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Evaluating the Yield Response to Bio-Inoculants of *Vigna unguiculata* in the Kavango Region in Namibia

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Abstract

The Kavango region (Northern part of Namibia) were the study was carried out, is extensively involved in agriculture and is also known to be dominated by the sandy aerosols soils. The bad soils in the region, which have poor nutrients and water holding capacity, combined with a fast rate of climate change in the region has contributed in the reduction in yield of most crops grown in the area. The main aim of the study was to determine cowpeas response to bio-inoculants by assessing yield of the pulse. Six different cultivars of *Vigna unguiculata* (Cow pea) were evaluated for their response to bio-inoculants. These cultivars were subjected to 3 different treatments. One with chemical fertilizer, another with *Bradyrhizobium* strains (14-3) and (1-7) bio-inoculants and a third which was a negative control with no treatment. After 90 days post seeding the cultivars were harvested and different yield parameters assessed. The cowpeas that were subjected to the bio-inoculant treatments yielded a later 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 cheaper eco-friendly alternative to mineral fertilizers.

Keywords: Arenosols; Climate-change; Cultivars; *Vigna unguiculata*; Cowpea, Bio-inoculants; Fertilizer; *Bradyrhizobium*; Yield

Introduction

There is a general notion that the Kavango region could be the “Breadbasket” of Namibia if the fields in the area could be productive [1]. This belief is met with challenges due to the harsh climate and the Kalahari sands (arenosols) that dominate most of the area. *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 [2]. The constraints that affect agriculture in The Kavango are the same ones that affect most of the Northern areas of Namibia [1], with poor nutrition status and water retention of the soil, a variable climate and a far distance from the market being the major ones. People’s main source of livelihood in these area is small scale farming [3] despite the poor crop production capacity of the porous soils, hence the need to intervene and study how crops that are widely grown in these regions with harsh conditions can be improved so as to contribute in increasing the fertility of the soils. 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 with that are highly nutritious and are high-yielding [4]. 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 [4]. With Southern Africa having the highest population of undernourished people in the world [4], cowpea, which is one of the major grain legumes in the region [5], is favourable to be explored to prepare for this threatened food security. Legumes such as cowpea are known to be raw materials that are important in the balancing of the human diet due them being able to provide high proteins, vitamins, minerals and an important source of carbohydrates according to Kiim et al. [6]. They have been known to have multiple physiological effects such as the prevention of metabolic diseases like colon cancer and diabetics and also in the reduction of blood and glucose levels [7]. *Vigna unguiculata* has been reported to have a high amount of organic matter and generally multiuse properties hence its use by farmers as fodder to feed their animals [8]. *Pennisetum glaucum* (locally known as mahangu), which is one of Namibia’s staple foods, is widely cultivated in the Kavango region. There have been reports of mahangu yields in the area being lower than they were about 30 years ago [1] due to decreasing soil fertility, therefore an urgent need to intervene is required. Despite legumes having to be known to be of wide occurrence during traditionally set cropping settings, they are also deliberately used to manage the soil fertility by most small scale subsistence farmers [9]. Legumes do this by fixing atmospheric nitrogen into ammonia by the help of the nitrogenase enzyme and also by their incorporation into cereal based cropping systems which result into them increasing the soil fertility as was demonstrated by Zahran [10]. Nitrogen fixation is an important process for life forms on earth, biological processes such as the use of legumes, fix about 60% of the world’s nitrogen [10]. Thus, this process is a major source of nitrogen into soils especially for arid environments [11]. for example states that nitrogen fixation by rhizobia on soy bean production in Brazil results in an estimated save of about US$ 10 billion annually instead of the use of chemical fertilizer, this is by using *Bradyrhizobium* as a bio-inoculant. The outcome of this study will be significant in providing the subsistence farmers with bio-inoculants that are able to effectively fix biological nitrogen when in symbiosis with cowpea under a climate of low rainfall. 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.

Materials and Methods

The study was conducted by obtaining cowpea cultivars from Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ), these were namely (Figure 1) Nakare (Na), Lutembwe (Lu), I2, Bira (Bi), Shindimba (Shi), and Silwana (Si) respectively. Cowpea strains

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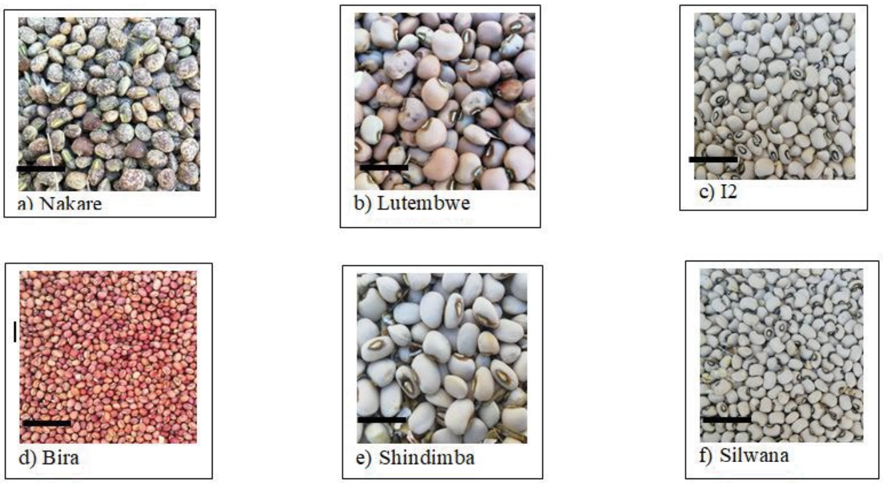

Figure 1: Cowpea cultivars that were used in the study with their indigenous names. Scale bar=1 cm in all the pictures (a) to (f).

Figure 2: Depicting the cowpea study field at Mashare just before harvesting.

Figure 3: Showing one Lutembwe shoot immediately after harvesting.

that are resistant to the 'witch weed' *Alectra vogelii*, which according to Mwaipopo [12] is a parasitic weed that is known to cause major constraints on legumes and most especially cowpea, where obtained from The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) these were named I2. The cowpea was grown under rain fed conditions so as to stimulate the natural environmental conditions of the area. The field was ploughed back and forth to homogenize the plot on which the planting was done. It was divided into 3 sections, with one section having a treatment of the 6 different cowpea cultivars plus nitrogen fertilizer, the other section having the cowpea cultivars plus bio inoculants and the third having only the cowpea cultivars minus any other treatment. This design is illustrated in the diagram below. The inoculant treatment was performed by getting a substantial amount of bacterial inoculant strains called *Bradyrhizobium* (1-7) and (14-3). The strains were grown fresh Modified Arabinose Gluconate Medium, with peat as a carrier, then packed in Whirl-Pack® sample bags. The bags were stored at room temperature with avoiding their exposure to direct sunlight for long hours. Just before planting the bio inoculant treatment, a small amount 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. Urea was added on the soils were the nitrogen fertilizer treatment was to be performed.

Yield assessment

Harvest data collection: The yield of the different cowpea cultivars under the three categories of namely: Nitrogen fertilizer, Bio inoculant and No treatment was assessed by comparing the physiological maturity of the different cultivars with the above-named treatments. This assessment was done by selecting 10 cow pea plants from the middle rows of a subplot avoiding the border plants. These were used to calculate the root dry matter, grain and plant dry matter and converted into yield per kilo hectare. Each subplot was expected to have has an estimated 120 plants. With some subplots recording only about half the amount or less during harvesting. This was due to some plants not being so adapted to the local soils and environment in the region and some having been dried up by the time the harvesting took place. 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 none inoculated plots were harvested first and for those subplots that had a few plants growing on them due to some having been dried or not germinated, for these, less than 10 middle plants were harvested such as 7 Nakare plants being excavated. This was followed by the harvesting of the bio inoculant treatment plots. 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.

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

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)
NAKARE Average Yield	383.33	225.21	1501.89	138.73	70.17	4678.22	2273.23	1262.9
SILWANA Average Yield	1125	225	15000	109.055	33.7525	2250.17	2401.86	1334.37
SHINDIMBA Average Yield	300	360	24000	339.24	80.31	5354.22	273.18	151.77
LUTEMBWE Average Yield	2500	1264.71	84313.9	105.3	27.65	1843.33	3560.66	1978.14
BIRA Average Yield	1800	990	66000	158.38	35.5	2366.33	4270.64	2372.58

Table 1: Average yield data per subplot from the negative control (non-inoculated plot).

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. Fertiliser Average Yield	2600	732.73	48848.58	118.905	32.53	2168.5	2264.78	1258.21
SHINDIMBA. Fertiliser Average Yield	517	517.5	34500	147.3	33.39	2226	463.97	257.76
NAKARE. Fertiliser Average Yield	966.67	172.07	11471.11	236.5	40.07	2671.33	1873.42	1040.79
SILWANA. Fertiliser Average Yield	2000	600	40000	110.28	29.13	1942.16	3703.37	2057.43
BIRA. Fertiliser Average Yield	2550	493	32866.67	104.38	39.7	2646.65	2460.98	1367.21
NIGERIAN Cultivar. Fertiliser Average Yield	350	105	7000	54.47	13.13	875.58	1652.95	918.31

Table 2: Showing the average yield data for the Subplots with fertilizer treatment.

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 stain 1-7 Average Yield	2100	966	64400	146.615	40.4025	2693.5	4901.745	2723.191667
LUTEMBWE+Bradyrhizobium strain 14-3 Average Yield	2350	1272.9175	84861.17	135.725	43.775	2918.33	4922.36	2734.64
NAKARE+Bradyrhizobium strain 14-3 Average Yield	850	578	38533.33	209.73	99.46	6630.67	8118	4510
NAKARE+Bradyrhizobium stain 1-7 Average Yield	300	427.5	28500	130.225	62.465	4164.33	4918.5	2732.5
SHINDIMBA+Bradyrhizobium stain 1-7 Average Yield	175	159.25	10616.67	188.8	53.32	3554.67	2921.4	1623
SILWANA+Bradyrhizobium stain 1-7 Average Yield	1900	1148.635	76575.67	129.95	49.965	3331	7185.49	3991.94
SILWANA+Bradyrhizobium strain 14-3 Average Yield	2300	1086.11	72407.33	168.425	41.985	2799	5844.51	3246.95
BIRA+Bradyrhizobium stain 1-7 Average Yield	550	403.335	26889	129.82	31.75	2116.67	3324.88	1847.16
BIRA+Bradyrhizobium strain 14-3 Average Yield	1100	407	27133.33	142.76	35.905	2393.67	2664.01	1480.01

Table 3: Showing the yield data from the bio-inoculant treated plots.

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	4.194	0	0.572
Intercept	73585686100	1	7.3586E+10	116.055	0	0.712
Treatment	1076062735	2	538031367	0.849	0.434	0.035
Cultivar_Name	20984435470	5	4196887093	6.619	0	0.413
Treatment * Cultivar Name	13659285540	8	1707410693	2.693	0.016	0.314
Error	29800844500	47	634060521	--	--	--
Total	1.78973E+11	63	--	--	--	--
Corrected Total	69687234570	62	--	--	--	--
a.R Squared=0.572 (Adjusted R Squared=0.436)						

Table 4: Tests of between-subjects' effects (Plant_Dry_Matter_Yield).

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. Lastly 100 seeds were selected, from the seeds obtained from the 40 pods, and weighed to get the 100 seed weight per subplot. The seed weight per subplot was also recorded and the above procedure was done for all the subplots.

Results

Harvesting

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.

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	0	0.603
Intercept	163213020.9	1	163213020.9	189.721	0	0.801
Treatment	22438450.78	2	11219225.39	13.041	0	0.357
Cultivar_Name	14938031.29	5	2987606.259	3.473	0.009	0.27
Treatment ˙Cultivar_Name	14217142.25	8	1777142.782	2.066	0.059	0.26
Error	40433110.91	47	860278.956	--	--	--
Total	308977495.6	63	--	--	--	--
Corrected Total	101833755.7	62	--	--	--	--
a.R Squared=.603 (Adjusted R Squared=.476)						

Table 5: Tests of between-subjects' effects (Grain_Yield).

Shoot biomass yield

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 (Figure 2) which was more effective by measuring the yield. The tables (Tables 1-3) that follow are the results of these comparisons in terms of the shoots, roots and the grain yield of each cultivar planted.

Data analysis

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. 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.

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

The p-value of treatment to Plant dry matter yield is 0.434, meaning there is no statistical difference between the treatments and shoot biomass yield at the 0.05 level of significance. On the other hand there is 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 Annex (Table 4). Therefore, there is 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.

Grain yield

For the grain yield statistical analysis, there is 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 is no statistical difference in means of the interaction of Treatment and Cultivar name with grain yield with a p-value of 0.059. Therefore for Treatment and Cultivar name, the null hypotheses that 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.

Are rejected at the 0.05 level of significance (Table 5).

Discussion and Conclusion

Yield in terms of shoot biomass

It is of utmost importance to assess the yield of the performed treatments and how these yield components respond so as 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 is no statistical difference in the obtained plant dry matter measurements across the three different treatments with a $p > 0.05$, this is despite there being an observed difference in the obtained mean values of the treatments. This study's findings in this regard correlates to a study done by Hungria et al. [13], who reported that the use of mineral fertilizer did not have any significant effect on the shoot dry biomass when applied at recommended rates but resulted in a significant increment when applied at 1.5 times the recommended amounts. These obtained results indicate that the use of nitrogen fertilizer to enhance shoot biomass of the bean is not beneficial. Andrade et al., [14] States that previous studies on 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 significant p-value less than 0.05. When subjected to all the three different treatments, the cultivar Lutembwe had the largest plant dry yield per hectare as compared to the other cultivars (Figure 3). Hence for farmers that would like to grow cowpea for forage use, this would be the recommended cultivar. This 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. With regards to the interaction between cultivar name and the type of treatment and their effect on shoot dry matter yield, at the 0.05 level of significance, there is a significant interaction between the factors and the shoot dry matter yield of 0.016. 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. Therefore with regards to the shoot biomass, with fertilizer treatment maximum yield is 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.

Grain yield

Cowpea grain yield is considered one of the most important parameters for farmers in terms of assessing how different treatments affect yield [15]. This is due to the proteins having a high protein content for consumption [14] in addition, the more the grain yield the more profits the farmers expects from the legumes and also this entails the farmer has a surplus to plant for the next season. There is a significant difference in the means of the different treatments and cultivars with relation to the grain yields. Our study reviewed a significant mean difference of 1604.14 based on the post hoc tests between the fertilizer treatment and the bio-inoculant treatment, with the bio-inoculant recording a higher grain yield. This is more than a 10% increase in grain yield which is a substantial as an indication that a treatment is working according to Ronner et al. [16]. Our findings are close to the 30% increase in grain yield by bio-inoculant application reported by Martins et al. [17]. 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 3619.45, 3621.25 and 2728.92 kg/ha respectively. Shindimba had the lowest grain yield, a less lower than the Nigerian cultivar. Therefore outcome of this study is significant in providing the subsistence farmers with bio-inoculants that are able to effectively fix biological nitrogen when in symbiosis with cowpea under a climate of low rainfall. 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.

References

1. Strohbach BJ, Petersen A (2007) Vegetation of the central Kavango woodlands in Namibia: An example from the Mile 46 Livestock Development Centre. Sou Afri J Bot 73: 391-401.
2. Mendelsohn J, Obeid S (2003) Sand and water: A profile of the Kavango region. Cape Town, Windhoek, Struik Publishers and RAISON.
3. Moray C, Game ET, Maxted N (2014) Prioritising in situ conservation of crop resources: A case study of African cowpea (*Vigna unguiculata*). Sci Rep 4: 5247.
4. Mendelsohn J (2009) Land use in Kavango: Past, present and future. Environmental protection and sustainable management of the Okavango River Basin (EPSMO) Project pp: 1-31.
5. Hamid S, Muzaffar S, Wani IA, Masoodi FA, Bhat MM (2014) Physical and cooking characteristics of two cowpea cultivars grown in temperate Indian Climate. J Sau Soci Agr Sci 15: 27-134.
6. Kiim YY, Woo KS, Chung HJ (2018) Starch characteristics of cowpea and mungbean cultivars grown in Korea.
7. Hoover R, Hughes T, Chung HJ, Liu Q (2010) Composition, molecular structure, properties and modifaication of luse starches: A review. Food Res Int 43: 339-413.
8. Phiri GK, Wellard K, Snapp S (2017) Agricultural systems in a changing world. In: Agr Sys pp: 3-15.
9. Kermah M, Franke AC, Adjei-Nsiah S, Ahiabor BDK, Abaidoo RC, et al. (2018) N2-fixation and N contribution by grain legumes under different soil fertility status and cropping systems in the Guinea savanna of northern Ghana. Elsevier 261: 201-210.
10. Zahran HH (1999) Rhizobium-Legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. Microbiol Mol Biol Rev 63: 968-989.
11. Leite J, Passos SR, Simoes-Araujo JL, Rumjanek NG, Xavier GR, et al. (2017) Genomic identification and characterization of the elite strains *Bradyrhizobium yuanmingense* BR 3267 and *Bradyrhizobium pachyrhizi* BR 3262 recommended for cowpea inoculation in Brazil. Brazil J Microbio 49: 703-713.
12. Mwaipopo BV (2014) Characterization of alectra vogelii (witch weed) strains using molecular markers in selected parts of malawi and tanzania.
13. Hungria M, Franchini JC, Campo RJ, Crispino CC, Moraes JZ, et al. (2006) Nitrogen nutrition of soybean in Brazil: Contributions. Canadian Journal of Plant Science 86: 927-939.
14. Andrade MMM, Stamford NP, Santos Carolina ERS, Freitas ADS, Sousa CA, et al. (2013) Effects of biofertilizer with diazotrophic bacteria and mycorrhizal fungi in soil attribute, cowpea nodulation yield and nutrient uptake in field conditions. Scientia Horticulturae 162: 374-379.
15. Horn L, Shimelis H, Sarsu F, Mwadzingeni L, Lainga MD (2017) Genotype-by-environment interaction for grain. Crop Science Society of China 6: 306-313.
16. Ronner E, Franke AC, Vanlauwe B, Dianda M, Edeh E, et al. (2016) Understanding variability in soybean yield and response to P-fertilizer. Field Crops Res 186: 133-145.
17. Martins LMV, Xavier GR, Range FW, Ribeiro JRA, Neves MCP, et al. (2003) Contribution of biological nitrogen fixation to cowpea: A strategy of improving grain yield in the semi-arid region of Brazil. Bio Fert Soils 38: 333-339.