

Nodulation of Legumes in Inland Valley Soils of Ghana

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Abstract

Because most African farmers cannot afford the purchase of chemical fertilizers to correct soil nutrient deficiencies, alternatives such as increased use of biological nitrogen fixation are most useful. For best results, sound recommendations are needed on the use of different legumes, their rhizobial requirement and management for optimum nitrogen fixation. In West Africa however, except for the highly popular legumes such as cowpea, soybean and groundnut, few reliable data are available on the abilities of other legumes to nodulate and fix nitrogen with naturally occurring strains of rhizobia in various soils and thus on the need for rhizobial inoculation. A screen-house study was conducted at the University of Ghana, Legon, to assess the potential of 13 commonly grown legumes in Ghana to form nodules with native rhizobia from eight inland valley soils and to identify those for which further research would be needed to enhance their nodulation. Two of the soils had indigenous rhizobia that induced formation of nodules on all the legumes evaluated. Rhizobia in the remaining soils induced the formation of nodules on more than 75% of the legumes studied. However, numbers of nodules plant⁻¹ on most of the legumes were low and were influenced by (i) the type of soil, (ii) legume and (iii) the soil x legume interaction. Population densities of rhizobia capable of forming nodules with

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legumes in the studied soils estimated by the most probable number method (MPN) ranged from very low (negligible to 1.7×10^1 cells g^{-1} soil for those that could form nodules with mucuna) to high (up to 1.7×10^5 cells g^{-1} soil and 3.1×10^5 cells g^{-1} soil for cowpea and green gram, respectively). Nodule number per plant correlated significantly with some important indices of soil fertility; namely %N, available NO_3^- and % organic C, with r values of 0.39*, 0.60* and 0.40*, respectively. Inoculation of mucuna increased nodulation by between 3 to 20-fold and N_2 fixation by 18 to 98 percent depending on the soil type. Non-inoculated mucuna absorbed a high proportion of its N (up to 60%) from the soil and may mine the soil of native N if harvested for seed. The low rhizobial populations and poor nodulation are indications for the necessity for inoculating many of these legumes in similar soils in West Africa.

Keywords: Nodulation, legumes, rhizobia, inland valley soils, nitrogen fixation

1. Introduction

The increasing human population and food demand in Africa require the development of technologies and agronomic practices that are sustainable. Such practices include the use of high yielding cultivars, mechanization, irrigation and most importantly the use of agrochemicals (Winteringham, 1984).

Nitrogen, is the most limiting mineral nutrient element for crop production in most soils in Africa. Although traditionally, soil N deficiency has been corrected by applying chemical fertilizers this practice is low in Africa, due to economic constraints of farmers. The Food and Agricultural Organization (FAO) of the United Nations has estimated a 10-fold increase in Africa's fertilizer consumption by the year 2020 if her food demand is to be met (FAO, 1997).

Matching food production with population growth in Africa, therefore calls for a systems approach that integrates all sources of plant nutrients, especially N, and crop production factors into a productive agricultural system that enhances soil fertility, crop productivity, and profitability (Roy and Braun, 1983). The importance of legumes in building and conserving soil fertility has been recognized since the beginning of agriculture (Nutman, 1965). Thus the dependence on nitrogen fertilizer can be reduced if methods for incorporating the full potential of N_2 fixing legumes into African farming systems are explored. Knowledge of factors that affect nodulation and nodule functioning in legumes is therefore of great importance. These include the legume genotype (Hardarson et al., 1984), population density of highly effective and competitive homologous strains of *Rhizobium* in the soil (Ikram and Broughton,

1981) and a favourable environment for growth of the plant, the *Rhizobium* as well as for effective functioning of the symbiosis (Vincent, 1965).

The presence or absence of a homologous *Rhizobium* with host legume in a soil is the first prerequisite to nodulation and biological nitrogen fixation (BNF) by legumes. While it is well known that a legume like cowpea (*Vigna unguiculata*) generally nodulates freely in most tropical soils, for high nodulation and N₂ fixation in some others (e.g. soybean, *Glycine max*), artificial inoculations with effective rhizobia are required. While data on the need for inoculation abound for some legumes and in some geographic locations, they are sparse for others. For west Africa for example, except for legumes like cowpea, soybean and groundnut (*Arachis hypogea*), few reliable data are available on the general occurrence of homologous rhizobia for many of the less popular legume species and cultivars used in this sub-region.

Screening of different legume genera (Gibson and Dreyfus, 1982), species (Nutman, 1984), and cultivars of a given species in a range of soil types for their ability to form nodules is the first step in determining the presence and possibly abundance of homologous rhizobia in a particular soil. It is only when insufficient numbers of highly effective rhizobia occur in a soil that inoculation of the soil or seed with proven laboratory-grown cells should be recommended (Vincent, 1965).

Screening rhizosphere soil of legumes for the presence and the abundance of highly effective native rhizobia in the soil is therefore very crucial and highly informative in efforts to stimulate greater exploitation of BNF as a possible substitute or supplement to N fertilizer. Such information is needed for formulating inoculation policies for enhancing nodulation and N₂ fixation in several promising legumes. In Ghana such information is lacking. Besides, since many soil types that occur in Ghana are found in other countries of the sub-region, it is likely that such information could be useful to farmers in the sub-region and possibly in many parts of Africa too.

To contribute towards the bridging of this knowledge gap a study was initiated in 1997 to evaluate 13 legume species and cultivars in eight inland valley soils in Ghana. Our objective was to assess the potential of these legumes to nodulate with native rhizobia, and to identify those for which rhizobial inoculation would be needed to enhance their nodulation. Of particular interest to us is mucuna (*Mucuna pruriens*) on which such information is lacking despite its promotion as a cover crop for soil fertility improvement.

Mucuna spp are in the family Fabaceae. They are herbaceous with an annual life cycle ranging from 100 to 290 days (Buckles, 1995). They have a spreading/trailing or climbing growth habit. Two species (*Mucuna pruriens* var *utilis* and *Mucuna sloanei*) are commonly grown (Kay, 1979). *Mucuna* has become one of the preferred leguminous cover crops in West Africa and Brazil where there is a renewed interest in green manure cover crops for the

improvement of soil fertility and control of *Imperata cylindrica*. In West Africa, Manyong et al. (1996) reported widespread adoption of mucuna cover crops in Benin. Researchers and Extension Agents in Ghana and other West African countries are promoting the use of mucuna as a cover crop in rotation with cereals and to reclaim degraded lands.

2. Materials and Methods

Soils, sampling sites and sampling details

Eight major inland valley (IV) soil series found in Ghana were used in this study. They are Changnalili (Plinthic Lixisol), Lima (Dystric Gleysol), and Volta (Dystric Gleysol) series, sampled from the Guinea savanna agroecological zone. The others are Hake, (Eutric Cambisol), Amo (Vertic Cambisol), Tefle (Dystric Vertisol), Akuse (Calcic Vertisol) and Bumbi (Calcic Vertisol) sampled from the Coastal savanna agroecological zone.

Soils from the Guinea savanna agroecological zone

The Guinea savanna zone has a mono-modal rainfall period, (March to October) punctuated by dry spells with a four months dry season. The annual mean rainfall is between 1000 mm and 1200 mm. Mean annual minimum and maximum temperatures are 24°C and 35°C, respectively. The vegetation is savanna grassland with *Panicum maximum* and may have variable amounts of trees, or shrubs. Volta, Lima and Changnalili soil series are the dominant rainfed rice soils in this area and are used mainly for rice and vegetables. The three soils together cover about 40–50% of the land area and are used for upland and rainfed lowland rice (Adu, 1957). Adu (1957), described Changnalili series as a shallow (15–30 cm sandy loam topsoil) soil overlying a poorly drained groundwater laterite and transitional between perfectly drained upland soils and Lima and Volta series in the floodplains. Volta and Lima series are deep loamy fine sand usually with ironstone layers at varying depth. They are poorly drained and subject to seasonal flooding for varying periods, but generally become dry during the dry season. Volta series is the major alluvial soil in the Guinea savanna zone and borders the floodplains of the major river of Ghana (Volta and its tributaries).

Soils from the coastal savanna

The coastal savanna has a bimodal rainfall regime with annual mean rainfall of between 500 mm and 1200 mm. The major rainy season is between

April and July and is followed by a short dry spell that runs till the end of August. The minor season is from September and ends in mid November. The mean annual minimum and maximum temperatures are 27°C and 32°C, respectively. The vegetation is savanna grassland with *Panicum maximum* and *Andropogon gayanus* as dominant plant species (Ahenkorah et al., 1993). The zone is important for the cultivation of rice and vegetables.

Hake, Amo, Tefle, Akuse, and Bumbi soil series, the major rice and vegetable soils in this agroecological zone were sampled from the Asutsuare irrigation project site for this study.

Hake, Amo and Tefle series are developed from recent mixed alluvium and belong to the Amo-Tefle associations. They occupy the mid-Volta flood plains and the lower Volta basin with Hake at the upper slope, Tefle at the valley bottom and Amo in between the two. The alluvial materials from which they were formed were transported from the higher catchment areas of the Volta basin.

Akuse series are black cracking heavy vertisolic clays developed from the weathering of basic granitiferous hornblende gneiss. Bumbi series on the other hand are developed from colluvial materials from surrounding uplands and occur within wide depressions (Antwi and Asiamah, 1987). Data on cation exchange capacities of Akuse and Bumbi reported by Acres (1985) suggest that montmorillonite is the dominant clay mineral.

Two common outstanding characteristics of these soils are:

- Poor internal drainage which renders them waterlogged during the rainy season for varying periods.
- All the soils were sampled from fields that have been left fallow for between 3 and 5 years and have no recent history of legume cultivation.

Ten soil-cores (0-20 cm depth) were randomly collected along two diagonal 50m transects from each soil series during the dry season in February. Soil cores from the same series were bulked and passed through a 2 mm sieve. Sub samples were taken for each soil series and stored in a refrigerator (4°C) for later chemical analysis and enumeration of rhizobia. The remaining soil was used for the assessment of nodulation of legumes, and assessment of the response of mucuna to inoculation.

Chemical properties and enumeration of rhizobial population

Soils were analyzed for pH in water (electrometric method), mineral N (Bremner and Keeney, 1965), total N (Bremner, 1960) organic carbon (Walkley and Black, 1934) available phosphorus (Bray 1 according to Bray and Kurtz, 1945) and particle size by Bouyoucos hydrometer method. Rhizobial

populations in the soils were estimated in growth pouches using the most probable number (MPN) method (Brockwell, 1980; Weaver and Frederick, 1972). Some important chemical properties of these soils and rhizobial densities in them are presented in Tables 1 and 2, respectively.

Table 1. Some physical and chemical properties of the inland valley soils used

Soil series	Chemical properties									
	Sand	Particle size (%)		Infiltr*	pH	Avail P	%N	Org C	NH ₄ ⁺ N	NO ₃ ⁻ N
	Silt	Clay		(mm/hr)		(mg/kg)	(%)	(%)	(mg/kg)	(mg/kg)
Changnalili	58	20	22		6.05	1.97	0.04	0.16	37.62	7.38
Lima	37	39	24		5.59	0.89	0.05	0.47	38.82	8.09
Volta	58	17	25		5.52	1.14	0.07	0.99	39.94	5.39
Hake	29	52	21		5.50	0.48	0.06	0.52	30.21	6.57
Amo	9	21	70		4.55	0.45	0.12	1.45	41.80	11.80
Tefle	4	21	75	4.34	4.80	0.46	0.11	1.59	25.94	16.99
Akuse	55	8	37	0.06	6.74	1.33	0.12	1.74	40.30	10.77
Bumbi	6	27	67	1.5	4.69	0.47	0.12	2.27	33.84	17.59
Lsd (0.05)					0.07	0.09	0.01	0.02	7.03	3.83

*Infiltration rate

Table 2. Rhizobial populations in the inland valley soils used (cells g⁻¹ soil, MPN method)

Soil series	Reference / test crop		
	Mucuna	Cowpea	Green gram
Changnalili	6	3,100	58
Volta	0	3,100	170
Lima	6	31	6
Bumbi	17	100,000	1,000
Tefle	6	170	31
Amo	6	170	31
Akuse	6	170,000	310,000
Hake	6	10	17

Experimental design

The study was conducted in 1.5 litre pots in a screen house at the Soil Science Department, University of Ghana Legon. Thirteen legumes consisting of two genera of grain legumes (*Vigna unguiculata* and *Phaseolus aureus* (mungbean)), and eight genera of forage/herbaceous legume species (*Lablab purpureus*, *Indigofera hirsuta*, *Mucuna pruriens*, *Crotalaria ratusa*, *Desmodium distortum*, *Mimosa pudica*, *Calopogonium mucunoides* and *Canavalia ensiformis*) were evaluated for their nodulation in the eight IV soils. For *Vigna unguiculata* and *Phaseolus aureus*, three and two cultivars, respectively were used. Six seeds of each legume were planted in triplicate in plastic pots containing 1.5 kg soil. Seeds were surface sterilized according to Somasegaran and Hoben (1994) before planting. Plants were thinned to two after emergence (10 days after planting). The pots were arranged randomly in a split plot design with soils as main plot and legumes as sub-plot treatments. Plants were watered daily with sterile distilled water placed in saucers beneath each pot and were harvested after 7 weeks of growth for nodule assessment.

A second pot experiment was conducted to estimate the effect of inoculation of mucuna on its nodulation, biomass yield and nitrogen fixation in three of the studied soil series (Volta, Akuse and Bumbi). Treatments were replicated three times with a non-inoculated treatment as the control. The study was conducted in 4 litre plastic pots filled with 3 kg soil into which four surface sterilized seeds (Somasegaran and Hoben, 1994) of mucuna or seeds of reference plants were planted. Luffa (*Luffa cylindrica*) and Cucumber (*Cucumis melon*) were the reference crops used for the calculation of N₂ fixed by either the isotope dilution (Fried and Middelboe, 1977) or total nitrogen difference (Leonard, 1943; Hatfield et al., 1974) methods. For the isotope dilution (ID) method, (NH₄)₂SO₄ enriched with 5 atom percent ¹⁵N was applied to each of the inoculated pots at a rate equivalent to 20 kgN ha⁻¹, one week after planting.

Rhizobial isolates used to inoculate mucuna were isolated according to Somasegaran and Hoben (1994), from mucuna nodules collected from the previous experiment. Prior to use, isolates were authenticated as rhizobia and evaluated for effectiveness. The three most effective isolates, designated M41, M51, and M76 were used in this study. Rhizobial cultures were grown in yeast manitol medium (YEM) at 28°C on an orbital shaker for 5 days to a cell density of between 10⁷–10⁹ colony forming unit (cfu) ml⁻¹. A cocktail of the three cultures was then constituted and one ml of the cocktail broth culture containing about 10⁷ cfu ml⁻¹ was inoculated onto each germinated seed a week after planting. Necessary precautions were taken to avoid cross contamination through seed, during watering, etc.

Data collected were, nodule number, nodule dry weight and aboveground shoot biomass.

All data were subjected to analysis of variance and means separated at ($P < 0.05$) by LSD, using the statistical package, Statitix. A correlation analysis was later performed to establish the relationship between soil chemical properties and nodulation.

3. Results

Soil chemical properties

The chemical properties of the various soils studied are shown in Table 1. Except for the Akuse and Changnalili soil series which were near neutral, all the soils were moderately to strongly acidic. Although all the soils were very low in available P, significant differences in available P among soils were found (Table 1). Available P correlated positively ($r = 0.769$) with soil pH ($P < 0.01$) but not with number of nodules. Soils sampled from the coastal savanna agroecological zone were significantly lower in available P but higher in % N, % organic C and available nitrate than those from the northern savanna agroecological zone. In terms of measured chemical properties, and the suitability of the soils for agriculture, Akuse series was the best for crop production.

Occurrence and abundance of rhizobia nodulating legumes in inland valley soils

Although almost all the inland valley soils contained indigenous rhizobia capable of forming nodules on the legumes studied, the variability in rhizobial numbers was great. Estimated rhizobial population for rhizosphere soil of three of the 13 legumes studied ranged from as low as six, to 1.7×10 cells g^{-1} soil for mucuna, to as high as 1.7×10 to 1.7×10^5 cells g^{-1} soil for cowpea and 10 to 3.1×10^5 cells g^{-1} soil for green gram (Table 2).

Nodulation of legumes

Except for canavalia (*Canavalia ensiformis*), mimosa (*Mimosa pudica*), desmodium (*Desmodium distortum*) and indigofera (*Indigofera hirsuta*), the various legumes formed nodules in every soil, although the numbers were generally low (Table 3). Canavalia and mimosa were nodulated only in 4 of the soils, while desmodium and indigofera formed nodules in six and seven, of the soils respectively.

The type of soil, legume and the soil X legume interaction were all significantly related to nodule numbers. The mean number of nodules for all

legumes (Table 3) was highest in the Tefle soil series (26 nodules plant⁻¹). This value is about 3 times the number of nodules recorded for the second best nodulating soil, the Akuse series, and about 17 times that of the Hake series, in which the lowest average number of nodules was formed (Table 3). Nodule number per plant correlated significantly with % N ($r = 0.39^*$), available NO₃⁻ N ($r = 0.6^*$) and % organic C ($r = 0.4^*$). The legumes nodulated better in the heavy textured soil series (Tefle, Akuse, Bumbi and Amo) than the light textured soil series (Lima, Changnalili, Volta and Hake).

Differences in nodulation among cultivars were observed within both *Vigna unguiculata* and *Phaseolus aureus* cultivars (Table 3). The *Vigna* cultivars, benkpla (an improved cv) and sanji (a local cv) had about 60% more nodules ($P < 0.05$) than another local cultivar, asedua. For phaseolus, the cultivar, green gram had about 70% more nodules than the cultivar, Yellow gram. *Crotolaria* and lablab were the best nodulators among the grain/forage legumes across the soils. Except for Tefle and Akuse soil series, nodulation of mucuna and mimosa was poor and on average hardly exceeded a nodule per plant. Mucuna however nodulated in all the soils while mimosa nodulated in only four of the soils.

Table 3. Nodulation of legumes in inland valley soils

Legumes	Inland valley soils series								Mean
	Chang	Lima	Volta	Hake	Amo	Tefle	Akuse	Bumbi	
<i>Vigna unguiculata</i> (benkpla)	8	17	9	3	7	30	25	10	13.9
<i>Vigna unguiculata</i> (Sanji)	6	10	6	5	10	38	16	10	12.9
<i>Vigna unguiculata</i> (Asedua)	2	4	3	3	5	35	10	6	8.4
<i>Phaseolus aureus</i> (Green gram)	5	5	3	0	6	41	15	1	9.4
<i>Phaseolus aureus</i> (Yellow gram)	5	3	4	1	2	13	12	6	5.5
<i>Crotolaria ratusa</i>	12	8	4	3	10	22	5	24	10.9
<i>Lablab purpureus</i>	7	5	6	3	3	30	13	11	9.6
<i>Calopogonium mucunoides</i>	5	8	5	1	17	18	11	5	8.7
<i>Desmodium distortum</i>	5	9	0	0	3	37	3	3	7.6
<i>Canavalia ensiformis</i>	0	1	0	0	11	43	0	3	7.3
<i>Indigofera hirsuta</i>	7	4	2	0	5	6	3	16	5.3
<i>Mucuna pruriens</i>	1	2	1	1	1	11	4	1	2.6
<i>Mimosa pudica</i>	1	0	0	0	0	10	3	1	1.9
Mean (soil)	4.9	5.8	3.3	1.4	6.1	25.8	9.1	7.6	

Lsd (0.05) soil = 1.7; Lsd (0.05) soil × legume = 2.21; Lsd (0.05) legume = 2.21.

Table 4. Effect of *Rhizobium* inoculation on symbiotic properties of mucuna in three inland valley soils in Ghana

Soil series	Inoculation	Nodule No. plant ⁻¹	Nodule wt. plant ⁻¹ (mg)	Shoot dry wt. (g)	Shoot N (%)
Volta	Non-inoculated	1.2	13.3	4.26	0.99
	Inoculated	23.3	153	8.75	1.28
Akuse	Non-inoculated	7.3	58	4.32	1.36
	Inoculated	21.6	176	6.16	1.53
Bumbi	Non-inoculated	2.2	40	6.13	1.35
	Inoculated	16.2	41	5.75	1.70
Lsd		6.8	37.3	0.63	0.08

Table 5. Effect of *Rhizobium* inoculation on nitrogen fixation by mucuna in three inland valley soils in Ghana

Soil series	Inoculation	Total N (mg plant ⁻¹)	Method			
			Isotope dilution N fixed (% mg/plant)		Total N difference N fixed (% mg/plant)	
Volta	Non-inoculated	42.35	ND	ND	39	17.03
	Inoculated	112.11	68.24	76.4	77.3	86.70
Akuse	Non-inoculated	58.39	ND	ND	58.6	34.48
	Inoculated	94.29	55.80	55.2	74.5	70.40
Bumbi	Non-inoculated	82.92	ND	ND	40.75	34.53
	Inoculated	98.11	39.4	38.13	49.85	49.73
Lsd		9.93	3.02	5.8	9.99	9.93

Nodulation and N₂ fixation by Mucuna

Type of soil and inoculation significantly affected nodule abundance, nodule weight, above ground shoot weight and shoot N-content in mucuna (Table 4). Inoculation increased nodulation and nodule dry weight between 3 to 20 and 0 to 10-fold, respectively. The highest effect of inoculation was observed on Volta series and the least on Bumbi. Although increased nodule number with inoculation in Bumbi soil series did not enhance nodule dry weight, it resulted

in more than 25% increase in shoot N content (Table 4). Across soils, the proportion of N fixed in non-inoculated mucuna, estimated by the total nitrogen difference method (TND), averaged 46% compared to 67.2% with inoculation. Using the ^{15}N isotope dilution technique, inoculated mucuna was estimated to have fixed on average of 54.4% of its total N content (Table 5). However, trends in N_2 fixation measurements in the different soils were not affected by the method (the isotope dilution or total nitrogen difference methods) of measuring the process.

4. Discussion

Nodulation of legumes

The generally poor nodulation of mucuna and many of the legumes in this study may to a large part be attributable to the low rhizobial populations in these soils. According to Theis et al. (1991), for effective nodulation, the number of rhizobia per gram soil should not be less than 50 cells. In this study, population densities of rhizobia nodulating mucuna in all the studied soils were less than 50 cells g^{-1} soil. However, the populations of rhizobia nodulating two of the legumes, cowpea and green gram were more than 50 cells g^{-1} soil in 75 and 50 percent of the studied soils, respectively (Table 2). These results may explain why cowpea does not respond to inoculation in most tropical soils (Ezedinma, 1964; Doku, 1969; Kang et al., 1977). The low rhizobial numbers and poor nodulation of mucuna found in these soils are similar to those reported by Sanginga et al. (1996) for mucuna grown in soils in Benin. The much greater ($>2\times$) nodulation of mucuna in the inoculated treatments (Table 4) suggests that low rhizobial populations in these soils limit nodulation.

Several reasons may account for the scarcity of specific rhizobia in a particular soil. Low soil pH is known to affect negatively the size of native rhizobia population (Barber, 1980; Lindstrom et al., 1985; Wood and Cooper, 1988; Evans et al., 1993) and also the survival and persistence of inoculant strains (Hiltbold et al., 1985; Carter et al., 1995). Woomer et al. (1988) found that extreme soil temperature, especially heat, affects growth and survival and consequently the size of rhizobial population. Osmotic fluctuations frequently encountered in drying and wetting of soil, are often very demanding in microbial communities resulting in a poor survival and population decline (Boonkerd and Weaver, 1982; Mary et al., 1994; Leung and Bottomley, 1994).

Although it is tempting to suggest that alternating seasonal flooding and drying conditions prevalent in inland valley soils would severely affect rhizobial populations, the fact that high counts of rhizobia occurred for cowpea in these soils makes this a less attractive reason than the cropping

history and native vegetation. Cowpea is native to this sub-region, while others, like mucuna, for which the rhizobial counts are low, are recent introductions. The presence however of rhizobia nodulating these new introductions suggests that either the rhizobia are naturally part of the indigenous population or some native legumes are serving as sources of inoculum.

The poor nodulation of most of the studied legumes suggests that inoculation may be necessary not only for increased nodulation and nitrogen fixation in mucuna but most importantly, also for most of the studied legumes in these soils. This finding is crucial for West Africa since inoculant use is not common and there is a great need to increase the contribution of BNF to enhance crop yields.

A positive correlation of nodule numbers with available soil nitrate is contrary to the general rule that nodulation correlates negatively with available soil nitrate (McNeil, 1982; Kosslak and Bohlool, 1985; Abaidoo et al., 1990; Rai, 1992; Wiersma and Orf, 1992). The positive correlation with available $\text{NO}_3^- \text{N}$ could be explained by the rather low levels of the available $\text{NO}_3^- \text{N}$ in the soils used (Table 1). Nitrate levels in most of the soils were lower than the recommended starter dose of nitrogen ($20\text{--}30 \text{ kg ha}^{-1}$, equivalent of $9\text{--}13 \text{ mg kg}^{-1}$ soil) to be applied to nitrogen deficient soils (Oghophorie and Pate, 1971), intended for legume cultivation. Studies to test the hypothesis that nodulation of legumes in these soils could be increased by applying starter nitrogen are planned.

Nodulation and N_2 fixation of Mucuna

Because a limited quantity of ^{15}N fertilizer was available it was decided to label only soils grown with inoculated seeds to be able to assess the potential for N_2 fixation of mucuna in these soils. Compared to the total nitrogen difference method (TND), the ID method gave lower values of % N derived from fixation. Assuming the ID method is more reliable (Danso et al., 1986; Danso, 1986; Fried et al., 1983), then this suggests that the reference crop must have underestimated the total amount of N absorbed by the fixing crop (Danso, 1985; Rennie and Rennie, 1983). Since it is likely that the reference crop affected all the estimates to the same extent then it should be possible to reliably rank treatments on the basis of relative N_2 fixation values estimated by the TND method. For this, the results clearly, indicate the advantage of rhizobial inoculation for increasing N_2 fixation in mucuna. These results indicate that either the native soil rhizobia were not highly effective, or/and their numbers were too low for high levels of nodulation and N_2 fixation. Certainly the population of native rhizobia was low, as shown earlier (Table 2). Thus it is not surprising that nodule numbers increased between 3 and 20-fold with inoculation of the three soils (Table 4).

Based on the ID method, % N derived from the atmosphere (% Ndfa) in inoculated plants ranged from just under 40% in the Bumbi soil series to almost 70% in the Volta soil series, demonstrating that the type of soil influences N₂ fixation of mucuna. With an average contribution of less than 55% of the total plant N from BNF, even when it is inoculated, suggests that mucuna derives a high proportion (almost half) of its total N requirement from the soil. This is still a high dependence on soil N, and leaves room for exploring avenues for decreasing the proportion of N derived from the soil by mucuna and has significant implications for growers relying on mucuna for soil input of N in crop rotations.

5. Conclusion

Rhizobial populations and nodulation of many legumes in inland valley soils were found to be low. To increase rhizobial numbers and nodulation of legumes in these soils, there may be a need for inoculation as shown with the case of mucuna. Screening for genotypes that will nodulate better under present conditions and/or enhancing the soil condition (soil pH, phosphorus, starter nitrogen, etc) may be other possible options. Because of the low level of nitrogen in these soils, the cultivation of legumes therein could require the application of starter nitrogen, the rates of which need to be determined for the various legumes and soils.

Given the importance of mucuna in the improvement of cropping systems in West Africa, we recommend that detailed studies on N-fixation of mucuna, the effectiveness of the native rhizobia and the response of mucuna to inoculation be conducted. At present, results of our study suggest that mucuna draws a high proportion of its N from the soil pool.

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