Response of grain legumes to rhizobial inoculation in two savanna soils of Nigeria

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Three inoculation trials with soybean, cowpea and groundnut were conducted on Eutric Cambisols (EC) and Rhodic Nitisols (RN) in a greenhouse. Five rhizobial inoculants: MAR 1495, TSBF Mixture, Legumefix, HiStick and IRJ 2180A were tested on each crop to determine their response to soil type and ability to form symbiotic relationship with the crops. Generally, response to inoculation was found to be significantly higher (P < 0.05) in EC than RN. In soybean and groundnut trials, highest nodulation in both soils was recorded by strain MAR 1495 followed by TSBF Mixture and these were significantly higher (P < 0.05) than other inoculants and control. A similar trend, though only in EC, was observed in N uptake and in nitrogen fixation but no significant difference was observed in dry matter yield. Cowpea trials did not show response to inoculation nor was there difference between the soils. Instead, control treatment surpassed all the inoculated treatments in nodulation at P < 0.05. Nitrogen uptake and N₂ fixation of control also surpassed those of inoculated treatments. Rhizobia strains MAR 1495 and TSBF Mixture showed similar ability to improve the productivity of soybean and groundnut thus can be used as common inoculants for the two crops.

Key words: Rhizobial inoculation, Eutric cambisols, Rhodic nitisols, nodulation, dry matter yield, N uptake, N₂ fixation.

INTRODUCTION

Continuous depletion of nitrogen (N) from the soil pool by processes such as volatilization, leaching and, perhaps most importantly, removal of nitrogen-containing crop residues from the land results in the decline of soil N reserves in agricultural soils. Replenishment has depended largely on the addition of inorganic fertilizers: MAR 1495, TSBF Mixture, Legumefix, HiStick and IRJ 2180A were tested on each crop to determine their response to soil type and ability to form symbiotic relationship with the crops. Generally, response to inoculation was found to be significantly higher (P < 0.05) in EC than RN. In soybean and groundnut trials, highest nodulation in both soils was recorded by strain MAR 1495 followed by TSBF Mixture and these were significantly higher (P < 0.05) than other inoculants and control. A similar trend, though only in EC, was observed in N uptake and in nitrogen fixation but no significant difference was observed in dry matter yield. Cowpea trials did not show response to inoculation nor was there difference between the soils. Instead, control treatment surpassed all the inoculated treatments in nodulation at P < 0.05. Nitrogen uptake and N₂ fixation of control also surpassed those of inoculated treatments. Rhizobia strains MAR 1495 and TSBF Mixture showed similar ability to improve the productivity of soybean and groundnut thus can be used as common inoculants for the two crops.

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Integration of legumes in cropping system can serve as an alternative to inorganic N fertilizers. This is achieved via the symbiosis between legumes and rhizobia. This symbiosis alone accounts for more than 20% of global...
biological nitrogen fixation and has been calculated to contribute 45-50 million tones of fixed N to agriculture each year (Giller, 2001). Biologically fixed nitrogen via rhizobia-legume symbiosis has therefore been recommended for the sustenance of traditional agriculture (Peoples et al., 1995a; Postgate, 1998). In many soils however, the native rhizobia are not adequate in either strain number, quality or effectiveness to enhance biological nitrogen fixation (FAO, 1984). Similarly, legume hosts differ in the range of partners with which they form symbioses. Some legumes nodulate with a restricted number of rhizobial strains or species while others nodulate with a wide range of fast- and slow-growing rhizobia. In addition, factors such as high soil temperature (Giller, 2001), nutrient deficiencies (Beck and Munns, 1984; Watkin et al., 1997; O'Hara, 2001), low levels of soil moisture (Boonkerd and Weaver, 1982), low pH (<5.5), low clay and organic matter (Dudeja and Khurana, 1989; De Mallaro and Izaguirre, 1994) adversely affect rhizobial survival. This suggests that soils varying in soil fertility status will respond differently to rhizobial inoculation. Therefore research efforts on effective management of soil fertility variability are required to derive maximum benefits from inoculation. The aim of this study was to identify rhizobia strains that are capable of establishing symbioses with different grain legumes, examine their contribution to dry matter yield, biological nitrogen fixation and compare the effect of soil type on the grain legumes' response to inoculation.

### MATERIALS AND METHODS

**Greenhouse**

**Soil sample collection and preparation**

Two bulk soil samples collected from farmer's fields (0-20 cm) at Mokwa (Southern Guinea savanna latitude 9°17'N and longitude 5°03'E) and Shanono (Sudan savanna latitude 12°03'N and longitude 7°59'E) were used for the experiments. According to FAO classification (2006), Mokwa soil is classified as Rhodic Nitisols (RN) while Shanono soil is classified as Eutric Cambisols (EC). The soils were air-dried, sieved through 4mm mesh and weighed into PVC (8 liters) tubes according to their bulk densities. 10.5 kg and 9.5 kg soils of RN and EC respectively were used in each tube. Earlier, the weighed soils were put in a polythene bag and appropriately mixed with nutrient solutions. The soils were then poured into the tubes and left for 24 h to equilibrate. The nutrient solutions used were calculated based on the optimum nutrient concentration in the plants' tissue.

**Treatments and experimental design**

The greenhouse experiments included three inoculation trials with soybean (TGx 1448-2E), cowpea (IT90K-277-2) and groundnut (Samnut 21) as test crops. Treatments included: Control (minus strains, minus mineral N), Reference (minus strains, plus mineral N) and five rhizobial strains namely: 1495MAR, IRJ 2180A, Legumefix, Histick and TSBF mixture (TSBF 442 + TSBF 531 + TSBF 560). Rhizobial cells in the strains were determined before use (Table 1). Each trial was laid down in a completely randomized design (CRD) on two soils collected from Shanono (Sudan savanna) and Mokwa (southern Guinea savanna). All the treatments were replicated three times.

**Trial management and data collection**

All the seeds were surface sterilized before planting by immersing them in 95% ethanol for ten seconds and then in 0.5% sodium hypochlorite solution for three minutes and then followed by rinsing with sterile water six times. Ten seeds of soybean and cowpea while five seeds of groundnut were planted in the PVC (8 liters) tube and were thinned down a week after to two for soybean and cowpea and one for groundnut. The peat based inoculants were coated to seed using gum arabic as a sticking agent while the liquid inoculants were prepared in a yeast ma...tual nitrogen in the plants' tissue.

**Table 1. Rhizobial cells in the strains used.**

| Strain   | CFU/ml | Cells/ml | Formulation |
|----------|--------|----------|-------------|
| MAR 1495 | >103   | >10^3    | Liquid      |
| TSBF Mixture | 332   | 3.32 x 10^8 | Liquid      |
| Legumefix | 196   | 1.96 x 10^7 | Peat        |
| HiStick  | 4      | 4.00 x 10^5 | Peat        |
| IRJ 2180A | 2     | 20       | Liquid      |

**Growth chamber**

**Experimental set up**

An experiment was set up in a growth chamber to estimate the total viable rhizobia in the experimental soils using plant infection method (Most Probable Number [MPN] technique). The host plants...
used were soybean and groundnut which were cultured in "growth 
pouches" (Somasegaran and Hoben, 1985). The seeds were 
surface sterilized as described above and transferred to a sterile 
germination tray. The seeds were then pre-germinated inside 
incubator at 30°C for 72 h. Upon emergence of the radicle, the 
pre-germinated seeds were taken to the growth chamber and 
transferred aseptically into growth pouches containing 75-100 ml of 
sterilized nitrogen-free nutrient solution (Woomer et al., 1988). The 
pouches were put into a rack for support. A week later, a six step 
five-fold soil dilution series was carried out and inoculated onto the 
root zone of the cultured plants.

Preparation of the serial dilution

A diluent solution was first prepared by dissolving 0.125 g KH2PO4 
and 0.05 g MgSO4.7H2O in 1000 ml of distilled water in order to 
increase the osmotic potential and stirred with magnetic stirrer. Four 
hundred milliliters (400 ml) of the diluent was measured out to a 250 
ml Erlenmeyer flask and 20 ml each to 6 different 125 ml 
Erlenmeyer flask labeled 51, 53, 54, 55 and 56. The diluents 
were then sterilized by autoclaving at 121°C for 15 min. Hundred 
gram (100 g) (dry weight) of each of the experimental soils was 
placed in the 250 ml Erlenmeyer flask and mixed in a rotary 
shaker set at high agitation level for 20 to 25 min. Five milliliters 
(5ml) of the soil aliquot from the 250 ml Erlenmeyer flask was 
pipetted out and transferred to 125 ml Erlenmeyer flask labeled 51 
which is the first dilution step. The dilution continued up to 56 level.

Inoculation

Serially diluted soils were taken to the growth chamber where the 
legume host plants were cultured. The plants were inoculated with 
1 ml of each level of dilution in four replicates.

Soil chemical analyses

Soil pH was determined in water on 1:1 soil/ water ratio (IITA, 
1982). Organic Carbon was determined by Chromic acid digestion 
(Heanes, 1984) and Total N was determined using autoanalyzer 
(Bremmer and Mulvaney, 1982). Available P was determined using 
Mehlich-3 extraction method (Mehlich, 1984). Cation Exchange 
Capacity was determined by saturation with 1 N ammonium acetate 
and extraction of ammonium with 2 M potassium chloride (TSBF, 
1993). Exchangeable acidity was determined by titration method 
after extraction with 1N KCl (Anderson and Ingram, 1993) while 
ECEC was determined by summation of exchangeable cations and 
exchangeable acidity. Soil particle size analysis was done by the 
ydrometer method (Bouyoucos, 1951).

Plant analyses

The plant samples were digested in hot sulphuric acid solution with 
Selenium catalyst using a method adapted from Novozamsky et al. 
(1983). 0.1 g of plant sample was weighed into digestion tube and 
2.5 ml sulphuric acid- selenium mixture was added to the samples 
and allowed to digest for 1 h at 100°C. The tubes were brought out 
of the digestion block and allowed to cool for 5 to 10 min. 
Afterwards, 1 ml and then 2 ml more of hydrogen peroxide were 
added and the tubes were placed back on the digestion block and the 
temperature was increased to 300°C and then condensed bottles were used to cover the tubes. The temperature was again 
increased up to 330°C for 2 h or more until a clear solution was 
obtained in the tubes. The samples were allowed to cool for 24 h 
and made up to 50 ml with distilled water. After digestion, the 
samples were read colorimetrically in an auto-analyzer for determination of N. The Berthiol (indophenol reaction) for the N 
analysis method was adapted from the method of Searle (1984). The 
ammonium ion reacts with phenol to form an indophenol blue 
dye. The blue dye is proportional to the concentration of ammonia 
in the solution and is measured at a wavelength of 630 nm.

Ureide analyses

The %Pfix was measured by determining the concentrations of 
ureide N, amino N, and NO3-N in shoot/petiole, as described by 
Peoples et al. (1989). The proportion of ureide-N, which is a 
reflection of %Pfix, was obtained using the following equations:

\[ \text{Total N in Sap} = (\text{ureide-N + NO}_3^-+\text{Amino-N}) \]

\[ \text{Relative ureide index} = \frac{\text{Ureide} - \text{N}}{\text{Total N in Sap}} \times 100 \]

But ureide contains 4N atoms, thus ureide-N is calculated as 4 \times 
ureide molar concentration. Therefore,

\[ \text{Relative ureide index} = \frac{4 \times \text{ureide}}{(4 \times \text{ureide} + \text{amino acid} + \text{nitrate})} \times 100 \]

The corresponding values for %Pfix were then calculated from the 
following equation.

\[ X = 1.4 + 0.31p + 0.0057p^2 \]

(for plants in the vegetative and flowering stages) Where, p is the 
proportion of plant N from N2 fixation, and x is the relative 
abundance of ureides in extracts of the shoot axis. 

Subsequently, the amount of nitrogen fixed per plant was 
estimated using the equation proposed by Peoples and Craswell 
(1992) as follows:

\[ \text{Amount of N fixed} = \text{Total N in Biomass} \times \%\text{Pfix} \]

It is important to mention that the N2 fixation for groundnut could not 
be determined by ureide analysis because groundnut is not a 
ureide exporter. Instead, the N difference method was used 
whereby maize was used as the reference plant to estimate BNF.

Statistical analysis

All the statistical analyses were carried out using SAS 9.2 software 
(SAS, 2008). Data were subjected Analysis of Variance (ANOVA) 
using Proc GLM. Standard error of difference derived from Least 
Square means (lsmeans) was used as means separation 
parameter.

RESULTS

Soil chemical analysis and most probable number

The physical and chemical analyses of the experimental 
soils have shown that both soils are sandy loam in 
texture. EC is slightly acidic while RN is moderately acidic 
(Table 2). The organic carbon content of both soils
**Table 2.** Physicochemical analyses of the experimental soils.

| Property               | Unit | Test value |
|------------------------|------|------------|
| pH (H₂O)               |      | 6.30       |
| Organic C              | g/kg | 4.15       |
| Total N                | g/kg | 0.35       |
| Mehlich-3 P            | mg/kg| 14.03      |
| Exchangeable Cations    | Cmol(+)kg |           |
| Ca                     |      | 2.77       |
| Mg                     |      | 1.18       |
| K                      |      | 0.38       |
| Na                     |      | 0.83       |
| Exchangeable Acidity   | Cmol(+)kg | 0.08       |
| ECEC                   | Cmol(+)kg | 5.23       |
| Sand                   | %    | 75         |
| Silt                   | %    | 12         |
| Clay                   | %    | 14         |
| Textural Class         |      | Sandy loam |

**Table 3.** Rhizobial counts in the experimental soils.

| Host Specie   | Microsymbiont     | Counts (cells/g) |
|---------------|-------------------|------------------|
| Glycine max   | B. japonicum      | 1.10 x 10²       |
| Arachis hypogae| Bradyrhizobium sp | 2.83 x 10³       |

is very low. Also, the total N contents are much lower than critical level of 1.5 g kg⁻¹. However, Mehlich-3 extractable P and effective cation exchange capacity (ECEC) were higher in EC than in RN.

The MPN counts of rhizobia in the experimental soils indicated that the native soybean rhizobia are very low in EC while RN is devoid of the native soybean rhizobia (Table 3). This clearly shows that the experimental soils have little or no N fixing ability for soybean. The cowpea rhizobia on the other hand, occurred relatively in high density in EC whereas appreciable number occurred in RN indicating the effectiveness of the soils in the N fixing potential for cowpea and groundnut.

**Nodulation**

Soybean planted on EC responded to inoculation more than on RN and difference between them was highly significant (P < 0.01). The percentage contrast between the soils in nodulation was 69.2%; EC being higher (Table 4). Highly significant difference (P<0.0001) between the treatments was also observed in the nodule number. The percentage variations observed between the treatments ranged from 29 to 497% higher in nodule number over the uninoculated control. The soil x treatment interaction was also significant (P<0.05) in the nodule number. 100% of the treatments performed better in EC compared to RN (Figure 1). All the strains recorded a significantly higher nodulation in EC compared to RN. Analysis of nodule dry weight did not show significant variation (p=0.058) between the soils. However, highly significant difference (P<0.0001) was observed between the treatments (Table 4). Percentage contrasts between the strains over uninoculated control range from 33 to 252%. Soil x treatment interaction was not significant.

Nodulation in cowpea did not show significant difference between the two soils (Table 4). Highly significant difference was however observed between treatments (P < 0.0001). The control treatment recorded highest nodule number and significantly differed (P < 0.001) from all other treatments inoculated with rhizobia. Also, highest nodule dry weight was recorded by the control treatment though only significantly higher than where mineral N was applied.

In groundnut trial, analysis of nodule number has shown that there was significant difference (P<0.01) between the soils (Table 4). EC recoded a higher
Table 4. Response of grain legumes to nodulation following inoculation.

| Treatment           | Soybean | Cowpea | Groundnut |
|---------------------|---------|--------|-----------|
|                     | Nodule number | Nod Dry weight (mg/plant) | Nodule number | Nodule Dry weight (mg/plant) | Nodule number | Nodule Dry weight (mg/plant) |
| Soil                |         |        |           |         |        |           |           |
| EC                  | 36.27   | 153    | 44.06     | 90      | 214.13 | 341       |
| RN                  | 21.44   | 110    | 31.73     | 90      | 169.52 | 300       |
| Mean                | 28.86   | 131.5  | 37.9      | 90      | 191.82 | 320       |
| SED                 | 0.08    | 0.02   | 7.81      | 20      | 20.19  | 30        |
| Inoculants          |         |        |           |         |        |           |           |
| MAR 1495            | 83.58   | 230    | 44.25     | 111     | 244.67 | 470       |
| TSBF Mixture        | 64.83   | 280    | 49.25     | 102     | 274.5  | 350       |
| Legumefix           | 16.67   | 150    | 48.58     | 118     | 225.17 | 410       |
| HiStick             | 33.67   | 220    | 43.58     | 113     | 212.17 | 430       |
| IRJ 2180A           | 18.08   | 110    | 35.83     | 84      | 239.67 | 320       |
| Control             | 14      | 80     | 81.47     | 172     | 209.67 | 370       |
| Reference           | 0       | 0      | 0.25      | 8       | 43.33  | 60        |
| Mean                | 28.86   | 131.5  | 37.9      | 90      | 191.82 | 320       |
| SED                 | 0.15    | 0.04   | 15.51     | 44      | 40.37  | 60        |
| Soil x Inoculant    |         | **     | NS        | NS      | NS     | NS        |

Significant value than RN (respectively, 214.13 and 169.52). The percentage contrast is 26.3% with EC being higher. Highly significant difference (P<0.0001) was also observed among the treatments. Highest nodule number was recorded by the treatment TSBF Mixture followed by MAR 1495. Significant difference was not recorded among the inoculated and uninoculated control. No significant difference was observed in soil x treatment interaction. Analysis of nodule dry weight revealed that there was significant difference (P<0.01) between the soils (Table 4). EC soil differed significantly higher than RN with magnitude of 13.6%. Also, there was highly significant difference (P<0.0001) among the treatments. Highest nodule dry weight was recorded by MAR 1495. No significant difference in the soil x treatment interaction was observed.

**Dry matter yield**

Significantly different dry matter yield (DMY) was recorded between the soils in soybean and groundnut trials while no significant difference was observed between the soils in the cowpea trial (Table 5). The percentage differences between the soils in terms of DMY are 12% and 14% for soybean and groundnut trials respectively. No significant difference was observed among the treatments for both soybean and groundnut trials. In the cowpea trial, control treatment was higher in dry matter yield than most of the treatments though the difference was not statistically significant (Table 5). In all the grain legumes, soil x treatment interaction was not significant.

**Nitrogen uptake**

The results of N uptake by the grain legumes are shown in Table 6. Response to inoculation was significantly higher in EC than RN in both soybean and groundnut trials. Percentage contrasts between the soils in terms of N uptake were 38
Table 5. Dry matter yield (g plant$^{-1}$) in grain legumes following inoculation.

| Treatment | Soybean | Cowpea | Groundnut |
|-----------|---------|--------|-----------|
| Soil      |         |        |           |
| EC        | 9.26    | 7.5    | 17.67     |
| RN        | 8.27    | 6.65   | 15.55     |
| Mean      | 8.77    | 7.08   | 16.68     |
| SED       | 0.35    | 0.72   | 0.89      |
| Inoculants|         |        |           |
| MAR 1495  | 8.41    | 7.1    | 17.68     |
| TSBF Mixture | 8.55 | 7.87   | 16.38     |
| Legumefix | 8.15    | 5.64   | 17.412    |
| HiStick   | 8.26    | 7.71   | 16.82     |
| IRJ 2180A | 8.91    | 6.03   | 15.22     |
| Control   | 9.25    | 7.8    | 16.18     |
| Reference | 9.82    | 7.38   | 16.57     |
| Mean      | 9.08    | 7.08   | 16.68     |
| SED       | 0.65    | 5.67   | 1.67      |
| Soil x Inoculant | NS | NS | NS |

NS = Not significant.

and 20% respectively for soybean and groundnut trials; EC being higher in each case. Response was not observed between the soils in the cowpea trial. A significant difference ($P < 0.05$) was also observed among the treatments in soybean where strains MAR 1495 and TSBF Mixture were at variance with other strains and control. Percentage differences between these strains over uninoculated control were 41 and 31% for MAR.
### Table 6. Plant N uptake (mg N plant\(^{-1}\)) in grain legume following inoculation:

| Treatment          | Soybean | Cowpea | Groundnut |
|--------------------|---------|--------|-----------|
| **Soil**           |         |        |           |
| EC                 | 280.75  | 209.12 | 633.02    |
| RN                 | 203.87  | 221.08 | 526.88    |
| Mean               | 242.31  | 215.1  | 579.95    |
| SED                | 16.59   | 20.57  | 30.96     |
| **Inoculants**     |         |        |           |
| MAR 1495           | 297.25  | 189.3  | 611.89    |
| TSBF Mixture       | 275.95  | 194.54 | 579.67    |
| Legumefix          | 222.19  | 164.5  | 621.62    |
| HiStick            | 197.79  | 234.33 | 575.33    |
| IRJ 2180A          | 209.68  | 192.44 | 515.01    |
| Control            | 210.17  | 269.76 | 561.72    |
| Reference          | 283.14  | 260.81 | 594.38    |
| Mean               | 242.31  | 215.1  | 30.96     |
| SED                | 31.04   | 37.18  | 187.7     |
| **Soil x Inoculant** |     |        |           |
| Significance       | *       | NS     | *         |

NS = Not significant; * Significant at \( P < 0.05 \).

1495 and TSBF Mixture respectively. Strain MAR 1495 was 5% greater than reference treatment. No significant N uptake was recorded among the treatments of both cowpea and groundnut. While significant soil x treatment interaction was not recorded in cowpea trials, soybean and groundnut trials revealed a significant (\( P < 0.05 \)) soil x treatment interaction (Table 6). Figure 2 represents the soil x treatment interaction chart. It shows that the treatment performed better in EC compared RN. Strains MAR 1495, TSBF Mixture, Legumefix and IRJ 2180A
were significantly higher in EC compared to RN. HiStick, control and reference did not show significant difference between the soils. However, these treatments were 5, 18 and 4% respectively better in EC than RN. The soil x treatment interaction in groundnut trials is shown in Figure 3. 71.4% of the treatments have higher N uptake in EC than in RN. Strains MAR 1495, TSBF Mixture, IRJ 2180A and reference treatment were significantly higher in EC compared to RN. These are 53, 52, 39 and 38% respectively.

Biological nitrogen fixation (BNF)

The amount of BNF estimated in the soybean trial showed that there was highly significant difference ($p<0.0001$) between the two soils. EC differed by 84% over RN (Table 7). There was also a highly significant difference ($P< 0.01$) among the treatments with strains MAR 1495 and TSBF Mixture fixing a significant amount over the other strains and the uninoculated control. Since the Reference treatment did not nodulate, it was concluded that it had 0% N2 fixation. The soil x treatment interaction was also highly significant ($P< 0.01$) as shown in Table 7. The interaction table (Table 8) had shown that BNF estimated in EC are 100% better than in RN. All the inoculated treatments of EC varied significantly higher than RN. These represent 187, 88, 79, 46 and 86% increment in the BNF estimated for MAR 1495, TSBF Mixture, Legumefix, HiStick and IRJ 2180A respectively. Even in the uninoculated treatment where no significant difference was observed, EC was 29% higher than RN. Cowpea trial did not show variation or improvement in BNF due to soil type. Among the treatments, the uninoculated control performed better than the inoculated treatments and was significantly higher than strain IRJ 2180A (Table 7). The percentage contrasts of the control over inoculated treatments ranged from 6 to 36%. No soil x treatment interaction was recorded.

Groundnut trials, like in soybean, showed significant difference between the soils. EC was better than RN with magnitude of 53%. There was no significant difference in the treatment effect. However, highly significant soil x treatment interaction was recorded (Table 7). Strains MAR 1495, TSBF Mixture and IRJ 2180A recorded a significantly higher BNF in EC as compared with RN. This was 53, 53 and 40% greater BNF in EC over RN for MAR 1495, TSBF Mixture and IRJ 2180A respectively.

DISCUSSION

The soil physical and chemical properties depict the characteristics of typical savanna soils which are very low in organic carbon and total N contents (Jones and Wild, 1975; Okalebo et al., 1993). On the other hand higher Mehlich-3 extractable P of EC falls within the medium range of fertility class while that of RN is very low in
Table 7. $N_2$ fixation (mg N plant$^{-1}$) in grain legume following inoculation:

| Treatment       | Soybean | Cowpea | Groundnut |
|-----------------|---------|--------|-----------|
| Soils           |         |        |           |
| EC              | 193.49  | 168.02 | 546.61    |
| RN              | 105.25  | 169.14 | 466.9     |
| Mean            | 149.37  | 168.58 | 506.76    |
| SED             | 9.02    | 19.67  | 22.44     |
| Inoculants      |         |        |           |
| MAR 1495        | 189.46  | 173.09 | 535.61    |
| TSBF Mixture    | 183.11  | 170.14 | 508.77    |
| Legumefix       | 128.14  | 148.12 | 548.29    |
| HiStick         | 130.24  | 185.18 | 504.65    |
| IRJ 2180A       | 132.8   | 130.33 | 450.55    |
| Control         | 132.5   | 204.63 | 492.66    |
| Mean            | 149.37  | 168.58 | 506.76    |
| SED             | 15.62   | 33.38  | 38.87     |
| Soil x Inoculant| **      | NS     | **        |

NS = Not significant; ** Significant at $P < .01$.

Table 8. Interaction between soil type and rhizobial inoculants on BNF (mg N plant$^{-1}$) in soybean and groundnut trials.

| Treatment       | Soybean | Groundnut |
|-----------------|---------|-----------|
|                 | EC      | RN       | EC      | RN       |
| MAR 1495        | 280.95  | 97.97    | 648.04  | 423.19   |
| TSBF Mixture    | 239.17  | 127.06   | 614.76  | 402.77   |
| Legumefix       | 164.39  | 91.88    | 549.69  | 546.9    |
| HiStick         | 154.5   | 105.97   | 479.53  | 529.78   |
| IRJ 2180A       | 172.61  | 92.98    | 525.86  | 375.24   |
| Control         | 149.33  | 115.67   | 461.8   | 523.51   |
| Mean            | 193.49  | 105.26   | 546.61  | 466.9    |
| SED             | 22.09   | 54.97    |         |          |

SED = Standard error of difference of Means

fertility (Enwezor et al., 1990). The ECEC of both soils are generally low (Marx et al., 1999) but EC contains more exchangeable cations than RN which suggests that the fertility status of EC is higher than that of RN. The low MPN results for native soybean rhizobia suggest that inoculant strains may play a larger role in the nodule formation for soybean and consequently $N_2$ fixation. Conversely, the inoculants strains would have to compete with the native population of rhizobia for nodule occupancy in cowpea and groundnut.

Higher nodulation observed on soybean in EC could be due to lower soil pH of RN. Soil pH has been widely reported to influence nodulation because it can induce deficiency in some essential nutrients such as P and Mo (Giller, 2001). These nutrients also affect the distribution of rhizobia (Peoples et al., 1995b). The consequence could be reduction of number and sizes of nodules (Marschner, 1995). Working with pea, Rice et al. (2000) reported that the nodule number and nodule weight increased with increasing soil pH. Results of nodulation among the treatments indicate that some strains could be of higher quality than others. Some of the rhizobia strains used in the trials did not show significant difference compared with control while others recorded even lower nodulation. It is possible that the rhizobial cells died in storage before they were put to use. This could result due to high temperature normally experienced in the tropics. Boonkerd (1991) reported that temperature was critical to the survival of soybean rhizobia in peat with substantial number at 10°C than at 30°C. The peat inoculants used in these trials might have been in storage for months and in an erratic temperature conditions which could have...
affected the bacterial number and hence viability. The success of commercial inoculants is dependent on the number of viable bacteria available to participate in the infection process at the point of use (Catroux et al., 2001). In addition, Hiltbold et al. (1980) reported that nodulation of soybean was directly related to number of rhizobia with no nodulation by the product supplying <10^3 rhizobia/seed and abundant by about 10^5 to 10^6 rhizobia/seed. This corroborates our findings which show higher nodule number and weight in MAR 1495 and TSBF Mixture which have greater than 10^6 cells ml^{-1} of inoculants applied than other strains with lower concentrations. Treatment where 91 ppm pot^{-1} urea-N (reference) was applied suppressed nodulation completely. Availability of mineral N decreases or impedes nodulation of legumes (Abaidoo et al., 1990). In particular, Moawad and Shamseldin (2010) reported that high N dose of 80 ppm inhibited nitrogenase enzyme and nodulation in common bean. While some of the inoculants increased nodulation, dry matter yield was not significant in all the grain legumes. Low soil N (0.35 g kg^{-1}) and other nutrients in the experimental soils which were replenished with the application of mineral N contributed to the insignificant increase in biomass yield among the grain legume. Application of mineral N and P has been reported to increase shoot dry matter yield of grain legumes (Jemo et al., 2006; Shamseldin and Moawad, 2007). Conversely, it was interesting to observe that some of the strains had a great influence on groundnut and soybean N uptake. Significantly higher plant N uptake in EC than RN could be due to its higher available soil P which has been found to influence N uptake (Yusuf et al., 2005).

The response of cowpea to inoculation was almost insignificant in all the parameters measured. In fact, nodulation and N uptake were significantly higher in the control and the same trend was observed with dry matter yield and BNF though the difference was not significant compared to other treatments. This could be due to competition between indigenous population and the inoculants which may culminate in antagonism. In other words, during their interaction, a phenomenon of ‘nodule blocking’ has occurred. Winarno and Lie (1979) demonstrated that a strain that was unable to nodulate a particular cultivar pea was shown to suppress nodulation completely by otherwise nodulation incompetent strain. Thus, even ‘non-symbiotic’ strains of rhizobia that may be abundant in the soil (Segovia et al., 1991) may be able to exact a very specific effect on competitive outcome.

Profuse literatures have shown that cowpea seldom responds to inoculation. The crop is a very promiscuous legume host (Ahmad et al., 1981; Ranga Rao et al., 1985) and Bradyrhizobium strains with which it can form effective nodules are normally present. Thus, cowpea and some other tropical legume have rarely been found to respond to inoculation unless they are grown in a soil where the conditions are not conducive for the survival of rhizobia (Giller, 2001). In soils where naturalized rhizobial populations are high (>10^8 Rhizobium bacteria g/m soil), introduction of new strains can be difficult and often unsuccessful (Thies et al., 1991; Brockwell et al., 1995). These reports strongly confirmed the fact that occurrence of high rhizobial population density in the experimental soils (Table 3) especially in EC was responsible for the failure of cowpea to respond to inoculation. Low soil pH and other nutrient status of the RN could have aggravated the situation. Lack of response to rhizobial inoculation has been attributed to low soil pH (Vinuesa et al., 2003; Shamseldin and Werner; 2004, 2005; Shamseldin, 2007).

Conclusion

Our study shows that soils varying in fertility status will respond differently to rhizobial inoculation. Research efforts on effective management of soil fertility variability are therefore required to derive maximum benefits from inoculation. Of all the strains evaluated, MAR 1495 and TSBF Mixture ranked highest thus could be further evaluated on a wider range of soils for incorporation into the existing cropping system. Future studies should also focus on quality assessment of both laboratory and commercial inoculants, not relying solely on manufacturers’ claims in order to avoid the use of substandard products. There is also the need to intensify efforts on identifying elite strains of rhizobia that would perform better than ineffective native population and under adverse soil conditions such as low pH.

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