Determination of Rhizobial Population of Soils from North Western Nigeria for Biological Nitrogen Fixation on Soybeans

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***Abstract*—A study was conducted to estimate the rhizobial population of soils for biological nitrogen fixation on soybeans (*Glycine max*) by the Most Probable Number (MPN) technique. Soils were collected from seven locations namely; Albasu, Bichi, Garko, Gaya (Sudan savanna), Giwa, Soba and Z/kataf (northern guinea savanna). The result showed that Garko has the least MPN rhizobia/g ($0.61x10^1$) and Z/kataf has the highest MPN rhizobia/g ($7.65x10^3$). The soils collected from northern guinea savanna have the highest MPN rhizobia/g compared to the soil collected from Sudan savanna.**

***Index Terms*—soybeans, rhizobia, most probable number, sudan, northern guinea savanna**

I. Introduction

Rhizobium is a soil habitat bacterium, which is able to colonize the legume roots and fixes atmospheric nitrogen symbiotically. The morphology and physiology of *rhizobium* will vary from free-living condition to the bacteroid of nodules. They have seven genera and highly specific to form nodule in legumes, referred to as cross inoculation group. *Rhizobium* inoculant was first made in USA and commercialized by private enterprise in 1930s [1]. Rhizobia elicit the formation of specialized organs, called nodules, on roots or stems of their hosts, in which they reduce atmospheric nitrogen and make it available to the plant. Symbiotic nitrogen fixation is an important source of nitrogen, and the various legume crops and pasture species often fix as much as 200 to 300 kg nitrogen per hectare [2]. Globally, symbiotic nitrogen fixation has been estimated to amount to at least 70 million metric tons of nitrogen per year [3].

The symbiosis between the root-nodule bacteria of the genus Rhizobium and legumes results in the fixation of atmospheric nitrogen in root-nodules. This symbiotic relationship is of special significance to legume husbandry as seed inoculation with effective strains of Rhizobium can meet the nitrogen requirements of the legume to achieve increased yields. Obviously, such a phenomenon is of world-wide interest because it implies lesser dependence on expensive petroleum based nitrogen fertilizers for legumes. In all regions of the world where food consumption exceeds production or where nitrogenous fertilizer has to be imported, leguminous crops have a special relevance. Self sufficiency for nitrogen supply and the high protein and calorific values of food, forage and feed legumes make them increasingly attractive. Greater use of legumes can have a significant beneficial impact in tropical countries where population increase and food production are most out of balance, and where the purchasing power for imported fertilizers is least adequate [4].

Many legumes (alfalfa, clover, peas, beans, lentils, soybeans, peanuts and others) contain symbiotic bacteria called *Rhizobia* within nodules of their root systems. These bacteria have the special ability of fixing nitrogen from atmospheric, molecular nitrogen (N_2) into ammonia. The Ammonia is then converted to another form, ammonium (NH_4^+), usable by (some) plants. This means that the root nodules are sources of nitrogen for legumes, making them relatively rich in plant proteins.

Annually, there are about 120- 160 million tons of atmospheric nitrogen (N_2) fixed in to nitrogen fertilizer forms through naturally biological nitrogen fixing process globally [5]. This amount of fertilizer is estimated two times as much as that of chemical production process in the world. In the nodule relationship between rhizobia and legume trees, the nitrogen fixing is done by the rhizobia; by which trees are supplied with nitrogen sources for their growth vice versa bacteria will obtain hydrate carbon for their existence. This relationship is essential for nitrogen cycle in nature and for sustenance of yield and productivity of crops as well as for ecosystem sustainability [6].

II. Materials and Methods

A. Collection of Soil Samples

Soil samples were collected from farm land that legumes were previously planted. Twenty soils were used for the research; the soils were collected from seven local government areas, four from Kano state and three from Kaduna state. The soils are Garko I, II and III, Bichi I, II

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and III, Gaya I, II and III, Albasu I, II and III, Giwa I, II and III, Soba I, II and III, and Z/kataf I and II. The GPS of the sites were also taken and recorded [4].

B. Preparations of Legume Seeds

The Legumes Seeds (TGX 1448- 2E) were selected and rinsed in 95% ethanol for 10 seconds to remove wax material and trapped air. Thereafter, the seeds were then soaked in a solution of 1% sodium hypochlorite for 4 minutes; this was then followed by rinsing with sterile water five times to remove residual disinfectant [7].

C. Development of Seedlings

Water agar was prepared by dissolving 14g of dehydrated medium in 1litre of water followed by sterilization. The prepared seeds obtained in 3.2 above were then planted on the water agar and allowed for 3-5days to germinate. Seedlings with good root development and free from contamination were selected and transplanted at 2cm depth in an experimental pot containing the soil sample obtained in 3.1 above. The setup was then irrigated daily for a period of two months (8weeks) after which the seedlings were harvested for nodules recovery [4].

Enumeration of rhizobia by most probable number (MPN) technique.

Preparation of physiological solution

Eight gram (8g) of sodium chloride (NaCl) was dissolved in one liter (1L) sterile distilled water and then mixed by shaking to form a solution.

Serial dilution

One hundred grams (100g) of each soil was dissolved in 400ml of sterile physiological solution in a sterile bottle. The content was mixed using a magnetic stirrer for twenty minutes and this gave the initial dilution of each soil. Six (6) bottles each containing 8ml of sterile physiological solution were labeled as; 5^{-1}, 5^{-2}, 5^{-3}, 5^{-4}, 5^{-5} and 5^{-6} respectively. Using a sterile micropipette 2ml of the initial soil dilution was transferred aseptically in to the bottle labeled 5^{-1} and then mixed carefully by shaking. From the first dilution (5^{-1}), two (2)ml was transferred with sterile pipette in to the second bottle labeled (5^{-2}) and mixed by shaking . From the second dilution (5^{-2}) 2ml was transferred to the bottle labeled 5^{-3} and mixed gently by shaking, this gave the third (5^{-3}) dilution. The procedure was repeated using 4^{th}, 5^{th} and 6^{th} dilutions which gave 5^{-4}, 5^{-5} and 5^{-6} dilutions respectively [8].

The enumeration of the rhizobial population was carried out by the most probable number (MPN) techniques in the screen house of International Institute of Tropical Agriculture (IITA) Kano station, in accordance with [8]. Six hundred pots were sterilized and then filled with sterilized sand. Soybean seedlings with good root development germinated in the lab were selected and one seedling was transplanted in to each of the pots, and irrigated with water daily and with Jensen's solution ones a week. The pots were grouped in to twenty blocks with each block containing twenty six pots, the pots were labeled in to six dilutions (5^{-1}, 5^{-2}, 5^{-3}, 5^{-4}, 5^{-5} and 5^{-6}) with each dilution containing four replicate pots plus two control pots. Then 1ml was taken from each of the serially diluted soil and inoculated in to the corresponding labeled pots in each block. Each block was inoculated with one soil to determine MPN rhizobia of the soil. The set up was irrigated for five weeks after which the roots of the soybeans were washed and the presence or absence of nodules was recorded for each dilution of each soil. Codes were derived from the nodules scoring which were compared to the MPN Table to get the estimated number of rhizobia presence in the soils.

III. Results

The result of the physicochemical parameter of the soils from various sampling sites showed the pH to range between 5.00 in Z/kataf to 6.03 in Albasu. Percentage organic carbon range between 0.30 in Garko to 0.69 in Giwa and Soba. Percentage nitrogen range between 2.41 in Albasu to 2.98 in Giwa and Soba. The textural classes of the soils showed that the soil sampled at Albasu, Bichi, Garko and Gaya were sandy loam and those sampled at Giwa and Soba were clay loam and that of Z/kataf was sandy clay loam as seen in Table I.

The result of the most probable number (MPN) of rhizobia per soil range between 6.1 ($0.61x10^1$) to 7653.5 ($7.65x10^3$) MPN/g as seen in Table II.

TABLE I. Mean Organic Carbon, Nitrogen Content, pH, Olsen P, Particle Sizes and the Textural Classes of the Sampled Soils

SOIL LOC	pH (H_2O) 1:1	%OC	%N	Olsen P (ppm)	PARTI-CLE % Sand	SIZE %Silt	%Clay	TEXTURAL CLASS
Albasu	**5.97**	**0.31**	**0.05**	**2.41**	**72.53**	**14.00**	**13.47**	**Sandy loam**
Bichi	**5.83**	**0.37**	**0.06**	**2.87**	**72.53**	**12.67**	**14.80**	**Sandy loam**
Garko	**5.77**	**0.30**	**0.06**	**2.91**	**76.53**	**10.67**	**12.80**	**Sandy loam**
Gaya	**6.03**	**0.44**	**0.07**	**3.48**	**76.53**	**10.00**	**13.47**	**Sandy loam**
Giwa	**5.40**	**0.69**	**0.08**	**2.98**	**42.53**	**29.20**	**28.27**	**Clay loam**
Soba	**5.40**	**0.69**	**0.08**	**2.98**	**42.53**	**29.20**	**28.27**	**Clay loam**
Z/kataf	**5.00**	**0.65**	**0.08**	**2.44**	**51.20**	**20.20**	**28.60**	**Sandy clay loam**

Key: LOC; Location, OC; organic carbon, N; Nitrogen, P; phosphorus.

TABLE II. MEAN MOST PROBABLE NUMBER (MPN) OF RHIZOBIA OF THE SOILS

Soil location		Most probable number
		(MPN) of Rhizobia/g
Albasu		6.3
Bichi		7.3
Garko		6.1
Gaya		14.6
Giwa		91
Soba		446.6
Z/kataf		7653.5

IV. DISCUSSION

A. Soil Analysis

The result showed that the pH of the soils sampled at Albasu, Bichi,Garko and Gaya is moderately acidic and that of Giwa and Soba is strongly acidic while that of z/kataf is very strongly acidic. The organic carbon and Nitrogen content of the soils sampled from northern guinea savanna (Giwa, Soba, Z/kataf) was higher than that of the soils sampled from Sudan savanna (Albasu, Bichi, Garko, Gaya), hence soils sampled from northern guinea savanna has higher organic matter and nitrogen content than soils from Sudan savanna. The olsen P content of all the soils sampled was found to be low according to [9] soil fertility ranking (Table I).

According to the result the textural class of the soils sampled at Sudan savanna was sandy loam and that of the northern guinea savanna was clay loam and this indicated that the soil sampled at northern guinea savanna has finer texture, high clay contents, high nutrient and water retention within the soils than the soil sampled at Sudan savanna. From the result the exchangeable cations of all the soils sampled from both Sudan savanna (Albasu, Bichi, Garko, Gaya) and northern guinea savanna (Giwa, Soba, Z/kataf) were within the same range. In all the soils sampled, the concentration of Ca is low and that of Mg, K, and Na is medium according to [9] soil fertility ranking (Table I). High nitrogen and organic carbon content of the soils sampled at Soba, Giwa and Z/kataf could be due to the textural classes of the soils. Soils with high clay content have finer texture and as a result have higher water and nutrient retention capacity.

B. Most Probable Number (MPN) Rhizobia in the Soils

Based on the Most Probable Number (MPN) of rhizobia per gram of each soil, the result showed that Z/kataf has the highest rhizobial MPN/g followed by Soba followed by Giwa, followed by Gaya, Bichi, Albasu, and Garko have the least MPN/g (Table II). The high MPN values in Z/kataf, Soba and Giwa could be due to the high N_2 content, high organic carbon content and textural class of the soils as shown in the soil analysis (Table I). The higher the MPN/g of a soil the higher the fertility of the soil, since rhizobia play significant role in biological nitrogen fixation.

V. CONCLUSION

Base on the result obtained soils sampled from the Albasu, Bichi,Garko and Gaya (northern guinea savanna) have higher Most probable number (MPN) of rhizobia per gram than the soils sampled from Giwa, Soba and Z/kataf (Sudan savanna). The high nitrogen and organic carbon content of the soils sampled at Soba, Giwa and Z/kataf was due to the textural classes of the soils. Soils with high clay content have finer texture and as a result have higher water and nutrient retention capacity. Therefore there is need to inoculate the soils in order to increase its fertility.

VI. RECOMMENDATION

Since native (indigenous) rhizobia are present in the soils which contain effective strains of *rhizobia* and nodulate legume crops to induce Biological Nitrogen Fixation (BNF) there is need to be planting promiscuous varieties of soybeans which can be nodulated by any species of rhizobia present in the soils.

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