ||||||||||  ||||||| | || ||||
||||||||||||||||||||||||||||
Sbjct  570
GATAATGAAGGGTCCGCGCAGCAATCTGCTACACGACCCAACTCGGATCGATCTCCTCTT  511

Query  343  TACGATGTCAGAAAACACGCAGTTCACTGTCTATCCAGACTAG-
TGAATGCGAAGTGATG  401
            |||||||||||||||||||| |   ||||||||||||||||  |
||||||||||||
Sbjct  510
TACGATGTCAGAAAACACGCAATCTGCTGTCTATCCAGACTAGCAGGATGCGAAGTGATG  451

Query  402  TTTCGCGGACGACGGT-
CAAGATCTCGGTGATCAAACCATCTGGTGGAGCCAGACGGGAT  460
            ||||||||||||||| || ||||||||
||||||||||||||||||||||||||||||
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Sbjct  450
TTTCGCGGACGACGGTGCACGATCTCGGCGATCAAACCATCTGGTGGAGCCAGACGGGAT  391

Query  461
CGAACCGACGACCTCATGCTTGCAAAGCACGCGCTCTCCCAGCTGAGCTATGGCCCCGTA  520

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Sbjct  390
CGAACCGACGACCTCATGCTTGCAAAGCACGCGCTCTCCCAGCTGAGCTATGGCCCCGTA  331

Query  521  ACCAGAAGACGAAGCGCCTCTCTAAGTCCGACGTCGCCCTTCGGGCTATGC--
CGGACAC  578
            ||||||||||||||  ||  ||  |   || | |  |||||| ||  | | ||  ||| |
|
Sbjct  330  ACCAGAAGACGAAGTGC-TCGATTGATCAGCC--CGCCCT-CGC-CAAGGCTACGG-
CGC  277

Query  579  A-CTTCGCTTCATCGTCTTGCATGGCAGTGCCGCGC-GAAGC--C-
AACGGCGAAGCGTG  633
               |  | || |||| | | | | |||   |||  || |||||  | ||  ||||||
||
Sbjct  276  GGCAGC-CTCCATC-T-T-GAA-GGCTC-
GCCTAGCCGAAGCAGCGAAGCGCGAAGGCTG  223

Query  634
GTGGGCCTGGGAAGACTTGAACTTCCGACCTCACGCTTATCAAGCGCGCGCTCTAACCAA  693

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Sbjct  222
GTGGGCCTGGGAAGACTTGAACTTCCGACCTCACGCTTATCAAGCGCGCGCTCTAACCAA  163

Query  694  CTGAGCTACAAGCCCCTAACGC-TATCC  720
            |||||||||||||||||||| |||||
Sbjct  162  CTGAGCTACAAGCCCCTAACGCATATCC  135
```

Range 2: 1 to 109GenBankGraphics Next Match Previous Match First Match

Alignment statistics for match #2

Score	Expect	Identities	Gaps	Strand
185 bits(100)	1e-43	106/109(97%)	0/109(0%)	Plus/Minus

```
Query  782
GCAGCCCCGGCGCGTGTTCGTCCGCGAAGAAAGAGAAACGAAGACGGCGAAATCGCGCCA  841
            |||| ||| ||||||||||||||||||||||||||||||||||||||||||||
|||||
Sbjct  109
GCAGGCCCAGCGCGTGTTCGTCCGCGAAGAAAGAGAAACGAAGACGGCGAAATCCCGCCA  50

Query  842  ATGCAGCTCAACGATCTGGCGATCTGTTGGCCACTGATGTTTCTAAAAC  890
            ||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  49   ATGCAGCTCAACGATCTGGCGATCTGTTGGCCACTGATGTTTCTAAAAC  1
>KM378507.1 Bradyrhizobium kavangense strain 14-3 16S-23S ribosomal RNA
intergenic spacer, partial sequence
GTTTTAGAAACATCAGTGGCCAACAGATCGCCAGATCGTTGAGCTGCATTGGCGGGATTTCGCCGTCTTC
GTTTCTCTTTCTTCGCGGACGAACACGCGCTGGGCCTGCAACGCAGGATCCGGGGTCCTTAACGGGATAT
GCGTTAGGGGCTTGTAGCTCAGTTGGTTAGAGCGCGCGCTTGATAAGCGTGAGGTCGGAAGTTCAAGTCT
TCCCAGGCCCACCAGCCTTCGCGCTTCGCTGCTTCGGCTAGGCGAGCCTTCAAGATGGAGGCTGCCGCGC
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CGTAGCCTTGGCGAGGGCGGGCTGATCAATCGAGCACTTCGTCTTCTGGTTACGGGGCCATAGCTCAGCT
GGGAGAGCGCGTGCTTTGCAAGCATGAGGTCGTCGGTTCGATCCCGTCTGGCTCCACCAGATGGTTTGAT
CGCCGAGATCGTGCACCGTCGTCCGCGAAACATCACTTCGCATCCTGCTAGTCTGGATAGACAGCAGATT
GCGTGTTTTCTGACATCGTAAAGAGGAGATCGATCCGAGTTGGGTCGTGTAGCAGATTGCTGCGCGGACC
CTTCATTATCTCCGGATCATTTTCGGCGCTCGCTTCCGGCCGTGAGGTTGGATGCGAGTTGTAAAATGAT
CCTTTTAGCGAAGCTTGACCGCCTCGCTATCGGAACGATCTTACGAAGCAAGCTGGTCTTTCTAGTCAAT
GTCCGGCTGTACGTAGCATTCATCGAGGGTGCGCGCCTGGGGGATTTCGGTCTCCCGGCAATCGTAGAGC
CAACATTCTGCCGAGTGTGTGGACATTGATAATGAGAGCAATCAAGTGCCTTAAGGGTGTTCGGTGGATG
CCTTGGCGCTGAGAGGCGATGAAGGACGTGCTACGCTGCGATAAGCCGTGGGGAGCTGC
```

Bradyrhizobium ferriligni strain CCBAU 51502 16S-23S ribosomal RNA intergenic spacer and 23S ribosomal RNA gene, partial sequence
Sequence ID: KJ818105.1Length: 840Number of Matches: 1
Related Information
Range 1: 1 to 840GenBankGraphics Next Match Previous Match

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
1421 bits(769)	0.0	819/841(97%)	12/841(1%)	Plus/Minus

```
Query  6
AGCGTAGCACGTCCTTCATCGCCTCTCAGCGCCAAGGCATCCACCGAACACCCTTAAGGC  65

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Sbjct  840
AGCGTAGCACGTCCTTCATCGCCTCTCAGCGCCAAGGCATCCACCGAACACCCTTAAGGC  781

Query  66
ACTTGATTGCTCTCATTATCAATGTCCACACACTCGGCAGAATGTTGTCTGCACAACTGC  125
            ||||||||||||||||||||||||||||||||||||||||||||||
|||||||||||||
Sbjct  780
ACTTGATTGCTCTCATTATCAATGTCCACACACTCGGCAGAATGTAGTCTGCACAACTGC  721

Query  126  C-----ATTG-A-----
AGGCACGCATCCTCGATGAATGCGATTGCACAGCCGGACATTG  174
            |     |  | |     ||||||||||||||||||||||||||||
|||||||||||||
Sbjct  720
CCGAGGACCGAAGTCCCAGGCACGCATCCTCGATGAATGCGATTGCGCAGCCGGACATTG  661

Query  175
ACTAGAAAGACCAGCTTGCTTCGTAAGATCGGCCCGATAGCGAGGCGGTCAAGCTTCGCT  234

||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  660
ACTAGAAAGACCAGCTTGCTTCGTAAGATCGGCCCGATAGCGAGGCGGTCAAGCTTCGCT  601

Query  235
AAAAGGGATCATTTACAACCCGCATCCAATCTTGCGATTGGAAGCGAGCGCCGAAATGAT  294
            ||||||||||||||||||||||||||||||||  ||||||
||||||||||||||||||
Sbjct  600
AAAAGGGATCATTTACAACCCGCATCCAATCTCTCGATTGAAAGCGAGCGCCGAAATGAT  541
```

```
Query  295
CCCGGAGATAATGAATGGCTCAGGCGAATTGCTTCGCTTGATCGACACTCGGATCGATCT  354
            |||||||||||||||||||||||||||||||||||||||||||||||| |
|||||||||||||
Sbjct  540
CCCGGAGATAATGAATGGCTCAGGCGAATTGCTTCGCTTGATCCAAACTCGGATCGATCT  481

Query  355
CCTCTTTACGATGTCAGAAAACACGCACGTCACCGTCCATCCGGACCAATGAGTGCGAAG  414

||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  480
CCTCTTTACGATGTCAGAAAACACGCACGTCACCGTCCATCCGGACCAATGAGTGCGAAG  421

Query  415
TGATGTTTCGCGGACGATATGAACGAGGCATTATCGAGCTTCAATTCCATCTGGTGGAGC  474
            ||||||||||||||||||
||||||||||||||||||||||||||||||||||||
Sbjct  420  TGATGTTTCGCGGACGAT-
TGAACGAGGCATTATCGAGCTTCAATTCCATCTGGTGGAGC  362

Query  475
CAGACGGGATCGAACCGACGACCTCATGCTTGCAAAGCACGCGCTCTCCCAGCTGAGCTA  534

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Sbjct  361
CAGACGGGATCGAACCGACGACCTCATGCTTGCAAAGCACGCGCTCTCCCAGCTGAGCTA  302

Query  535
TGGCCCCGTACCAGAAGACGAATGCTCCGCACTCGATCAAAATGGTGGGCCTGGGAAGAC  594

||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301
TGGCCCCGTACCAGAAGACGAATGCTCCGCACTCGATCAAAATGGTGGGCCTGGGAAGAC  242

Query  595
TTGAACTTCCGACCTCACGCTTATCAAGCGCGCGCTCTAACCAACTGAGCTACAAGCCCC  654

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Sbjct  241
TTGAACTTCCGACCTCACGCTTATCAAGCGCGCGCTCTAACCAACTGAGCTACAAGCCCC  182

Query  655
TAACACGAGAGACATGCCGGCGCGCACTCGACCTGAAGTCGGTGCATCGCACAGCGCTCA  714
            ||||||||||||||||||||||||||||||||||||||||||||||
||||||||||||
Sbjct  181
TAACACGAGAGACATGCCGGCGCGCACTCGACCTGAAGTCGGTGCACCGCACAGCGCTCA  122

Query  715
GCCCCTGGCGCGTGTTCGTCCGCGAAGAAAGAGAAACGAAGACGGCGAAATCCCGCCAAT  774

||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121
GCCCCTGGCGCGTGTTCGTCCGCGAAGAAAGAGAAACGAAGACGGCGAAATCCCGCCAAT  62
```

```
Query  775
GGAGCTCAACAATCCTGAAACTGTTGGCCCCTGAATGTTTCTAAAACGATCCGATAGATA  834

||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61
GGAGCTCAACAATCCTGAAACTGTTGGCCCCTGAATGTTTCTAAAACGATCCGATAGATA  2

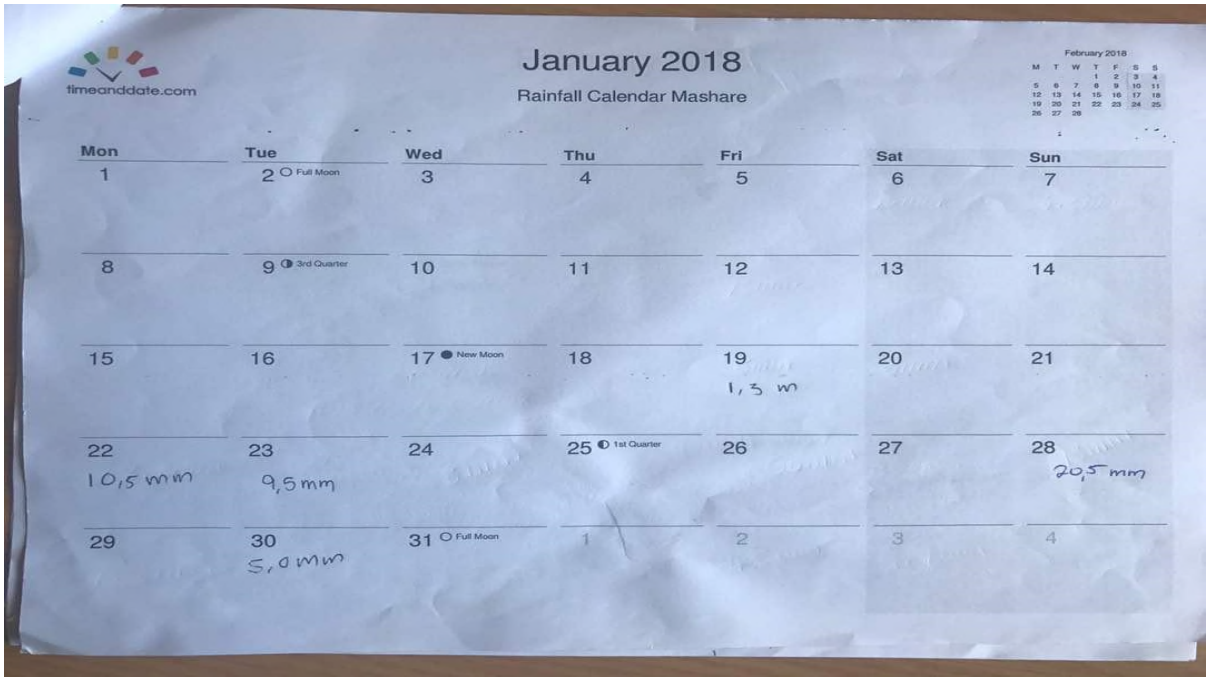
Query  835  G  835
             |
Sbjct  1    G  1
```

GenBank: KJ818105.1

GenBank Graphics PopSet

```
>KJ818105.1 Bradyrhizobium ferriligni strain CCBAU 51502 16S-23S
ribosomal RNA intergenic spacer and 23S ribosomal RNA gene, partial
sequence
CTATCTATCGGATCGTTTTAGAAACATTCAGGGGCCAACAGTTTCAGGATTGTTGAGCTCCATTGGCGGG
ATTTCGCCGTCTTCGTTTCTCTTTCTTCGCGGACGAACACGCGCCAGGGGCTGAGCGCTGTGCGGTGCAC
CGACTTCAGGTCGAGTGCGCGCCGGCATGTCTCTCGTGTTAGGGGCTTGTAGCTCAGTTGGTTAGAGCGC
GCGCTTGATAAGCGTGAGGTCGGAAGTTCAAGTCTTCCCAGGCCCACCATTTTGATCGAGTGCGGAGCAT
TCGTCTTCTGGTACGGGGCCATAGCTCAGCTGGGAGAGCGCGTGCTTTGCAAGCATGAGGTCGTCGGTTC
GATCCCGTCTGGCTCCACCAGATGGAATTGAAGCTCGATAATGCCTCGTTCAATCGTCCGCGAAACATCA
CTTCGCACTCATTGGTCCGGATGGACGGTGACGTGCGTGTTTTCTGACATCGTAAAGAGGAGATCGATCC
GAGTTTGGATCAAGCGAAGCAATTCGCCTGAGCCATTCATTATCTCCGGGATCATTTCGGCGCTCGCTTT
CAATCGAGAGATTGGATGCGGGTTGTAAATGATCCCTTTTAGCGAAGCTTGACCGCCTCGCTATCGGGCC
GATCTTACGAAGCAAGCTGGTCTTTCTAGTCAATGTCCGGCTGCGCAATCGCATTCATCGAGGATGCGTG
CCTGGGACTTCGGTCCTCGGGCAGTTGTGCAGACTACATTCTGCCGAGTGTGTGGACATTGATAATGAGA
GCAATCAAGTGCCTTAAGGGTGTTCGGTGGATGCCTTGGCGCTGAGAGGCGATGAAGGACGTGCTACGCT
```

Mashare recorded rainfall Data

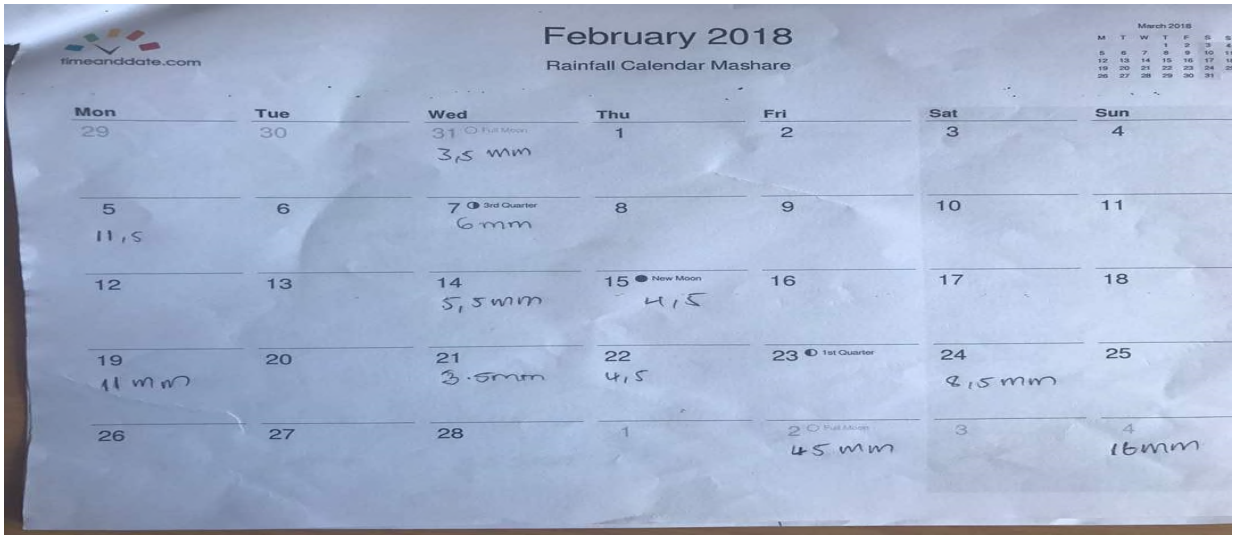


January 2018

Rainfall Calendar Mashare

timeanddate.com

Mon	Tue	Wed	Thu	Fri	Sat	Sun
1	2 Full Moon	3	4	5	6	7
8	9 3rd Quarter	10	11	12	13	14
15	16	17 New Moon	18	19 1,3 m	20	21
22 10,5 mm	23 9,5 mm	24	25 1st Quarter	26	27	28 20,5 mm
29	30 5,0 mm	31 Full Moon	1	2	3	4

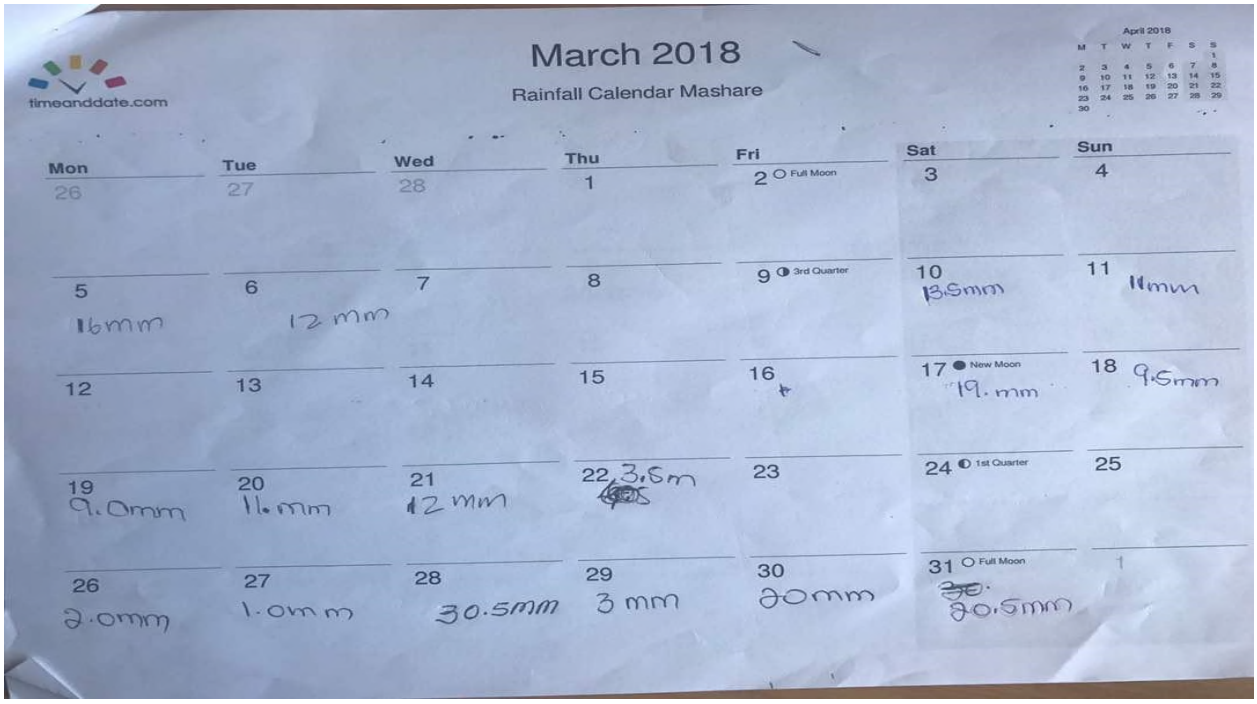


February 2018

Rainfall Calendar Mashare

timeanddate.com

Mon	Tue	Wed	Thu	Fri	Sat	Sun
29	30	31 Full Moon 3,5 mm	1	2	3	4
5 11,5	6	7 3rd Quarter 6 mm	8	9	10	11
12	13	14 5,5 mm	15 New Moon 4,5	16	17	18
19 11 mm	20	21 3.5mm	22 4,5	23 1st Quarter	24 8,5 mm	25
26	27	28	1	2 Full Moon 45 mm	3	4 16mm

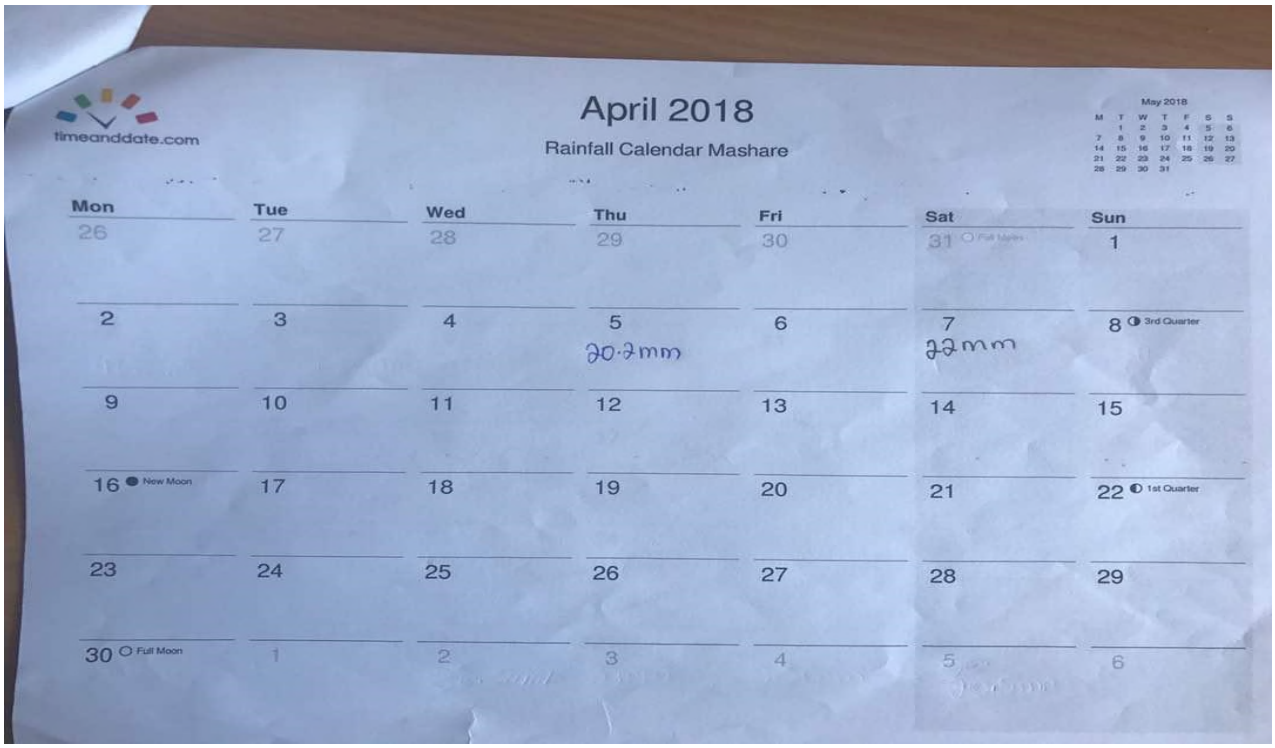


March 2018

Rainfall Calendar Mashare

timeanddate.com

Mon	Tue	Wed	Thu	Fri	Sat	Sun
26	27	28	1	2 ○ Full Moon	3	4
5 16mm	6 12 mm	7	8	9 ◑ 3rd Quarter	10 13.5mm	11 11mm
12	13	14	15	16	17 ● New Moon 19. mm	18 9.5mm
19 9.0mm	20 11.0mm	21 12 mm	22 3.5m	23	24 ◐ 1st Quarter	25
26 2.0mm	27 1.0mm	28 30.5mm	29 3 mm	30 20mm	31 ○ Full Moon 30. 20.5mm	1



April 2018

Rainfall Calendar Mashare

timeanddate.com

Mon	Tue	Wed	Thu	Fri	Sat	Sun
26	27	28	29	30	31 ○ Full Moon	1
2	3	4	5 20.2mm	6	7 22mm	8 ◑ 3rd Quarter
9	10	11	12	13	14	15
16 ● New Moon	17	18	19	20	21	22 ◐ 1st Quarter
23	24	25	26	27	28	29
30 ○ Full Moon	1	2	3	4	5	6