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# $N_2$ Fixation by Grain Legume Varieties as Affected By Rhizobia Inoculation in The Sandy Loam Soil Of Sudano-Sahelian Zone of North Eastern Nigeria.

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**ABSTRACT:** Rhizobium – legume symbiotic association contributes considerable amount of N in tropical soils. However, low rainfall and high temperature in Sudano-Sahelian region of Northeastern Nigeria may affect the Rhizobial population in the soil. Therefore, the influence of Rhizobia inoculation on $N_2$ fixation by cowpea (Vigna unguiculata (L.) Walp.), groundnut (Arachis hypogaea L.) and bambara groundnut (Vigna subterranean L.Verdc.) was evaluated under field condition in Maiduguri. The seeds of the crops were inoculated with their Rhizobium strains and grown for 50 days, after which they were harvested and the amount of N fixed was measured. The results showed that rhizobia inoculation increased the amount of N fixed by 46% over the control. Cowpea differed significantly ($P<0.05$) from groundnut and bambara groundnut. Cowpea fixed 42.68 $KgNha^{-1}$ while groundnut and bambara groundnut fixed 27.19 and 32.53 $KgNha^{-1}$, respectively. Cultivation of Bornoji red cowpea variety inoculated with Rhizobium will alleviate soil N level.
**Key words**: $N_2$-fixation; grain legumes; rhizobia; inoculation

## INTRODUCTION

Majority of soils in Sudano- Sahelian zone of north eastern Nigeria are sandy and inherently low in N fertility (Rayar, 2000). High cost of chemical fertilizers and their non-availability at proper time in the region have renewed the interest of farmers on biological nitrogen fixation. Biological $N_2$ – fixation provides continuous supply of N for plant growth in situ, add organic matter to the soil and is economically viable. The direct availability of the fixed N to the host plant allows it to grow in environments that are low in N and at the same time reduces losses from denitrification, volatilization and leaching, improving the sustainability of agricultural system that involves such symbiosis. Rhizobium – legume symbiotic association contributes considerable amount of N in tropical soils.. According to Dakora and Keya (1997), grain legumes fix about 15-210 kgN/ha seasonally in Africa. Studies in humid regions of western Nigeria have shown that cowpea can fix 66-120 kgN/ha in 57 days (Awosaike *et al.,* 1990). Yusuf *et al,* (2006) reported that cowpea fixed 16-34kgN/ha and soybean between 41 and 50kgN/ha in the northern Guinea Savanna zone of Nigeria. Studies conducted under semi-arid conditions (Rayar, 1986a) indicated that groundnut can fix about 65 – 100 kgN/ha/year. Grain legumes are therefore involved in rotation and intercropping systems in order to increase the yield of cereal crops. Reported yield responses to previous legume crops are mainly in the range of 50-80% increase over yields in cereal-cereal sequence. Legumes also increase the efficiency with which water is used (Rifat,2005). The amount of N fixed by *Rhizobium*-plant association varies considerably with the environmental factors such as soil temperature, moisture, and pH which affect the host-plant (macrosymbiont) and the *Rhizobium* (microsymbiont) (Rayar, 2000). Rhizobial population densities tend to be low under dry soil conditions and to increase as the moisture stress is relieved (Tate,1995). High soil temperatures in tropical and subtropical areas are a major problem for biological nitrogen fixation of legume crops (Michiels *et al.,* 1994). For most R*hizobia,* the optimium temperature range for growth in culture is 28 to $31^0$C. The low rainfall and high temperature in Sudano-Sahelian zone of north eastern Nigeria may affect the population and activity of R*hizobia* in the soil. Information on the effect of R*hizobia* inoculation on legume crops in this region is lacking. Therefore, this study aimed at evaluating the effect of

*Rhizobia* inoculation on $N_2$ fixation by cowpea, groundnut, and bambara groundnut.

## MATERIALS AND METHODS

A two- year field experiment was conducted during the 2005 and 2006 cropping seasons at the Department of Soil Science Teaching and Research Farm, University of Maiduguri, located on latitude $11^0$ $51^1$N,and longitude $13^0$ $15^1$ E. Maiduguri falls in the sudano-sahelian agroecology with semi-arid climate. The total amount of rainfall for the 2005 and 2006 were 830 and 423mm, respectively. The mean annual temperatures for the years were $33^0$C and $34.6^0$C, respectively. The soil has been classified as *Typic ustipsamment* (Rayar, 2000).

The treatments consisted of three legume cultivars: Bornoji red (cowpea), KoljiKanuri (groundnut) and Mallum Karekare (bambara groundnut); a sorghum var. Paul Biya ( as a reference crop) and three *Bradyrhizobium* strains (collected from the same legume cultivars during the previous experiment and preserved according to Clayet – Marcel (1989). Seed inoculation was done as described by Montage and Saint Marcary (1989). This was conducted using a factorial experiment and the treatments were arranged in randomized complete block design (RCBD), replicated three times. A pre-plant soil sample of the site aws taken and analyzed for some physico-chemical properties in accordance with VanReeuwijt (1992).The site was ploughed, harrowed and laid out into plot sizes of 4 m long and 3 m wide. Three seeds were sown per hole according to BOSADP (1993) recommended spacing. Seedlings were thinned down to two per hill one week after sowing. Weeding was done with hoe. At fifty days after sowing, the crops were harvested from a 1 $m^2$ (quadrant) area within the plots by cutting the shoots at ground level and digging out the roots with their nodules. Post harvest soil samples were also taken from each plot and analyzed for total N as described by van Reeuwijk (1992). The number of nodules was counted and the plant materials were oven dried at $65^0$C, weighed and analyzed for the N concentrations (Marr and Cresser, 1983).

The amount of N fixed and percent N derived from biological fixation were estimated by the equations of Mary *et al.,* (1995).

$$\text{Total N in plants} = \frac{\text{Dry matter weight X \%N in plants}}{100}$$

$$\text{N fixed ( NDFA)} = \text{Total N in legume-Total N in reference crop}$$

$$\text{\%NDFA} = \frac{\text{Total N in legume – Total N in reference crop x 100}}{\text{Total N in legume}}$$

Where, NDFA means nitrogen derived from fixation.

The data collected were subjected to analysis of variance (ANOVA) and significant differences were compared using Duncan's multiple range test at 5% probability level (Gomez and Gomez. 1984).

In the second cropping season, the plots of the previous season were maintained in their respective positions. Each plot was cultivated separately with hoe and its treatments (crop and inoculants) were repeated. However, all other cultural practices as well as soil, plant and statistical analysis were the same.

## RESULTS AND DISCUSSION

The soil of the experimental site is sandy loam in texture and neutral in reaction with low organic carbon (< 5 g/kg soil), total N (<1 g/kg soil) and available P (<10 mg/kg soil) according to FPDD (2002) soil fertility ratings (Table 1).

**Table 1.** Texture and some selected chemical characteristics of soil of the experimental site 2005

| Parameter | Values |
|---|---|
| Sand (g/kg) | 700 |
| Silt (g/kg) | 150 |
| Clay (g/kg) | 150 |
| Texture | Sandy loam |
| pH(1:2.5,$H_2O$) | 6.71 |
| Organic carbon (g/kg) | 4.40 |
| Total nitrogen (g/kg) | 0.50 |
| Available phosphorus (mg/kg) | 5.30 |

The effects of *Rhizobium* inoculation on cowpea, groundnut and bambara groundnut in the year 2005 and 2006 cropping seasons are presented in Tables 2 and 3, respectively. The results of the cropping seasons followed similar trends but the values of 2006 were higher than those of 2005.This could probably due to residual effects of Rhizobial inoculation which masked the effects of temperature and rainfall. Averaged over the two years, the results presented in Table 4 shows that seeds inoculation with suitable R*hizobium* strains enhanced the performances of crops significantly as reflected by the interactions between crops and Rhizobia. This positive responses of the legumes as a result of Rhizobium inoculation could probably due to low population densities of the indigenous Rhizobia, which in turn might be as a result of low rainfall and high temperature in this zone. Studies conducted in savanna zone of Benin Republic (Hounguandan *et al*., 2000) showed that the response of mucuna to inoculation was inversely related to the population of indigenous Rhizobium in the soil, and that there was only a response when the population was < 5 Rhizobia cells/g soil. Sanginga *et al.*, (1996b) observed a similar relationship between the Rhizobial population and inoculation for soybean in the Savanna zone of Nigeria. High soil temperature in the tropical areas is one of the major problems affecting symbiotic nitrogen fixation in legumes. The optimum temperature range for growth of most Rhizobia is 28 to $31^0$C and many are unable to grow at $37^0$C ( Michiels *et al.,* 1994). According to Graham (1992), rhizobial survival in soil exposed to high temperatures is greater in soil aggregates than in non- aggregated soil, which is the dominant soil in this zone. Zahran (1999) reported that the survival of a strain of *Bradyrhizobium* of Cajanus in a sandy loam soil was very poor and it did not persist to the next cropping season when the soil moisture content was about 2.0 to 15.5%.These indicate the need for seeds inoculation with effective R*hizobium* for adequate $N_2$-fixation in Sudano-Sahelian regions. Rhizobium inoculation is a cheaper and usually more effective agronomic practice for ensuring adequate nitrogen supply. The results also showed significant ($P<0.05$) differences among the crops in their responses to the inoculation. In cowpea, inoculation caused increase in nodule number by 21%, N content by 33% and amount of N fixed by 38% over the control. These results concur with the reports of Chude *et al*. (2006) and Uzoma *et al*. (2006) that cowpea responded positively to *Rhizobium* inoculation in savanna soils of Nigeria. Rabie and Al-Humiany ( 2004) observed that a mixed inoculation of $N_2$-fixing bacteria, P-solublizing bacteria and vesicular-arbuscular mycorrhizal fungus is more effective than $N_2$-fixing bacteria alone on cowpea in calcareous soil. In contrast, Ahmad *et al*. (1981) observed that cowpea rarely respond to *Rhizobium* inoculation in soils already containing R*hizobia* which nodulate such crops.

A positive response to the *Rhizobium* inoculation was also observed in groundnut; N content increased by 32% and amount of fixed N by 39% over the control. This results were in line with Ataur Rahman (2006) and Manisha and Bhadoria (2008),who observed significant responses of groundnut to Rhizobia inoculation. In contrast, Badiane and Gueye (1992) showed that inoculation did not improve the performance of groundnut. The inefficiency of the inoculation may have been caused by the presence of indigenous *Bradyrhizobia* that were more efficient than the strain used in the experiment. Similarly, El-tum Hassan(2003) did not observe a significant effect of foreign R*hizobium* on groundnut in Western Sudan. The positive result obtained in this study could be because indigenous *Rhizobia* strains were used for inoculation.

**Table 2.** Effect of rhizobium inoculation on nodulation, nitrogen content and $N_2$ fixation by cowpea, groundnut and bambara groundnut in 2005 cropping season.

| Crops | No *Rhizobium* | Inoculated with *Rhizobium* | Mean |
|---|---|---|---|
| | | Nodules/plant | |
| Cowpea | 37.67 | 43.00 | 40.33 [b] |
| Groundnut | 46.00 | 49.67 | 47.83 [b] |
| Bambara groundnut | 24.00 | 42.67 | 33.33 [c] |
| Mean | 35.89 [b] | 45.11 [a] | |
| SE± | 1.43 (rate) | | |
| SE± | 1.75 (crop) | | |
| SE± | 2.47 (interaction) | | |
| | | Total N in plant (kg/ha) | |
| Cowpea | 38.56 | 41.24 | 39.90 [a] |
| Groundnut | 13.39 | 17.11 | 15.25 [b] |
| Bambara groundnut | 19.05 | 27.72 | 23.38 [b] |
| Mean | 23.67 [a] | 28.69 [a] | |
| SE± | 3.44 (rate) | | |
| SE± | 4.22 (crop) | | |
| SE± | 5.97 (interaction) | | |
| | | N-fixed (kg/ha) | |
| Cowpea | 35.84 | 37.08 | 36.46 [a] |
| Groundnut | 9.00 | 12.71 | 10.85 [b] |
| Bambara groundnut | 14.65 | 23.59 | 19.12 [b] |
| Mean | 19.83 [b] | 24.46 [a] | |
| SE± | 3.56 (rate) | | |
| SE± | 4.35 (crop) | | |
| SE± | 6.16 (interaction) | | |
| | | % NDFA | |
| Cowpea | 87.46 | 88.19 | 88.19 [a] |
| Groundnut | 66.21 | 62.88 | 62.88 [b] |
| Bambara groundnut | 74.13 | 79.14 | 79.14 [a] |
| Mean | 75.93 [a] | 77.56 [a] | |
| SE± | 3.09 (rate) | | |
| SE± | 3.79 (crop) | | |
| SE± | 5.36 (interaction) | | |
| | | % Soil N | |
| Cowpea | 0.065 | 0.075 | 0.070 [a] |
| Groundnut | 0.051 | 0.051 | 0.051 [b] |
| Bambara groundnut | 0.046 | 0.084 | 0.065 [ab] |
| Mean | 0.054 [b] | 0.070 [a] | |
| SE± | 0.004 (rate) | | |
| SE± | 0.005 (crop) | | |
| SE± | 0.006 (interaction) | | |

Means in columns and rows followed by similar letter(s) are not significantly different at the 5% probability level of the Duncan's Multiple Range Test (DMRT).

**Table 3.** Effect of Rhizobium inoculation on nodulation, nitrogen content and $N_2$ fixation by cowpea, groundnut and bambara groundnut in 2006 cropping season.

| Crops | No *Rhizobium* | Inoculated with *Rhizobium* | Mean |
|---|---|---|---|
| | | Nodules/plant | |
| Cowpea | 36.65 | 47.00 | $41.53^{b}$ |
| Groundnut | 49.00 | 54.67 | $51.83^{a}$ |
| Bambara groundnut | 32.00 | 41.67 | $36.83^{b}$ |
| Mean | $39.22^{b}$ | $47.78^{a}$ | |
| SE± | 1.99 (rate) | | |
| SE± | 2.43 (crop) | | |
| SE± | 3.44 (interaction) | | |
| | | Total N in plant (kg/ha) | |
| Cowpea | 42.60 | 66.53 | $54.56^{a}$ |
| Groundnut | 42.09 | 56.33 | $49.21^{a}$ |
| Bambara groundnut | 40.95 | 64.07 | $52.51^{a}$ |
| Mean | $41.88^{b}$ | $62.31^{a}$ | |
| SE± | 2.73 (rate) | | |
| SE± | 3.34 (crop) | | |
| SE± | 4.72 (interaction) | | |
| | | N-fixed (kg/ha) | |
| Cowpea | 36.92 | 60.85 | $48.89^{a}$ |
| Groundnut | 36.42 | 50.65 | $43.54^{a}$ |
| Bambara groundnut | 35.27 | 58.39 | $46.83^{a}$ |
| Mean | $36.20^{b}$ | $56.63^{a}$ | |
| SE± | 2.73 (rate) | | |
| SE± | 3.34 (crop) | | |
| SE± | 4.72 (interaction) | | |
| | | % NDFA | |
| Cowpea | 86.58 | 91.29 | $88.94^{a}$ |
| Groundnut | 86.12 | 89.95 | $88.03^{a}$ |
| Bambara groundnut | 86.11 | 90.94 | $88.53^{a}$ |
| Mean | $86.27^{b}$ | $90.72^{a}$ | |
| SE± | 0.51 (rate) | | |
| SE± | 0.62 (crop) | | |
| SE± | 0.88 (interaction) | | |
| | | % Soil N | |
| Cowpea | 0.079 | 0.098 | $0.089^{a}$ |
| Groundnut | 0.070 | 0.098 | $0.084^{a}$ |
| Bambara groundnut | 0.065 | 0.079 | $0.072^{a}$ |
| Mean | $0.073^{b}$ | $0.092^{a}$ | |
| SE± | 0.004 (rate) | | |
| SE± | 0.004 (crop) | | |
| SE± | 0.008 (interaction) | | |

Means in columns and rows followed by similar letter(s) are not significantly different at the 5% probability level of the Duncan's Multiple Range Test (DMRT)

**Table 4.** Effect of Rhizobium inoculation on nodulation, nitrogen content and fixation by cowpea, groundnut and bambara groundnut,combined for two years

| Crops | Not Inoculated | Inoculated | Mean |
|---|---|---|---|
| | | Nodules/plant | |
| Cowpea | 37.17 | 45.00 | 41.08 [b] |
| Groundnut | 47.50 | 52.17 | 49.83 [a] |
| Bambara groundnut | 28.17 | 42.17 | 35.17 [b] |
| Mean | 37.61 [b] | 46.44 [a] | |
| SE± | 2.35 (rates) | | |
| SE± | 2.88 (crops) | | |
| SE± | 4.07 (interaction) | | |
| | | Total N in plant (kg/ha) | |
| Cowpea | 40.58 | 53.89 | 47.23 [a] |
| Groundnut | 27.74 | 36.72 | 32.23 [b] |
| Bambara groundnut | 30.00 | 45.89 | 37.95 [b] |
| Mean | 32.77 [b] | 45.50 [a] | |
| SE± | 2.18 (rates) | | |
| SE± | 2.67 (crops) | | |
| SE± | 3.78 (interaction) | | |
| | | N-fixed (kg/ha) | |
| Cowpea | 35.55 | 48.97 | 42.68 [a] |
| Groundnut | 22.71 | 31.68 | 27.19 [b] |
| Bambara groundnut | 24.96 | 40.99 | 32.98 [b] |
| Mean | 27.74 [b] | 40.55 [a] | |
| SE± | 2.20 (rates) | | |
| SE± | 2.69 (crops) | | |
| SE± | 3.80 (interaction) | | |
| | | % NDFA | |
| Cowpea | 87.02 | 90.11 | 88.56 [a] |
| Groundnut | 76.16 | 74.75 | 75.46 [b] |
| Bambara groundnut | 80.12 | 87.55 | 83.83 [a] |
| Mean | 81.10a | 84.13[a] | |
| SE± | 1.51 (rates) | | |
| SE± | 1.84 (crops) | | |
| SE± | 2.61 (interaction) | | |
| | | % Soil N | |
| Cowpea | 0.072 | 0.086 | 0.079 [a] |
| Groundnut | 0.061 | 0.075 | 0.068 [b] |
| Bambara groundnut | 0.051 | 0.082 | 0.067 [b] |
| Mean | 0.062 [b] | 0.081 [a] | |
| SE± | 0.005 (rate) | | |
| SE± | 0.006 (crops) | | |
| SE± | 0.008 (interaction) | | |

Means in columns and rows followed by similar letter(s) are not significantly different at the 5% probability level of the Duncan's Multiple Range Test (DMRT).

The results al so showed that inoculation resulted in increase in nodule number by 52%, N accumulation by 53% and N fixed by 64% over the control in bambara groundnut. These results were in line with the reports of Gueye (1992) and Kishinevsky *et al*.,(1996) that Rhizobium inoculation caused a significant increase in nodule dry weight, N accumulation, N fixation and percentage N derived from fixation in bambara groundnut. A greater performance was obtained with double inoculation with *Rhizobium* strain and *Glomus mosseae ( Gueye, 1992).* The author further pointed out that bambara groundnut has a high nitrogen-fixing potential provided that an effective *Rhizobium* strain is used and that the plant nutrient requirements other than nitrogen are met.

When the crops are compared, cowpea differed significantly (P<0.05) from groundnut and bambara groundnut. Cowpea fixed 42.68 $KgNha^{-1}$ while groundnut and bambara groundnut fixed 27.19 and 32.98 $KgNha^{-1}$, respectively.

**Conclusion**: Based on the results of this study, it could be concluded that in the sandy loam soil of Sudano-Sahelian zone of northeastern Nigeria seed inoculation significantly improved nodulation, $N_2$-fixation and N secretion by cowpea, groundnut and bambara groundnut. The crops differed significantly in their responses to Rhizobium inoculation. Cowpea fixed the highest amount of N (42.68 kg/ha). Therefore, cultivation of Bornoji, red cowpea variety inoculated with compatible Rhizobium strain will improve soil N fertility and reduce fertilizer cost and environmental pollution in this agroecological zone.

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