

NODULATION PROMISCUITY OF SOYBEAN GENOTYPES.

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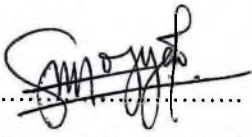
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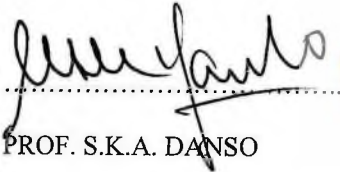
DECLARATION

I hereby declare that, except for reference to work of other researchers which have been duly cited, this work is the result of my own original research and that this thesis has neither in whole or in part been presented for another degree elsewhere.



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DEDICATION

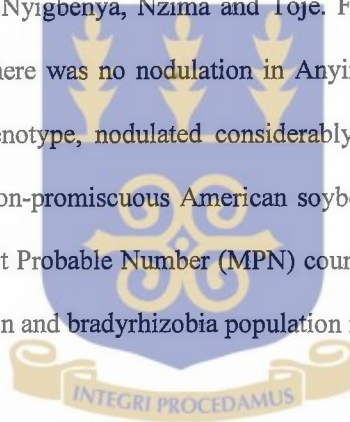
To my father, Aaron Agyei Attua and my entire family.



ABSTRACT

The objective of the study was to obtain information on the existence of *Bradyrhizobium japonicum* strains in Ghanaian soils, evaluate their effectiveness with the view to improving nodulation, nitrogen fixation and yield potential of soybean.

Eight soil series were screened for nodulation capabilities of soybean using six cultivars (four promiscuous and two non-promiscuous). The soils were Adenta, Akuse, Anyinase and Bekwai; the rest were Hatso, Nyigbenya, Nzima and Toje. Four cultivars nodulated in four soils and one in five soils. There was no nodulation in Anyinase, Bekwai and Nzima soils. Bragg, a non-promiscuous genotype, nodulated considerably well contrary to documented reports in the literature that non-promiscuous American soybean genotypes do not normally nodulate in tropical soils. Most Probable Number (MPN) counts carried out established some relationship between nodulation and bradyrhizobia population in the soils used for the studies.



Symbiotic effectiveness test carried out on 60 selected isolates from the screening experiment showed that 15% of the isolates were highly effective, 65% ineffective and 20% moderately effective.

Inoculation studies were carried out on three soybean cultivars namely Bragg (Non-Promiscuous American genotype), Bengbie (Promiscuous) and TGx (Promiscuous) using five isolates from the screening experiment and two imported isolates from Thailand in the Bekwai

soil. Generally inoculation led to improvement in shoot dry matter and total N, although the levels were different among the cultivars and isolates and thus showing that plant genotypes and bradyrhizobia strains significantly influenced inoculation response.

Inoculation and N fertilization response carried out on four soybean cultivars, Bragg, Bengbie and TGx and Non-nodulating soybean genotype, in Adenta and Bekwai soils showed better nodulation and percent N-fixed in Adenta than Bekwai. This could be attributed to the higher bradyrhizobia count in Adenta than in Bekwai. Total N fixed was however higher in Bekwai than Adenta. This means that other factors inherent in Bekwai had enhanced plant growth and total N accumulation, and hence total nitrogen fixed. Bekwai soil had higher nitrogen, organic matter and phosphorus and was thus more capable of providing nutrients and plant requirement for better plant growth than Adenta. The higher nodulation, percent and total N fixed at the 10 kg N/ha rate than at 100 kg N/ha application could be attributed to the depressing or inhibitory effects that inorganic nitrogen fertilizers have on nodulation and nitrogen fixation.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Nitrogen is frequently the most limiting nutrient for high crop yields in the tropics, where food production depends mostly on the natural fertility of the soil. Closely associated with this problem is the global shortage of dietary protein, which is most acute in the tropics, and where the cost of animal protein products is often beyond the purchasing power of many people. Protein deficiency has thus been linked to the predominantly carbohydrate diet of the population in the tropics.

1.1.1 Inorganic Fertilizer

Traditionally, nitrogen fertilizers have been used to improve nitrogen fertility of the soil. However, there are many factors militating against the use of nitrogen fertilizers. These include, high fertilizer cost due mainly to the high cost of production and high transportation charges. The high production cost of fertilizers is due primarily to the enormous amount of fossil energy (crude oil) required for their production, a situation which has worsened in recent times of escalating crude oil prices. In addition, the fact that these fertilizers are not manufactured locally and have to be in most cases imported from far away Europe and North America further inflates the cost. This situation is not likely to change favourably in the foreseeable future. In fact according to Bumb (1994), price hike of fertilizer in Ghana was 29000% compared to what pertained a decade earlier, a situation that caused the reduction of fertilizer use on rice by more than 60%. Apart from the initial high cost of fertilizers, local infrastructure is in such a poor

state that, purchase, transport and delivery are serious drawbacks to sustainable food crop production in the tropics (Ayanaba, 1977).

There are equally serious problems associated with actual application of fertilizer in the field. Nitrogen fertilizers when applied in large quantities have the tendency of causing pollution and in some cases acidification of soils (Tisdale and Nelson, 1975). Low pH is potentially detrimental to agricultural production because it adversely affects the growth of plants and the survival of beneficial microorganisms in the soil. Low pH also disrupts the balance of some soil nutrients. Also there is the possibility of nitrogen fertilizers leaching beyond the root zone or being washed away by erosion, thereby rendering them unavailable to plants (Bartholomew, 1977). Furthermore, fertilizer elements can end up in water bodies causing ecological problems to aquatic life and also to humans. For example, nitrates from fertilizer that find their way through leaching and erosion into water bodies, cause algal blooms, consequently leading to oxygen depletion upon the death and decomposition of the algae in such water bodies (Alexander 1971). It is also known that the consumption of plants with excessive uptake of nitrate can cause a disease called methaemoglobinaemia (Alexander 1971 and Keeney 1982). Finally large amounts of energy (crude oil) could be saved and put to other uses if alternatives or supplements to fertilizers were to be used.

1.1.2 Biological Nitrogen Fixation

The problems associated with inorganic fertilizers that have been mentioned above warrant the search for other alternatives to nitrogen fertilizers. This need has led to the intensification of research into biological nitrogen fixing systems as alternatives or supplements to chemical

fertilizers, as recommended by Date (1975). Not only is biologically fixed nitrogen (BNF) inexpensive compared with inorganic fertilizers; fixed nitrogen is also not plagued with many of the disadvantages such as pollution and health hazards that are associated with the non-judicious use of inorganic fertilizers. The escape of biologically fixed nitrogen into the environment in the form of nitrates is very minimal. Reports from FAO (1978) estimate that globally, biological nitrogen fixation contributes about three times as much nitrogen as supplied by inorganic fertilizer. It has to be recalled that natural nitrogen fixation pre-dates the use of artificially made nitrogen fertilizers and there is a lot of prudence in reverting to this source of nitrogen in this era of environmental consciousness when there is a lot of aversion for most things chemical.

Biological nitrogen fixation therefore appears to be the most promising alternative or supplement to inorganic fertilizers (Hardarson *et. al.*, 1987). However, the amount of nitrogen fixed in legumes depends on factors such as effectiveness of the symbiosis between the host and the (*Brady*)*Rhizobium* strain, the species, and even cultivar of the legume, among other things. In order to realize the maximum benefits of the symbiosis, there is the need to ensure that the appropriate *Rhizobium* capable of nodulating the host plant and fixing nitrogen is present in the soil.

1.1.3 Soybean

Soybean occupies a premier position among crops, being the most important source of both protein concentrates and vegetable oil (de Haen, 1994). In a situation where plant sources contribute about 70% of the world's protein needs, or even more in developing countries (Rachie and Roberts, 1974), the importance of soybean cannot be overemphasised. de Haen, (1994) predicted that about 628 million people would become seriously undernourished by the

end of the twentieth century, most of them in tropical countries and that the demand for affordable protein and energy-rich food in these countries was already high and continued to increase.

Soybean demonstrates exceptional potential for minimizing protein deficiency in both human and animal nutrition in tropical developing countries (Rachie and Roberts, 1974). Moreover, soybean and cowpea are widely grown in the tropics and the amount of nitrogen fixed by these important plants is very substantial (Alexander, 1977).

Soybean is an important source of food, feed and oil. It is said to be a whole meal, because it contains all the major food requirements in satisfactory combinations. It produces high quality oil (about 21%) a protein content of about 40% and carbohydrate of about 34% (Scott and Aldrich, 1983). Soya oil is highly digestible and contains no cholesterol while the protein is superior with essential amino acid distribution similar to milk. It could therefore serve as a good substitute for meat and fish. In addition soybean is the most cultivated crop legume in the world in terms of total production and international trade (Source: Humanity Development Library, Legon.).

Since soybean is capable of symbiotic nitrogen fixation with *Bradyrhizobium*, an intelligent manipulation of the soybean-*Bradyrhizobium* relationship will go a long way in alleviating the problem of food shortage in the tropics and protein deficiency in the whole world.

There are two main soybean genotypes, the American soybean genotype also known as the non-

promiscuous genotype and the Asian genotype referred to as the promiscuous genotype (Pulver *et. al.*, 1978; Roughley *et. al.*, 1980). The higher yielding American genotype does not nodulate readily with the indigenous bradyrhizobia in tropical soils, and therefore there is frequently the need to inoculate it for adequate nodulation and nitrogen fixation (Nangju, 1980; Pulver *et. al.* 1982). Cultivars of Asian origin have however been reported to nodulate freely in many tropical soils (Anon, 1982).

The main focus of this study is to obtain information on the existence of soybean bradyrhizobia in Ghanaian soils, to evaluate their effectiveness and to improve upon the nodulation, nitrogen fixing and yield potential of both promiscuous and non-promiscuous soybean genotypes.

1.2 Objectives.

- ❖ To assess whether inoculation is necessary for nodulation, increased nitrogen fixation and yield of promiscuous and non-promiscuous soybean genotypes.
- ❖ To isolate bradyrhizobia from soybean genotypes with the aim of identifying effective and competitive strains for inoculation studies.
- ❖ To examine whether some bradyrhizobial isolates from the promiscuous type would nodulate and be effective on the non-promiscuous genotype.

1.3 Hypotheses

- ❖ That highly effective and competitive *Bradyrhizobium japonicum* strains occur in soils in

Ghana.

- ❖ That some *B. japonicum* isolates obtained from promiscuous soybean genotypes are capable of nodulating non-promiscuous soybean genotypes
- ❖ That *B. japonicum* inoculation on seed or into soil is necessary for higher nodulation, increased nitrogen fixation and enhanced yield of promiscuous and non-promiscuous soybean genotypes.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Background.

Soybean has great potential in the tropics as a source of oil and high protein food (Ranga Rao *et al.*, 1982), and has also been identified as an affordable source of protein and oils especially for rural dwellers in many African countries (Abaidoo, 1997). In Ghana, soybean is the most important source of plant protein and edible oil (Awuku *et al.* 1991, Giller and Wilson, 1993) The protein content obtained from soybean is about twice that of meat, cowpea or limabean and four times that of cereals (Awuku *et al.* 1991). It is therefore not surprising that Africa used to import soybean to meet its protein needs in the past (Abaidoo, 1997). However, the imports had to be curtailed due mainly to balance of payment problems (Abaidoo, 1997). The only option left was for the African countries to resort to the production of soybean locally. In Ghana, currently the crop is intensively cultivated within and around the Tono Irrigated Project site.

Nitrogen has long been recognised, as the key to soil fertility and major constraint to crop production (Nye and Greenland, 1960). Large amounts of nitrogen are required for good soybean production (Cattelan and Hungria, 1994). But the element is a very limited nutrient in tropical and African soils, and in Ghana the deficiency is widespread (Nye and Greenland, 1960) to the extent such that, local production of the legume would become sustainable only when soil nitrogen is supplemented with nitrogen from fertilizer or biological nitrogen fixation (BNF) (Cattelan and Hungria, 1994).

Several factors make the addition of fertilizers to tropical soil a poor management practice. The efficiency of fertilizer utilization by soybean is usually less than 50% (Cattelan and Hungria, 1994). Moreover, nitrogen fertilizer is very expensive, which explains why the dependence of soybean only on this source of N would lower the profit of farmers, at times they may not make any profit at all (Cattelan and Hungria, 1994) especially in Ghana which is a non-fertilizer producing country. Ecological problems have also been reported to be associated with fertilizer use.

From the foregoing it is evident that N fertilizer does not hold much promise for Africa and the most sustainable nitrogen source is biological nitrogen fixation.

2.2 Biological Nitrogen Fixation.

Biological nitrogen fixation refers to the symbiotic and non-symbiotic fixation of atmospheric nitrogen by microorganisms into forms that plants can utilize, a process which raises high hopes of meeting the high nitrogen demand of the world (Dakora, 1977). Symbiotic nitrogen fixation involves mutual association between bacteria of the genus *Rhizobium* and legumes, which results in the fixation of nitrogen in the root nodules (Frobisher *et. al.*, 1974). Neither the legume nor the bacterium is capable of fixing nitrogen by itself. The rhizobia, when growing in nodules of legumes, convert nitrogen from the air into organic forms. Thus by the combined action of plant cells and bacterial cells, gaseous nitrogen is assimilated into simple nitrogenous compounds, such as amino acids and polypeptides in plants, bacteria and the surrounding soil (Frobisher *et. al.*, 1974). In the absence of rhizobia, and combined nitrogen in the soil, legumes die. However, if the right types of rhizobia are present, legumes are capable of thriving in nitrogen deficient soil, and in so doing, they enrich the soil as well (Frobisher *et. al.*, 1974). This

form of nitrogen fixation forms the basis for the recognition of the importance and application of legumes in agriculture as far back as the Greek Roman era (Tisdale and Nelson, 1956).

Rhizobia are unique among soil microorganisms in their ability to form nitrogen-fixing symbiosis with legumes. To enjoy the benefits of the symbiotic association, the rhizobia must exhibit saprophytic competence and also be able to out-compete other rhizobia for infection sites on legume roots (Somasegaran and Hoben, 1985). Generally rhizobia are capable of existence in the soil for a long time without contact with the host plant. However, the rhizosphere is a zone of increased microbial growth and activity compared to bulk soil. The roots of leguminous plants secrete soluble, organic nitrogenous compounds and simple soluble carbon compounds such as amino acids, malic acid, pentoses and phosphorus for use by microorganisms and other plants. The sloughing off of bark and root coverings and death of roots provide a rich source of carbohydrates and their derivatives to support a luxuriant flora of nitrogen fixers (Frobisher *et. al.*, 1974).

A well nodulated crop such as red clover may introduce as much as 120 kg of fixed N/ha into the soil. This nitrogen was estimated to cost around \$600 in 1973 in the form of commercial fertilizer (Frobisher *et. al.*, 1974). In fact it has been estimated that globally, BNF contributes between 139 and 170 million tons of nitrogen to plants each year (Burns and Hardy, 1975), a figure that is about three times the world annual nitrogen fertilizer production of 49.6 million tons (FAO, 1978). Duong *et. al.* (1984) have reported that biological nitrogen fixation facilitates the cultivation of soybeans on commercial scale with reduced nitrogen fertilizer inputs.

2.2.1 Brief Classification of *Rhizobium*.

The root nodule bacteria of the family Rhizobiaceae are genetically diverse and physiologically heterogeneous group of microorganisms that used to be classified together (as *Rhizobium*) by virtue of their ability to nodulate groups of plants of family Leguminosae.

In 1982, Jordan classified rhizobia into two genera and named them *Rhizobium* and *Bradyrhizobium* based on their growth rate. *Rhizobium* strains are considered fast growers and *Bradyrhizobium* slow growers. Since then further work by taxonomists have led to the discovery of three new genera that have been added to the two existing ones. These are *Azorhizobium* (Dreyfus *et. al.*, 1988), *Sinorhizobium* (de Lajudie *et. al.*, 1994) and *Mesorhizobium* (Lindstrom *et. al.*, 1995).

Soybean rhizobia belong to the genus *Bradyrhizobium* and have been referred to as *Bradyrhizobium japonicum* (Jordan 1982).

2.2.2 Cross Inoculation Groups

A cross inoculation group refers to a collection of leguminous species that develop nodules when exposed to bacteria obtained from the nodules of any member of that particular plant group. Therefore, a single cross-inoculation group ideally includes all host species that are infected by an individual bacterial strain (Alexander, 1977). Out of the over 20 cross inoculation groups identified, only seven have achieved prominence, and six have been sufficiently classified to the species status (Alexander, 1977). But the six species are not entirely distinct.

For example, the soybean and cowpea bacteria groups, commonly considered to be separate, contain many similar bacterial strains, and organisms isolated from soybean nodules frequently infect cowpea and vice versa, thus suggesting that at least some cowpea rhizobia may be varieties of the soybean rhizobia (Alexander, 1977). Bacterial strains that invade legumes outside their particular class and plants that they infect are examples of a phenomenon referred to as symbiotic promiscuity.

The validity of the cross-inoculation group system has not gone unchallenged because it has been found that many legumes are nodulated by rhizobia of other host-bacterial groups. The effect is that, the integrity of the cross inoculation concept as a system for determining relatedness among rhizobia strains has become compromised (Wilson, 1944; Bromfield and Barron, 1990) and is now in general disrepute. Its continued usage is on the basis of convenience and agronomic significance (Graham *et al.* 1991). Also it has some practical use for selecting rhizobial strains with potential for inoculant usage for particular legume crops (Mpepereki *et al.*, 1996).

2.2.3 Promiscuity of soybean genotypes.

Certain cultivars of *Glycine max* are nodulated by some strains of *Rhizobium* spp. (Cowpea miscellany) as well as their normal micro-symbiont, *Bradyrhizobium japonicum* (Leonard, 1923; Sears and Carroll, 1927; Van Rensburg *et al.*, 1976; Roughley *et al.*, 1980). Pulver, *et al.* (1978) also reported that locally-adapted cultivars of *G. max* in Nigeria, e.g. *Malayan*, nodulated promiscuously and fixed nitrogen with indigenous rhizobia whereas cultivars bred in U.S.A. e.g.

Bossier were more specific, nodulated sporadically, fixed little nitrogen and responded significantly to inoculation with *B. japonicum*. Nangju (1980), Pulver *et. al.* (1982) and Ranga Rao *et. al.*(1982) established that, although soybean has great potential in the tropics as a source of oil and high protein food, adequate nodulation and nitrogen fixation of high yielding cultivars bred in North America required inoculation with the appropriate *Bradyrhizobium japonicum* when grown in soils in which soybeans had not been previously cultivated.

The indication is, that the American soybean genotypes have specific bradyrhizobia species requirement and the compatible populations are seldom available in soils where the crop has not been previously grown, a demonstration of the classical cross-inoculation concept, where soybean would not nodulate with cowpea rhizobia population. By contrast, those resulting from breeding programmes possessing the promiscuity genotype, nodulate freely in many tropical soils (Anon, 1982). The American soybean types are therefore referred to as non-promiscuous genotypes. The Asian soybean genotypes on the other hand are able to nodulate promiscuously with native rhizobia and have therefore been referred to as promiscuous soybean types.

The specific relationship that exists between the legume and the rhizobia should be carefully considered as one of the key factors affecting the amount of nitrogen fixed. While soybeans for example require *B. japonicum* for effective nodulation and nitrogen fixation (Caldwell and Vest, 1968), others such as cowpea nodulate effectively with a range of indigenous bradyrhizobia populations (Sellschop, 1962) referred to as *Bradyrhizobium* species.

2.2.4 Inoculation

For optimum nitrogen fixation to be achieved in legumes, especially in soils where such legumes have not existed or been cultivated traditionally, there is the need to inoculate with specific effective strains of rhizobia.

Soybean is exotic to Africa (Hardley and Hymowitz, 1973) and generally considered to be a new crop in most tropical countries (Cattelan and Hungria, 1994), therefore their *B. japonicum* is either not present in the soil or they occur in very low populations (Cattelan and Hungria, 1994; Anon, 1975). It has been observed that where soybean has not been previously grown, there is generally a response to inoculation with *B. japonicum* especially with non-promiscuous cultivars (Cattelan and Hungria, 1994). Even where nodulated legumes are grown, there is a school of thought that there is the need to inoculate subsequent crops as an insurance against inoculation failure. This is done on account of the fact that populations of rhizobia decline rapidly in soils in the absence of the host plant, particularly in highly acidic soils. With low population, the expectation is that inoculation with efficient strains would increase crop yield. *B. japonicum* inoculation is therefore a necessary prerequisite for adequate nodulation and nitrogen fixation of soybeans in Africa.

But inoculation in Africa has not been without problems. In fact in the past, inoculation in Africa was generally considered not feasible since many countries were not sufficiently equipped to cope with the demands associated with the handling and usage of inoculum in the tropics (Ayanaba, 1977).

Some of the factors that militated against inoculation were:

- Lack of laboratory facilities for production, maintenance and distribution of inoculants.
- The absence of skilled personnel to man the inoculum production laboratories.
- Low survival rate of imported rhizobia strains in adverse tropical climate.
- Poor transportation and storage facilities (Ayanaba, 1977).

Over the years there have been some improvements in the requisite factors and conditions for inoculant production (Abaidoo, 1997), to the extent that some African countries such as Zambia and Uganda have successfully developed inoculum facilities which have promoted inoculum use in soybean production (Anon, 1993; Anon, 1996). Nevertheless, the fact still remains that, the level of inoculation in Africa is rather very low and unsatisfactory. A condition which might be partly due to lack of education and demonstration of the importance of inoculation to legume crop productivity. It is also worthy of mention that, the factors alluded to earlier as having contributed to inoculation failure in Africa are, by and large, much prevalent in many parts of Africa.

Even though Rhizobiologists may view the inability of Africa to adopt inoculation as a means of increasing legume crop productivity as a disappointing development, most farmers and agronomists prefer conditions and circumstances that make inoculation unnecessary and irrelevant. Perhaps, while the solution to the inoculation problems in Africa was being sought, albeit quite slowly, the breeding of soybean genotypes that would be susceptible to the nodulation with indigenous bradyrhizobia population already present in African soils was resorted to as an alternative means of improving soybean production (Abaidoo, 1997). This

approach is predicated on reports that some cowpea-miscellany rhizobia were capable of nodulating American soybean genotypes (Leonard, 1923; Sears and Carroll 1927). Furthermore, it had been observed that local soybean varieties in Nigeria and Tanzania (Pulver *et. al.* 1985) and Thailand (Na Lampong, 1976) nodulated freely with bradyrhizobia in the soils and did not respond to *B. japonicum* inoculation. This was in contrast with the non-promiscuous American soybean cultivars which nodulated sporadically, fixed little nitrogen and responded significantly to *B. japonicum* inoculation (Pulver *et. al.*, 1985). Pulver *et. al.* (1982) also reported that the indigenous bradyrhizobia that nodulated the local and American soybean cultivars in Nigeria were *Bradyrhizobium* spp. Furthermore, they attributed the insignificant response to inoculation exhibited by local soybean cultivars to their incompatibility with *B. japonicum* strains and the presence of the more compatible indigenous bradyrhizobia in Nigerian soils. Kueneman *et. al.* (1984) also observed that the marginal response of adapted soybean cultivars to *Bradyrhizobium japonicum* inoculation was indicative of the fact that *Bradyrhizobium* spp were capable of maintaining high soybean yields without inoculation or fertiliser nitrogen.

Motivated by these findings, Researchers at the International Institute of Tropical Agriculture (IITA) in Nigeria adopted, as guiding principle, the capability of soybean to nodulate effectively with indigenous *Bradyrhizobium* spp but not with *B. japonicum* (Kueneman *et. al.*, 1984). A working principle which operates on the following basic assumptions:

- That the improved soybean genotypes designated as Tropical Glycine cross (TGx), nodulate freely and effectively with *Bradyrhizobium* spp. populations.
- That the *Bradyrhizobium* spp. nodulating TGx exist abundantly in all African soils, therefore, soybean yields could not be limited by BNF without inoculation and inorganic

fertilizer N.

From the above principles and assumptions, it is not surprising that soybean breeding programmes in Africa for over two decades tended to rely on effectiveness of indigenous *Bradyrhizobium* spp. to supply N to meet soybean requirements (Abaidoo, 1997). Later research findings have however shown that, nitrogen fixation by indigenous bradyrhizobia could not meet the N demands of all soybean genotypes in all locations of cultivation. There were instances where Pal and Norman (1987) measured yield increases from fertilizer application in Northern Nigeria contrary to claims by Pulver *et. al.* (1985) that TGx soybean cultivars did not respond to N application; Pal and Norman (1987) therefore recommended the application of inorganic N fertilizer in split-applications at several growth and developmental stages of soybean to obtain maximum yields. Furthermore, they observed that the ability of TGx soybean cultivars to maintain moderate yields without fertilizer application or inoculation depended on the type of cultivar as well as the location of cultivation; an observation which has been confirmed by Okereke and Eaglesham (1992) and Abaidoo (1997). According to Abaidoo, field trials of soybean conducted in Nigeria (unpublished data) as well as observation from soybean fields in Nigeria and Ghana indicated that TGx soybean genotypes failed to nodulate adequately in some farmers fields though they had been previously cropped with cowpea, groundnut, bambara groundnut and pigeon pea. The fact that these tropical legumes had been previously cultivated on those fields may suggest the presence of adequate *Bradyrhizobia* spp. in the soil to infect the soybean genotypes that were planted.

One can therefore surmise that, high soybean productivity can be achieved by establishing the

presence of indigenous *Bradyrhizobium* spp. and/or *B.japonicum* populations in all locations of interest in Africa and ascertaining, their compatibility and effectiveness on various soybean genotypes. Where these native rhizobia species prove efficient in optimum nitrogen fixation, there may not be the need for inoculation. On the other hand, where they are found not to be efficient, there may be the need to introduce (through inoculation) more efficient and compatible strains selected either from a few local or imported bradyrhizobia. This idea is based on reports that, in situations where native rhizobia are often more competitive, yet less efficient nitrogen fixers than introduced strains (Johnson *et. al.*, 1964; Bergerson *et. al.*, 1971; Van Schreven, 1971) the native rhizobia tend to colonize most of the available sites for nodule formation. Also they form nodules that fail to sufficiently satisfy the nitrogen needs of the host because such symbiosis is ineffective in terms of nitrogen fixation. In other cases however, the introduced strains fail to obtain response due to the presence of high population of efficient indigenous strains present in the soil (Sellschop, 1962; Ayanaba and Nangju, 1973). For inoculation to yield its desirable objective in soils with large but ineffective rhizobia, there is the need for the introduced inoculum to be more competitive (Obaton, 1975). Looking at the other scenario where efficient native strains already exist, inoculation cannot offer any special advantage.

2.2.5 Effectiveness of Rhizobia Strains.

Rhizobia have generally been categorized into three groups according to their ability to fix nitrogen. These are, effective, moderately effective and ineffective. Far back in 1888, Hellriegel achieved fame by producing conclusive evidence that the apparent nitrogen fixation in leguminous plants occurred only when the plant was furnished with

root nodules. Further research over the years have revealed that the ability of rhizobia to induce and form nodules on their compatible legume host is not a sufficient condition for nitrogen fixation to proceed, though it is a necessary precondition (Giller and Wilson, 1993).

Singleton and Stockinger (1982) asserted that the strains of *Rhizobium* present in the soil might range from highly efficient symbionts (effective strains) to those that are capable of nodule formation but unable to reduce atmospheric nitrogen (ineffective strains). Effectiveness according to them followed a normal distribution pattern. From the foregoing, one can safely deduce that, while some rhizobia fix nitrogen in large quantities, others fix partially and yet others may live in nodules as non-fixing parasitic forms.

Evidently, there is the need therefore to do a thorough screening of rhizobia in the soil for effectiveness before embarking on any measure of inoculation process. Symbiotic effectiveness of indigenous rhizobia population is therefore an important parameter for the selection of strains for inoculant production. Also, it is a primary factor for the determination of incidence and magnitude of legume response to inoculation (Singleton and Travers, 1986, Thies *et. al.*, 1991). To conclude, one may re-echo the conviction held by Bromfield and Ayanaba (1980) as well as Danso (1988), that, the presence in the soil of the appropriate rhizobial strains that are highly effective is a prerequisite for nitrogen fixation in legumes and that where these are either absent or ineffective, rhizobial inoculation is necessary to ensure nitrogen fixation.

There is the prevalence of ineffective indigenous strains in the average soils against which inoculant strains have to compete for nodulation (Owiredu, 1980). Many researchers associated some legume failures with the large population of ineffective native rhizobia present in soils (Leonard, 1930; Johnson *et. al.*, 1964; Holland, 1970; Labandera and Vincent, 1975). In soils

with large population of inefficient native strains, the strong competitive ability of the native strains accounts for the lower rate of nodules formed by the introduced strains, often accounting for between only 0 and 17% of all the nodules formed (Johnson *et. al.*, 1964 and Ham *et. al.*, 1971).

2.2.6 Soil Rhizobia Population

Nodulation and nitrogen fixation occur in legumes when the appropriate rhizobia are present in the soil and in adequate numbers. Also, population density of indigenous rhizobia contributes immensely towards competition for nodule occupancy and response to inoculation. Information on rhizobia numbers would go a long way in helping rhizobiologists to assess the need or otherwise for inoculation among other things. However, data on the population sizes of cowpea and soybean rhizobia in tropical soils are lacking (Munlongoy and Ayanaba, 1986). The limited available information suggests a population range of 10^3 to 10^4 rhizobia per gram soil for native food legumes (Zengbe, 1980; Munlongoy and Ayanaba, 1986; Danso and Owiredo, 1988). According to Danso (1992), by most standards, these numbers should be adequate for nodulation. Estimates of bacteria numbers vary according to the means of determination (Alexander 1977). Enumeration of rhizobia is normally done by the most probable number (MPN) method (Alexander, 1965) using plastic pouches (Weaver and Frederick, 1972). Other methods include the immunofluorescence technique (Schmidt *et. al.*, 1968).

The most probable number infection test is based on the assumption that organisms are randomly distributed and that the presence of one *Rhizobium* cell is capable of inducing nodulation on an appropriate host (Woomer *et. al.*, 1988).

2.2.7 Nodule Formation and Development.

Rhizobium infects legume roots leading to the development of nodules within which nitrogen fixation takes place.

Nodule formation is a multistep process with four main stages: (1) Pre-infection (2) Infection and nodule organogenesis (3) Nodule functioning and maintenance (Vincent, 1980), and (4) Nodule senescence.

Pre-infection starts when rhizobia are attracted by chemotaxis to the organic compounds (flavonoids) excreted by root hairs (Turgeon and Bauer, 1985; Nap and Bisseling, 1990; Gerahty *et al.*, 1992). The flavonoids are host specific and stimulate the multiplication of rhizobia besides acting as chemo-attractants and cause the rhizobia to become attached to the root hairs. Each flavonoid switches on nod genes in the bacterial cell, which causes it to synthesize Nod factors. Root hair deformation or curling occurs (Bauer, 1981) under the influence of Nod factors (Hirsch, 1992). Dazzo *et al.* (1984) suggested that the adhesion of the bacterial symbiont to the root surface is a critical step prior to the successful infection phase of the nodule development process. Following the attachment of the rhizobia to the root hair, invagination of the root hair takes place and the bacteria penetrate the root hair epidermis and enter the plant. Penetration is an active process during which the bacteria enter the plant through the tip of the root hair (Bauer, 1981; Dazzo *et al.*, 1984) provided these bacteria are the suitable strains. Penetration of root hair appears to be under control of both *Rhizobium* and the plant (Bauer, 1981). Only a small and variable proportion of the host root hairs become infected and about 60-99% of the infections abort during the process (Dart, 1977). It has also been observed that only certain discreet portions of the root are susceptible to infection (Bauer, 1981;). Mature

roots are however generally found not to be susceptible to infection (Dart, 1977).

The penetration of the root hair is followed by the formation of a hypha-like infection thread in which the rhizobia multiply enormously. The infection process causes cells in the root cortex to divide to form the nodule primodium. The infection threads enter the primodial cells and bacteria are released into the plant cell cytoplasm. Each bacterium becomes a bacteroid, and undergoes fission. The presence of the bacteria stimulates multiplication of plant cells around that area, with the resultant formation of a nodule tissue. The rhizobia within the nodule receive their nutrient supply from the plant.

During senescence, the supply of carbohydrate to the nodules is reduced. Nitrogenase activity and leghaemoglobin content decline and the nodule degenerates.

There are two main types of nodules; determinate and indeterminate. Generally, temperate legumes form indeterminate nodules while tropical legumes form determinate ones (Vance, 1983). Determinate nodules have a fixed life span as against the indeterminate ones, which grow for an infinite period of time. The type of nodule formed depends on the plant and not the *Rhizobium* strain (Dart, 1977).

There are different shapes and sizes of nodules, determined by soil conditions and *Rhizobium*-plant variety interaction (Lynch and Wood, 1989). The size may vary from 1mm. to over 1cm. The shapes are global, cylindrical, peanut, elongate and lobed, flattened or collaroid. Lynch and Wood (1989) have attributed the failure of bacteroids to persist in nodules as a major cause of nodule ineffectiveness. Generally, ineffective nodules have much shorter life span and of smaller size than those which are effective, but they are in greater number than the effective ones. The leghaemoglobin content of the effective nodules far exceed the ineffective ones and this is reflected in the interior colour of the effective being more pink while the ineffective range

from less pink to green in colour (Lynch and Wood, 1989).

2.2.8 Environmental Factors Affecting Biological Nitrogen Fixation.

Factors affecting nitrogen fixation have been extensively studied. The outcome of the studies generally shows that environmental factors affect the legume and *Rhizobium* individually or both components of the association as a whole. They may affect plant growth, the symbiotic association directly (Sprent *et. al.*, 1988) or they may affect the biochemistry of fixation indirectly. Environmental factors may be biological, chemical and physical. For purposes of this discussion they would be considered under biotic and abiotic factors.

Abiotic factors include moisture, aeration, nutrients, soil temperature, pH and salinity among others. Biotic factors result from competition and antagonism from other organisms living with the microsymbiont in the soil.

Considering the plant and the rhizobia, since water is a major component of protoplasm, adequate supply must be available for their vegetative development. But where moisture becomes excessive, microbial proliferation as well as root development are suppressed because the oversupply limits gaseous exchange and available oxygen supply (Alexander, 1977). Soil moisture may affect biological nitrogen fixation indirectly through plant growth, and directly through infection (Sprent, 1979) and nodule characteristics. Sinclair *et. al.* (1987) have shown that nitrogen fixation rates are more sensitive to moisture than any other plant physiological process (Alexander, 1977). Lack of oxygen to the host legume roots in waterlogged

environment may also result in reduced acetylene reductase activity and nodulation (Witty *et al.*, 1986). The detrimental effects of desiccation and high temperature on cowpea and soybean *Rhizobium* survival in soils are well documented (Boonkerd and Weaver, 1982; Hartel and Alexander, 1984; Munlongoy *et al.*, 1981). On the other hand, there is the evidence that *Rhizobium* has the ability to survive in desiccated soils (Van Schreven, 1970; Foulds, 1971; Danso, 1977). It is believed that the bacteria were able to achieve this due to their dependence on hygroscopic water surrounding the soil particle (Giltner and Langworthy, 1916).

Soil temperature affects the survival of *Rhizobium* and *Bradyrhizobium* in soil, with lower temperatures being more favourable than high temperatures (Bowen and Kennedy, 1959; Danso and Alexander, 1974; Danso, 1977). High soil temperatures may also affect survival of inoculated *Rhizobium* in tropical soils (Bowen and Kennedy, 1959). Hungria and Franco (1993) observed that nodules formed by effective strains at high temperatures (35 and 38°C) were ineffective. Hardarson *et al.* (1989) and Wadisirisuk *et al.* (1989) reported that deep placement of nodules enhanced nitrogen fixation in soybean. Piha and Munns (1987) also suggested that deeper-placed nodules might be more active in nitrogen fixation when top soil temperatures are high because the sub-soil temperatures are lower than the top-soil temperatures.

Strains of rhizobia differ in their ability to withstand high temperatures (Bowen and Kennedy, 1959; Marshall, 1956). The differences in tolerance may be due to adaptation of rhizobia to hot environment (Tuzimura *et al.*, 1963), age and number of cells, (Fred *et al.*, 1932) and type of soil (Marshall and Roberts, 1963). The seasonal fluctuations of rhizobial population in tropical soils is the result of high soil temperatures, large variations of soil moisture and low level of

organic matter (Obaton, 1975).

Soil acidity affects plant growth as well as bacterial occurrence and the survival of *Rhizobium*. Highly acidic or alkaline conditions tend to inhibit many common bacteria as the optimum for most species is near neutral pH. Nevertheless, there are reported cases of soils of pH 3 containing bacteria (Alexander, 1977). Low pH is common in many tropical soils and tends to severely limit the survival of introduced rhizobia and legume nodulation (Danso 1977; Danso and Alexander, 1974; Vincent and Waters, 1954). Acid and aluminium stresses are essentially bacteriostatic (Munns and Keyser, 1981). Aluminium toxicity is of importance to growth and survival of cowpea rhizobia (Hartel and Alexander, 1983). In 1984, F.A.O. reported that acidity, calcium, aluminium and manganese concentrations interact and affect both bacterial growth, root-hair infection and plant growth.

Salinity has been known to cause permanent water stresses due to high osmotic pressure and making some nutrients such as phosphorus, molybdenum, iron, boron, manganese and zinc unavailable to both legume plant and the rhizobia.

The availability of nutrients is essential for growth and multiplication of rhizobia (Fred *et. al.*, 1932). Nevertheless, lack of nutrients does not cause the rapid death of rhizobia in the soil (Chen and Alexander, 1971; Danso and Alexander, 1974)

High levels of available soil nitrogen have been shown to sufficiently supply the nitrogen need of nitrogen fixing plants and as a result inhibit biological nitrogen fixation.

There have been many studies of the effects of combined nitrogen on the physiology of the *Rhizobium*-legume symbiosis. One study by Anderson (1956) gave the indication that biologically fixed nitrogen and combined soil nitrogen seem to produce the same response in nitrogen fixing plants and the interaction between them is negative.

It has also been established that large amounts of applied nitrogen reduce root-hair infection (Munns, 1968; Dazzo and Brill, 1978), nodule number (Dart and Mercer, 1965), nodule mass (Summerfield *et. al.*, 1977), the nitrogen fixing activity of nodulated roots (Gibson, 1974), and the total amount of nitrogen fixed, (Allos and Bartholomew, 1959). The degree of inhibition however varies with the form of nitrogen compound (Dart and Wilson, 1970), the cultivar (Gibson, 1974), the species (Allos and Bartholomew, 1959), strain of *Rhizobium* (Pate and Dart 1961) and nutritional conditions (Pankhurst, 1981).

At low levels, the effects of applied nitrogen on symbiosis may be stimulative (Gibson and Nutman, 1960; Gibson, 1974).

Phosphorus deficiency is acute in soils of very low or very high pH (Sanyal *et. al.*, 1990). Both the host legume and *Rhizobium* require phosphorus for the normal establishment and functioning of the nitrogen fixing symbiosis (Robson *et. al.*, 1981).

Many biological factors affect the rhizobial population in the soil. Some microorganisms may antagonise *Rhizobium* by producing antibacterial metabolites. Others such as protozoa and bacteriophages act as predators and parasites respectively on rhizobia (Danso *et. al.*, 1975; Barnet, 1980; Roughley, 1985); while others may act as competitors. Root-nodule bacteria are known to persist better in sterile than non-sterile soil (Van Schreven, 1970; Danso and

Alexander, 1974). One can therefore deduce that biological agents are implicated in the decline of rhizobia numbers in the soil. Microorganisms add many compounds to their environment, some of which are produced to ward off potential competitors, parasites and predators. For instance Holland and Parker (1966) obtained a toxic water extract which inhibited *Rhizobium trifolii*. There are however reports of stimulation of some rhizobia by some soil microbes (Dixon, 1966). Some isolates of bacteria and actinomycetes were found to stimulate strains of *Rhizobium trifolii* (Hattugh and Luow, 1966). Among *Rhizobium* strains, it is reported that mixed inocula produced higher yields of shoot dry weight and total nitrogen in red clover than single strain inoculation (Hofer, 1945), showing some synergism among the strains.

2.3 Soybean-The Host Plant.

Soybean (*Glycine max.* L. Merrill) is a member of the Leguminosae family and of the order Papilionaceae. It is considered to have its origin in Northeastern China, although the genus has two major centres. One in Eastern Africa, the second in Australasia region with a secondary centre in China.

Since its domestication around 11th century BC in China soybean has been a staple food in eastern Asia (Hymowitz, 1970). It remained confined to Asia until the beginning of the 20th century when U.S.A. developed it into a major commercial crop. According to Anon (1982), all over the world soybean is the most important source of commercial oil and grain legume crop. Soya flour is being incorporated into weaning foods (Annan, 1998) and in Ghana this constitutes one of the major uses of soybean. Soya flour can also be used to improve the protein content of bread and gari (a staple food in most parts of West Africa and Ghana). Soybean can be processed into a variety of edible products such as soya milk, soyabean streak, soya sauce soya

flour and soybean yoghurt. There are reports that milk is being processed from soybeans in Ghana (Annan, 1998). Soybean is the most cultivated crop in terms of total production and international trade. In the United States of America it is the second most valuable crop, surpassed only by maize. The world's production has more than doubled over the last 20 years or so with global production in the latter part of the 1980s exceeding 100 million tons annually (Source: Humanity Development Library, Legon.).

The wet subtropics provide the best climate for the cultivation of soybean with annual temperatures of around 25°C and optimal rainfall of between 500 and 750 mm. The plant is extremely photoperiodic, most varieties flower with day-light less than 14 hours a day. Reduced growth and yield become prevalent when daylight is less than 12 hours.

Soybean is mainly cultivated for its seeds, commercially grown for human consumption, livestock food and the extraction of oil. The fruit is normally a short hairy pod, which it varies from 2 to 10cm in length and 2 to 4cm in width according to variety. Being an annual its quick growing habit and easy cultivation lend itself to subsistence farming, a farming system very much predominant in West Africa and the tropics as a whole (Source: Humanity Development Library, Legon.).

It has been mentioned earlier that large amounts of nitrogen are required for good soybean production. For a yield of 3000 kg/ha, 231 kg of N is required (Borkert and Sfredo, 1994). Soybean can use nitrogen released by mineralization, residual soil nitrogen, fertilizer N or

atmospheric nitrogen, which is converted to usable form in root nodules through symbiotic relationship between *B. japonicum* and soybean (Borkert and Sfredo, 1994). Like all legumes, the value of soybean lies in its ability to grow under a wide range of environment and may do well on poor soils without the need for supplemental nitrogen. The reason being that, though the soil is the primary source of N for many crops, soybean obtains 65-86% of its needs through the symbiotic process (Borkert and Sfredo, 1994). Therefore in most areas where soybean is now grown, production would be impractical without efficient symbiotic nitrogen fixation (Borkert and Sfredo, 1994).

But soybean does not nodulate satisfactorily in West Africa, perhaps due to the fact that it was recently introduced into the Sub-region. (Anon 1975; Cuttalan and Hungria 1987; Ayanaba, 1977). However there are reports of inoculation response by selected rhizobial strains (Bromfield and Ayanaba 1980; Owiredu and Danso, 1988; Annan 1998). Dennis (1975) reported of inoculation response in three Ghanaian soils and predicted soybean to become a promising legume in terms of nitrogen fixation. Work done by Owiredu and Danso (1988), also in Ghana, showed no nodulation in uninoculated Jupiter soybean (American genotype). However, few nodules were formed on Williams, also an American genotype, grown in the same soil. This seems to suggest that there are possible differences in the nodulating capacities of non-promiscuous soybean genotypes to nodulate with native *Bradyrhizobium* in tropical soils (Kueneman *et. al.*, 1984). According to Danso (1977), Danso and Alexander (1974) and Vincent and Waters (1954), low pH (a prevalent phenomenon in tropical soils) severely limits the survival of introduced rhizobia and legume nodulation. Other reports by Haque *et. al.* (1980) in Sierra Leone, on the other hand, attributed poor nodulation in soybean to high soil temperature

and moisture stress.

High acidity is common in many tropical soils and can be a stress to soybean. The specific causes of poor plant growth on acid soils may vary with soil pH, clay mineral type and amount, organic matter content and kind, level of salts and particularly, with plant species or genotype (Foy *et. al.*, 1978). Tropical soils should undoubtedly be limed in order to produce high yields. For example, Owiredu and Danso (1988) observed nodulation increase of 13 times when lime was pelleted on the inoculated seeds compared with direct-seed inoculation only treatment.

2.4 Measurement of fixed nitrogen

Soon after the discovery of biological nitrogen fixation, several attempts were made to measure the amounts of nitrogen fixed by plants. Out of these attempts emerged several methods for the estimation of fixed nitrogen. The relevance of these measurements is to gather information on gains and losses of nitrogen in agricultural soils since scarcity and excessive amount of nitrogen are of great interest to agriculture and the environment. Scarcity of nitrogen significantly affects crop yield because it is a major nutrient required by plants. On the other hand excess nitrogen in the soil leads to undesirable environmental consequences (Danso, 1995). In addition the measurement of biological nitrogen fixation is an important prerequisite in determining how environmental factors could be manipulated to optimize nitrogen fixation.

Several methods have been devised for the measurement of biological nitrogen fixation but it has to be mentioned that some of these methods are qualitative and hence of little use for

quantitative purposes (Danso, 1985). Several publications have discussed various methods for measuring BNF (Chalk, 1985; Danso, 1985; Danso, 1988; Danso *et. al.*, 1993; Denison *et. al.*, 1983; Kumarasinghe *et. al.*, 1992; Shearer and Kohl, 1986 and Streeter, 1979). For purposes of this review, a brief account of some of these methodologies would be discussed.

2.4.1 Nodule Assessment.

Nitrogen fixation in legumes takes place exclusively within nodules. Therefore the quantity and mass of nodules have been relied upon as indirect evidence of nitrogen fixation and to some extent the magnitude of nitrogen fixed (Westermann and Kolar, 1978; Weber, 1966). Nodule assessment is a quick and inexpensive method that does not require any highly skilled labour. Initial screening programmes involving several *Rhizobium* strains and legume varieties usually employ this quick method. However, it is not a valid technique because it is now a well-known fact that not all the nodules formed on a variety or species of legumes fix similar amounts of nitrogen. Some nodules are effective; others are moderately effective and the rest ineffective. Therefore the method cannot estimate the exact amount of nitrogen fixed.

2.4.2 Dry matter yield (DMY)

This is also another indirect way of estimating nitrogen fixed. Crop yield increases are due to the provision of nitrogen through fertilizers in nitrogen deficient soils (Rennie *et. al.*, 1982; Deibert *et. al.*, 1979; Weber, 1966). In nitrogen free medium containing all other nutrients, dry matter yield increases are attributable to nitrogen fixed and this is the principle on which the method is based. Some studies have reported of reliable estimates of nitrogen fixation by the DMY method (Haydock *et. al.*, 1980). The method is also useful in the initial screening of large

numbers of plants for nitrogen fixation. It's usefulness however is diminished in soils with high nitrogen, where plant yields may already be near their peak (Fried, 1978). In addition, significant yield responses are sometimes not attained in the presence or absence of nitrogen fixation because other limiting factors besides nitrogen do not permit the nitrogen fixed to be translated into increased yields. The method is not sensitivity enough for the detection of small differences in nitrogen fixation (Hardarson *et. al.* 1984). Another limitation is, that the method cannot be used on different varieties or species, as potential yields could genetically be different even under identical conditions.

2.4.3 Total Nitrogen difference (TND) method.

This is one of the oldest and simplest methods and has provided many valuable estimates of nitrogen fixation, upon which several management practices have been based (Danso, 1995). It measures BNF as the difference between the total nitrogen contents of plants that fix nitrogen and those that do not derive nitrogen from fixation.

$$\text{Ndfa} = \text{Total N (fixer)} - \text{Total N (non fixer)}$$

Where Ndfa is nitrogen derived from fixation.

The method is based on the assumption that, both the nitrogen fixing and non-fixing plants absorb equal amounts of soil nitrogen (Rennie and Rennie, 1983). This assumption may not hold in all situations since different plants may rarely have similar root morphological and physiological properties as well as absorbing their nitrogen from similar depths (Danso *et. al.*,

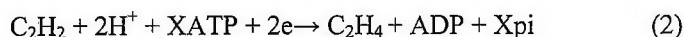
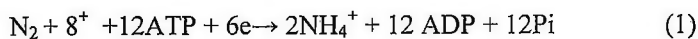
1986). This is a major limitation of the method (Danso, 1985). On the other hand several reports have demonstrated high correlation between TND estimates and those made by other BNF determination methods which are more sophisticated and expensive, including the most widely accepted ^{15}N labelling technique (Legg and Sloger, 1975; Broadbent *et. al.*, 1982). Studies by Patterson and LaRue (1983) and Rennie (1984) demonstrated that the TND method gives reliable estimates of nitrogen fixed in plants grown in soils in which initial nitrogen content is low because in such situations BNF is high compared with soil nitrogen uptake.

2.4.4 Acetylene Reduction Assay (ARA) Technique

This is also an indirect method for estimating biological nitrogen fixation. The main rationale underlying the procedure is that nitrogenase, the enzyme in procaryotic organisms involved in nitrogen fixation also converts acetylene to ethylene (Dilworth, 1966). Today, the ARA technique, as has been modified for current usage, is based on procedures outlined by Hardy and other researchers (1968). The method involves incubating the sample to be analysed in gas-tight chamber containing 0.03-0.1% (v/v%) acetylene for a few minutes to several hours. At the end of the incubation period, a gas sample from the incubation vessel is injected into a gas chromatograph fitted with a Porapak N or P column and assayed for ethylene production (Hardy *et. al.*, 1973).

The amount of ethylene produced could be used as a measure of nitrogenase or nitrogen fixing activity. On the other hand, the quantity of ethylene formed can be converted into total amount of nitrogen fixed by multiplying ethylene produced by a conversion factor of 3 (Hardy *et. al.*,

1973). The rationale is, that stoichiometrically, three pairs of electrons are used up during the conversion of N_2 to NH_4 , compared to a single pair of electrons used in the conversion of acetylene to ethylene, i.e.,



The following are the advantages of the ARA technique:

- ❖ The process is facilitated in view of the fact that no end products other than ethylene are produced.
- ❖ The ethylene produced is stable and storable, so it is possible to analyse the gas samples later.
- ❖ It is a highly sensitive and a less costly method.

The problems associated with ARA have been reviewed by many authors (Danso, 1985; Denison *et al.*, 1983; Minchin *et al.*, 1983; Witty and Minchin, 1988), some of which have been listed below:

- ❖ The technique involves short term assays whiles the BNF that it measures proceeds over long duration in crops. Therefore ARA measurements have to be extrapolated to cover periods over which no measurements were made.
- ❖ ARA measurements are normally not done on whole plants growing in the field. In the preparation of the plant samples, disturbances suffered by the nitrogen fixing system induce increased resistance to the flow of oxygen into nodules which adversely affects the rate of

acetylene conversion into ethylene (Minchin *et. al.*, 1986; Witty and Minchin, 1988).

- ❖ The samples used for ARA assays consist of uprooted plants and mostly some of the active nodules are lost in the uprooting process. This affects the amount of ethylene produced since it depends on the proportion of active nodules remaining on the plant after uprooting.

As a result of these shortcomings many reviewers and editors often reject papers that base their interpretations on ARA measurements (Vessey, 1994).

2.4.5 ¹⁵N-Methodology

The ¹⁵N methods have been classified into three main forms. These are: (1) The ¹⁵N labelled gas method, (2) The ¹⁵N isotope dilution method and (3) The A value method (Danso, 1995). The underlying principle behind all these methods is, that the nitrogen fixing plants are grown in soils or atmosphere containing ¹⁵N/¹⁴N ratio which is different from ¹⁵N/¹⁴N ratio (of 0.3663%) in the atmosphere. During fixation the incorporation of nitrogen from the atmosphere results in a different ¹⁵N/¹⁴N in plant tissues from that of the soil or any other substrate on which the plant grows.

The ¹⁵N isotope dilution method has two approaches (1) The ¹⁵N natural abundance method where the inherent ¹⁵N/¹⁴N ratios in some soils is higher than in the atmosphere (Amarger *et. al.*, 1979). Various nitrogen turnover processes such as denitrification, which has preference for ¹⁴N over ¹⁵N, bring about the higher ¹⁵N/¹⁴N ratio.

The second approach is where ¹⁵N-enriched inorganic or organic nitrogen is deliberately

added to soil to artificially widen the differences between the $^{15}\text{N}/^{14}\text{N}$ ratio of soil nitrogen and that of the atmosphere (Fried and Middelboe, 1977). In both cases the $^{15}\text{N}/^{14}\text{N}$ ratio of the plant tissue is lowered during the assimilation of unlabelled nitrogen (Danso *et. al.*, 1993) by the plant. However, in the case of the natural abundance the ^{15}N enrichment is rather very low, and calls for the use of highly sophisticated mass spectrometers well outside the reach of many laboratories in developing countries. The more the amount of nitrogen fixed the greater the dilution of $^{15}\text{N}/^{14}\text{N}$.

The ^{15}N -enriched fertilizer approach requires less sophisticated analytical capability; also, it is subject to fewer errors than the natural ^{15}N abundance approach (Ledgard *et. al.*, 1985). Therefore it is the most widely used in nitrogen fixation studies.

There are some practical problems and in some cases improper usage that have been identified with the use of the ^{15}N techniques. These have been revealed in the course of research on the increasing reliance on the technique. Questions being asked in the midst of all these criticisms have led to a closer examination of the methodology with the view to rectifying some of the anomalies of the techniques (Chalk, 1985). In some cases however, the limitations seem to have arisen from general lack of understanding of some of the basic principles of the methods (Vose and Victoria, 1986).

The accuracy of the BNF measurements using the isotope dilution approach depends on how the $^{15}\text{N}/^{14}\text{N}$ ratio assessed by the reference plant reflects that of the soil-derived N. This is the greatest source of error, especially for many studies where prior selection of suitable reference plant was not done or where the criteria for selection were not followed satisfactorily (Fried *et. al.*, 1983). In certain cases even prior selection may not work because the reference plant does

not perform satisfactorily under all environments (Chaiwanakupt *et. al.*, 1991; and Danso, 1991). There are situations where Danso (unpublished data) observed that seeds supposed to be non-nodulating isolines, upon inspection, nodulated profusely in some soils. Therefore the selection of an appropriate reference crop for each nitrogen fixing crop is of great importance (Fried *et. al.*, 1983; Wagner and Zapata, 1982)

A suitable reference plant must fulfil the following conditions:

- ❖ It should not fix N under the conditions of the study otherwise the nitrogen fixation estimate made shall be underestimated by the extent to which the reference crop fixed nitrogen.
- ❖ Both reference and nitrogen fixing plants should take up N from a similar zone even though they do not necessarily have to absorb the same quantity of soil N.
- ❖ Both reference and N fixing plants should have similar physiological growth patterns and both should be harvested at the same time.
- ❖ The tolerance levels of both reference and nitrogen fixing plants to crucial environmental stresses should be similar to ensure that conditions affecting active uptake of nitrogen of both plants would almost be equal.

Typical examples of some reference plants that have been used to assess soil $^{15}\text{N}/^{14}\text{N}$ ratios have been listed below:

- A non-nodulating legume isoline (Ruschel *et. al.*, 1982)
- An uninoculated legume (Fried *et. al.*, 1983). This is valid only in soils lacking effective *Rhizobium* strains for the legume in question.

- Legume inoculated with ineffective *Rhizobium* strains for the legumes in question.
- A non-legume, non-fixing plant e.g. cereal (Wagner and Zapata, 1982; Fried and Broeshart, 1975).

The advantages derived from the ^{15}N methodology are that:

Currently the ^{15}N soil enrichment technique is the most reliable for measuring N fixed in the field (Duhoux and Dommergues, 1985; Duque *et. al.*, 1985; Hardarson *et. al.*, 1984, Legg and Sloger, 1975; Rennie, 1982; Ruschel *et. al.*, 1979, 1982; West and Wedin, 1985).

The method is very useful because, at a single harvest, it can measure the integrated amounts of nitrogen assimilated by both green-house and field-grown plants as well as measuring the nitrogen contributed from soil or fertilizer sources (Danso, 1985; Fried *et. al.*, 1983; Vose and Victoria, 1986), thus making it possible to manipulate plants or nitrogen fixing systems for maximum nitrogen fixation.

The disadvantages of the method include the relatively high cost of ^{15}N fertilizers and equipment. There is also the need for highly skilled technicians to do the ^{15}N analysis. Recently the cost of ^{15}N fertilizers has gone down dramatically, and also, relatively inexpensive equipment such as micromass and emission spectrometer have been developed, solely for ^{15}N analysis.

CHAPTER THREE

3.0 MATERIALS AND METHODS.

3.1 Soil Sampling.

Soil samples collected from three ecological zones of Ghana were used to assess nodulation capabilities of soybean. The local names of these soils were, Adenta, Akuse, Anyinase and Bekwai soil series. The rest were Hatso, Nyibenya, Nzima and Toje series (Table 3.1). Adenta, Hatso, Nyibenya and Toje soils were collected from the University of Ghana Farms at Legon (Coastal Savanna zone, Fig 3.1). Bekwai and Nzima series were obtained from the University of Ghana Research Station at Okumaning near Kade in the Eastern region Semi-Deciduous Forest Zone, Fig 3.1). Akuse series was collected from the Agricultural Research Station of the University of Ghana at Kpong, also in the Eastern Region (Coastal Savanna zone, Fig 3.1), while Anyinase series was collected from Anyinase near Axim in the Western Region (Rainforest Zone, Fig 3.1).

All the soils were sampled from a depth of 0-15 cm from uncultivated soil. Each soil was air-dried and stones, roots and other plant parts contained in the soil removed. They were then pulverised using pestle and mortar, and sieved through a 2mm-mesh sieve.

3.2 Screening of soybean for nodulation capabilities.

3.2.1 Planting Material.

Six soybean cultivars were screened for their nodulation potential in eight Ghanaian soils. They were made up of four promiscuous and two non-promiscuous genotypes.

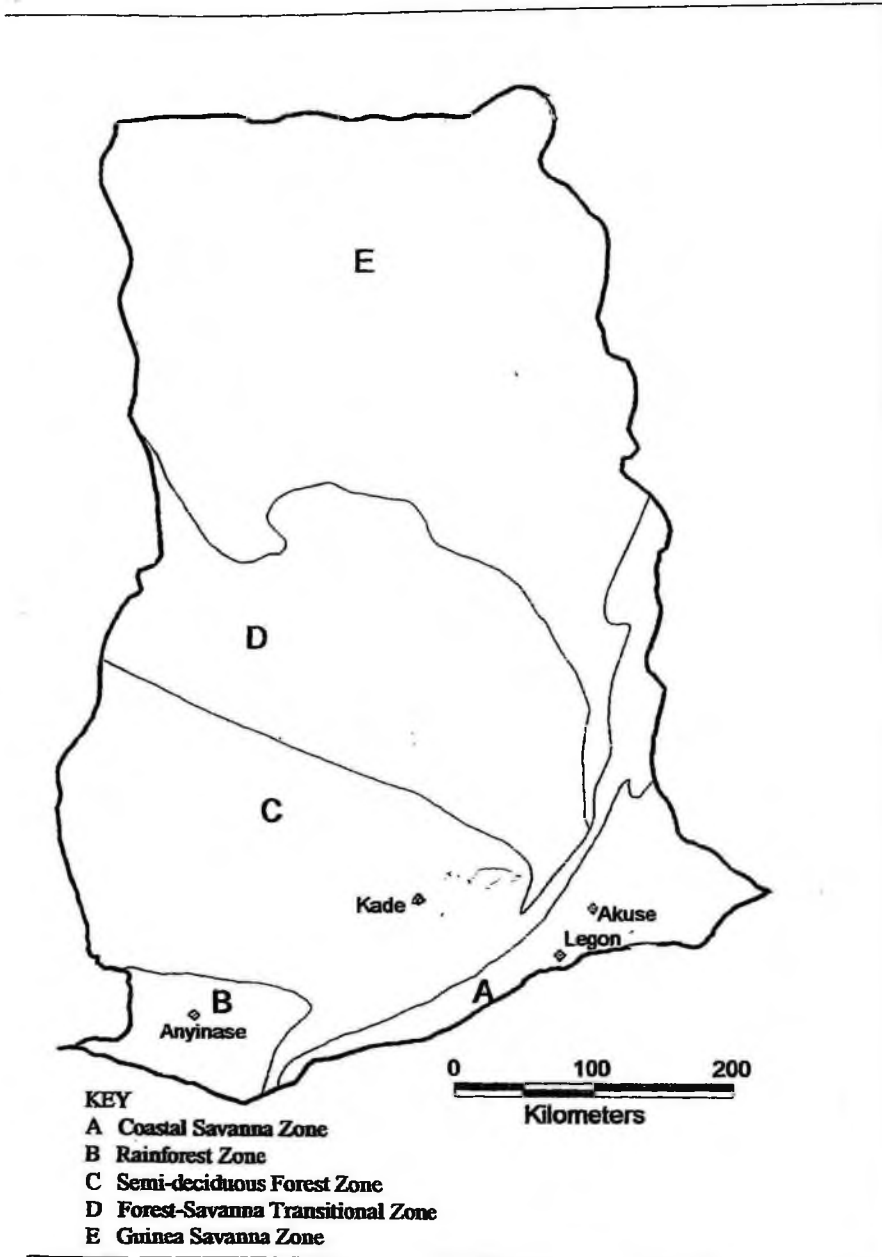


Fig. 3.1 Map of Ghana showing the ecological zones and sites where the soils were sampled.

Table 3.1 Classification of soils Studied.

SOIL SERIES	CLASSIFICATION	
	LOCAL	USDA
Adenta	Savanna Ochrosol	Typic Paleustalf
Nyigbenya	Savanna Ochrosol	Lithic Rhodustalf
Hatso	Savanna Ochrosol	NA
Akuse	Tropical Black Earths	Typic Calciustert
Anyinase	Forest Oxisols	Ultisols
Toje	Savanna Ochrosol	Typic Rhodustalf
Bekwai	Red Forest Ochrosols	Rhodic Paleudult
Nzima	Brown Forest Ochrosols	Rhodic Paleudult

Source: Extracted from Ph. D. & M.Phil. theses of Fening and Dowuona respectively.

Four of the soybean genotypes were obtained from seed store of the Crop Science Department of the University of Ghana and two of the genotypes obtained from the Savanna Agricultural Research Institute (SARI) in Nyankpala, near Tamale in the Northern Region of Ghana.

3.2.2 Pot Experiment.

The six soybean cultivars were planted in the eight soils using plastic pots, 28cm high with top interior diameter of 25cm and bottom interior diameter of 15cm. Three holes, each of diameter 0.5cm were perforated at the base of the pots to allow for drainage of excess water, but with the base covered with filter paper to prevent loss of soil. Each pot contained 1kg of soil. The seeds were surface-sterilised with 70% alcohol and rinsed with six washes of sterile distilled water before planting them at four per pot. Three days after germination seedlings were thinned to two per pot. There were three replicates for each soil and for each cultivar, using the split-treatment design, with soils being the main treatment. The experiment was carried out in the greenhouse and watered twice a day with distilled water. Harvesting was done 5 weeks after planting and nodulation assessment carried out after washing the roots under a gentle stream of water to free them of all soil particles. The nodules on each plant were counted and recorded, and some removed and used for *Rhizobium* isolation.

3.2.3 Isolation of Bradyrhizobia

Two nodules from each plant were taken from the screening experiment. They were surface sterilised by immersing in 70% alcohol in small beakers for 3 minutes and in 0.1% mercuric

chloride also for 3 minutes, after which the nodules were rinsed with 6 washes of sterile distilled water, as recommended by Somasegaran and Hoben (1985). The sterilised nodules were pulverized with a pair of heat sterilised, blunt tip forceps in a large drop of sterile distilled water in a petri dish. A loopful of the nodule suspension was streaked on yeast mannitol agar and incubated at 28°C. After restreaking colonies growing on YEM agar plates were transferred into eppendorf tubes containing agar, labelled, and stored in a refrigerator at 4°C for future work.

3.2.4 Authentication of Bradyrhizobia Isolates.

This was done to ensure that the isolates were pure cultures, and were thus still capable of forming nodules on soybean roots. Soybean seeds were surface sterilised and pregerminated on 1% (w/v) water agar and planted in growth pouches (Somasegaran and Hoben, 1985). Each sterilised 2-plants/unit growth pouch was inoculated with 1ml broth culture of an isolate (Somasegaran and Hoben, 1985). Those that were not inoculated served as control. The pouches were arranged randomly on a wooden rack with the experimental set-up in the greenhouse and the plants supplied with 50ml N-free nutrient solution (Broughton and Dilworth, 1970). The plants were harvested 28 days after planting, and the roots examined for the presence or absence of nodules.

3.3 Cross Inoculation Studies.

Specificity and promiscuity in symbiosis were studied in cross inoculation experiments. The ability of the soybean isolates to nodulate cowpea and groundnut varieties was examined. The

seeds of these legumes (cowpea and groundnut) were carefully selected by hand sorting. The aim was to obtain viable, undamaged, clean seeds of reasonably uniform sizes and colour for planting so as to reduce, as much as possible, variability among the seeds. The seeds were surfaced sterilised by immersion in 70% alcohol for 3 minutes and rinsed with 6 washes of distilled water. They were also immersed in 0.1% mercuric chloride for 3 minutes and rinsed with 6 washes of distilled water, then allowed to imbibe water by soaking in distilled water for 1 hour and then rinsed twice. Pregermination was done by transferring the seeds aseptically onto 1% (w/v) water agar in petri dishes and incubated at 28°C for 2 or 3 days, after which they were planted in sterilised growth pouches containing sterile N-free nutrient solution (Somasegaran and Hoben, 1985). Each growth pouch was inoculated with 1ml suspension of YMA broth culture of one of the isolates, which had been grown in culture bottles on a shaker for 5 days. Uninoculated growth pouches were set up as control. The growth pouches were randomly arranged on wooden racks in a greenhouse. The plants were supplied with sterile N-free nutrient solution throughout the growth period. Harvesting was done after 5 weeks and the plants examined and scored for the presence or otherwise of nodules.

3.4 Assessment of the Effectiveness of Bradyrhizobia Isolates.

This experiment was done using one cultivar of soybean named Bengbie. The growth medium was sand obtained from the Densu riverbed. The sand was flooded with 2N hydrochloric acid solution and left to stand for 3 days in large plastic containers and then rinsed thoroughly with tap water. The acid treatment was meant to digest any organic matter present in the sand and the rinsing done to get rid of excess acid until the pH was between 6.8 and 7.0. The sand, which was wet, was dried in the sun and then used to fill the top chamber of Leonard jars. Centrally placed

cotton wicks dipping into the sterile N-free nutrient solution irrigated the sand in the top chamber of the jars. The isolates were grown in culture bottles containing 50ml YEM broth on a shaker for 5 days (Somasegaran and

Hoben, 1985). The seeds were surfaced sterilised by immersion in 70% alcohol for 3 minutes and rinsed with 6 washes of sterile distilled water. They were also immersed in 0.1% mercuric chloride for 3 minutes and rinsed with 6 washes of distilled waters and allowed to imbibe water by soaking in distilled water for 1 hour and then rinsed twice. Pre-germination was done by transferring the seeds aseptically onto 1% (w/v) water agar in petri dishes and incubated at 28°C for 3 days, after which they were planted four per jar in the sterilised Leonard jars containing sterile N- free nutrient solution (Somasegaran and Hoben, 1985). After the seeds had fully germinated they were thinned to two plants per jar and each plant inoculated with 1ml suspension of YMA broth culture of one of the isolates grown in culture bottles on a shaker for 5 days. The two plants in the same jar were inoculated with the same isolate. Each jar was replicated two times. There were two separate uninoculated controls, one supplied with nitrogen and the other without nitrogen. The inoculated plants and the uninoculated ones without nitrogen were supplied with N-free nutrient (Broughton and Dilworth, 1970) solution while the uninoculated N-control received N-free solution to which nitrogen had been added (Somasegaran and Hoben, 1985).

The experiment was set up in the greenhouse and the jars arranged in completely randomised design. The plants were supplied with their respective nutrient solutions regularly throughout the period of growth. They were harvested 35 days after planting and nodule number, nodule dry weight and shoot dry weight records taken. In the process the shoots were severed from their

roots at the collar, put in labelled envelopes and oven-dried at 80°C for 72 hours after which their dry weights were taken.

The mean shoot dry weight (\bar{x}) was used to calculate the Effectiveness index E given by the following relation:

$$E = \frac{\bar{x}_i - \bar{x}_{To}}{\bar{x}_{Tn} - \bar{x}_{To}} \times 100$$

where \bar{x}_i is the shoot dry weight of inoculated strain, \bar{x}_{To} that of the uninoculated control and \bar{x}_{Tn} that of the nitrogen control.

CRITERIA FOR GROUPING ISOLATES.	
Group	Criterion
Ineffective Isolate	Isolate with effectiveness index less than 50%
Moderately Effective Isolate	Isolate with effectiveness index between 50%-80%
Highly Effective Isolate	Isolate with effectiveness index more than 80%

3.5 **Inoculation of Soybean Genotypes with Bradyrhizobia Isolates.**

This experiment was conducted in the Bekwai soil series using three soybean types, namely, Bragg (a non-promiscuous genotype), TGX 1303 and Bengbie (which are promiscuous). A non-nodulating soybean genotype was included for estimating nitrogen fixed by the ^{15}N isotope dilution method (Fried and Middelboe, 1977.)

Plastic pots used for the experiment were each filled with 1.2 kg of the sieved soil sample.. Seven bradyrhizobia isolates were used, five of them being indigenous isolates obtained from the screening experiment while the other two (J2 and J23) were standard tropical isolates received from Thailand. All the isolates were first streaked onto YEM agar plates and incubated for 3 days after which they were transferred into sterile YEM broth in culture bottles and grown aseptically (Vincent, 1970) at 28°C on a wrist-action shaker for 5 days.

The seeds were carefully selected by hand sorting to obtain viable, undamaged, clean seeds of reasonably uniform sizes and colour for planting so as to reduce as much as possible variability among the seeds. They were surface sterilised by immersion in 70% alcohol for 3 minutes and rinsed with 6 washes of distilled water. They were also immersed in 0.1% mercuric chloride for 3 minutes and rinsed with 6 washes of distilled water and then allowed to imbibe water by soaking in distilled water for 1 hour and then rinsed twice. Pre-germination was done by transferring the seeds aseptically onto 1% (w/v) water agar and incubated at 28°C for 3 days,

after which they were planted four plants per pot. They were thinned out to two plants per pot after germination. A micropipette (dispensette) was used to dispense 1ml culture of each isolate and inoculated onto each plant. The control plant for each genotype however did not receive any inoculation. Each treatment was replicated three times, and the pots arranged in a split-treatment design in the greenhouse. ^{15}N -labelled ammonium sulphate fertilizer was dissolved in sterile distilled water and applied to all treatments at a rate equivalent to 10 kg N/ha. This was done in three split-applications at 1 week, 3 weeks and 5 weeks after planting. The plants were watered daily with sterile distilled water and harvested 7 weeks after planting. All the shoots were severed from the roots around the collar, put into labelled envelopes and oven-dried at 80°C for 72 hours after which their dry weights were taken. The roots were washed thoroughly to get rid of adhering soil using a gentle stream of water from a hose. Nodules per plant were counted and recorded, then carefully wrapped in aluminium foil, oven dried at 80°C for 48 hours, and their dry weights taken.

3.6 Soybean Response to Inoculation and Nitrogen Fertilizer Application.

This experiment was conducted in Bekwai and Adenta soils. Three soybean types were used for the study; they were Bragg, TGx 1303 and Bengbie. In addition a non-nodulating soybean isolate was included as a reference crop. One isolate (isolate 38) was used to inoculate all treatments except the non-nodulating genotype, which was not inoculated. Half of the treatments received ^{15}N -labelled ammonium Sulphate fertilizer at a rate equivalent to 10 kgN/ha. The rest received ammonium sulphate fertilizer enriched by ^{15}N -atom excess at a rate equivalent to 100kgN/ha.

Table 3.2 *PHYSICOCHEMICAL PROPERTIES OF THE SOILS USED FOR THE INOCULATION STUDIES.*

SOIL SERIES	Bekwai	Adenta
CLASSIFICATION		
LOCAL	Red Forest Ochrosols	Savanna Ochrosols
USDA	Rhodic Paleudult	Typic Paleustalf
MATERIAL	Phyllite	Quartzite schist
pH	5.6	4.6
ORGANIC CARBON (%)	2.86	0.84
TOTAL N (%)	0.21	0.058
AVAILABLE	5.1	3.47
TOTAL P (mg/kg)	173	120

Isolate 38 was first streaked onto YEM agar plate and incubated for 3 days after which it was cultured aseptically at 28°C on a wrist-action shaker for 5 days in culture bottles containing YEM broth. The seeds were carefully hand-sorted to obtain clean viable seeds of uniform size and colour for the study. They were surface-sterilised by immersion in 70% alcohol for three minutes and washed in 6 changes of distilled water; they were also immersed in 0.1% mercuric chloride for 3 minutes and rinsed with 6 washes of distilled water. They were made to imbibe water by soaking in distilled water for 1 hour and then rinsed twice with distilled water. They were then transferred aseptically onto 1% (w/v) agar plates and incubated at 30°C in an incubator for 3 days. Four of the pre-germinated seeds were planted in each pot and

subsequently thinned to two per pot after they had fully germinated. Each treatment was replicated three times, and the pots arranged in a split-treatment design in the greenhouse. A dispensette was used to dispense 1ml of the culture of bradyrhizobia isolates and used to inoculate each plant. The two different rates of fertilizer were applied to their respective treatments by dissolving the required quantities of ^{15}N -labelled ammonium Sulphate fertilizer in sterile distilled water and applying them in 3 split-applications at 1 week, 3 weeks and 5 weeks after planting. The plants were watered daily until they were harvested 7 weeks after planting. All the shoots were severed from their roots around the collar and put into labelled envelopes, oven dried at 80°C for 72 hours, after which their dry weights were taken. The roots, contained in a sieve were washed thoroughly to get rid of the adhering soil, using a gentle stream of water from a hose. The nodules per plant were counted and recorded, carefully wrapped in aluminium foil, oven dried at 80°C for 48 hours, and their dry weights taken.

3.7 ^{15}N -Analysis

After the determination of the shoot dry weight, the plant shoots in the two previous experiments were milled and used for ^{15}N -determination. A little quantity (550 mg) of each of the milled samples was weighed into labelled Kjeldahl flasks. A loopful of selenium reaction mixture (catalyst) was added to each sample and mixed thoroughly. Eight millilitres of concentrated sulphuric acid were added to each sample and shaken vigorously to ensure that the samples were properly mixed with the acid.

The samples were digested using the digester for about 2 hours. About 5ml of distilled water

was then added to each sample in the flask followed by the addition of two drops of Tahiru indicator. Meanwhile 10ml of 0.1N hydrochloric acid had been discharged into conical flasks and labelled the same way as those of the Kjeldahl flasks. Two drops of Tahiru indicator were also put into each flask containing the hydrochloric acid. The digest was distilled and the gas that evolved collected into the 0.1N HCl. A back titration of 0.1N NaOH against the distillate was done and the volume of the acid for the back titration recorded for the determination of total nitrogen. The sample was vapourised using the evaporator until just about 2 ml or so was left. This was stored safely in a capped tube and kept in a refrigerator. The actual ^{15}N readings were done using the Emission spectrometer (NOI-6e) (Fieldler and Proksch, 1975).

3.8 Counting of rhizobia

The most probable number (MPN) method (Vincent 1970) also called the plant infection count was used to assess the rhizobial populations capable of infecting soybean in the eight soils used for the screening experiment.

The promiscuous soybean variety, Bengbie; was used for the enumeration. The seeds were carefully hand-sorted to obtain clean viable seeds of uniform size and colour for the study. They were surface-sterilised by immersion in 70% alcohol for 3 minutes and washed in 6 changes of distilled water and in 1% mercuric chloride for 3 minutes and subsequently washed in six changes of sterile distilled water. They were then allowed to imbibe water by soaking in distilled water for 1 hour and then rinsed twice (Somasegaran and Hoben, 1985). They were thereafter transferred aseptically onto 1% (w/v) water agar and incubated at 30°C until the radicles were 2cm long. The seedlings were planted in sterilised growth pouches, at two plants per pouch. The pouches used in the exercise were made of transparent heat-resistant polythene

(16 by 18cm) with paper wick liners (Somasegaran and Hoben, 1985). Each pouch contained N-free nutrient solution (Broughton and Dilworth, 1970). Tenfold dilutions of each soil suspension with four replicates per dilution were used to inoculate the pouches at 1ml per pouch. When the plants were 5 days old the growth pouches were reorganised and randomised on wooden racks and kept in the greenhouse. The plants were observed periodically and the nutrient solution replenished as and when necessary. Signs of nodulation were evident after 2 weeks. Twenty-eight days after inoculation, nodulation was assessed and the most probable numbers of rhizobia calculated (Vincent, 1970).

CHAPTER FOUR

4.0 RESULTS

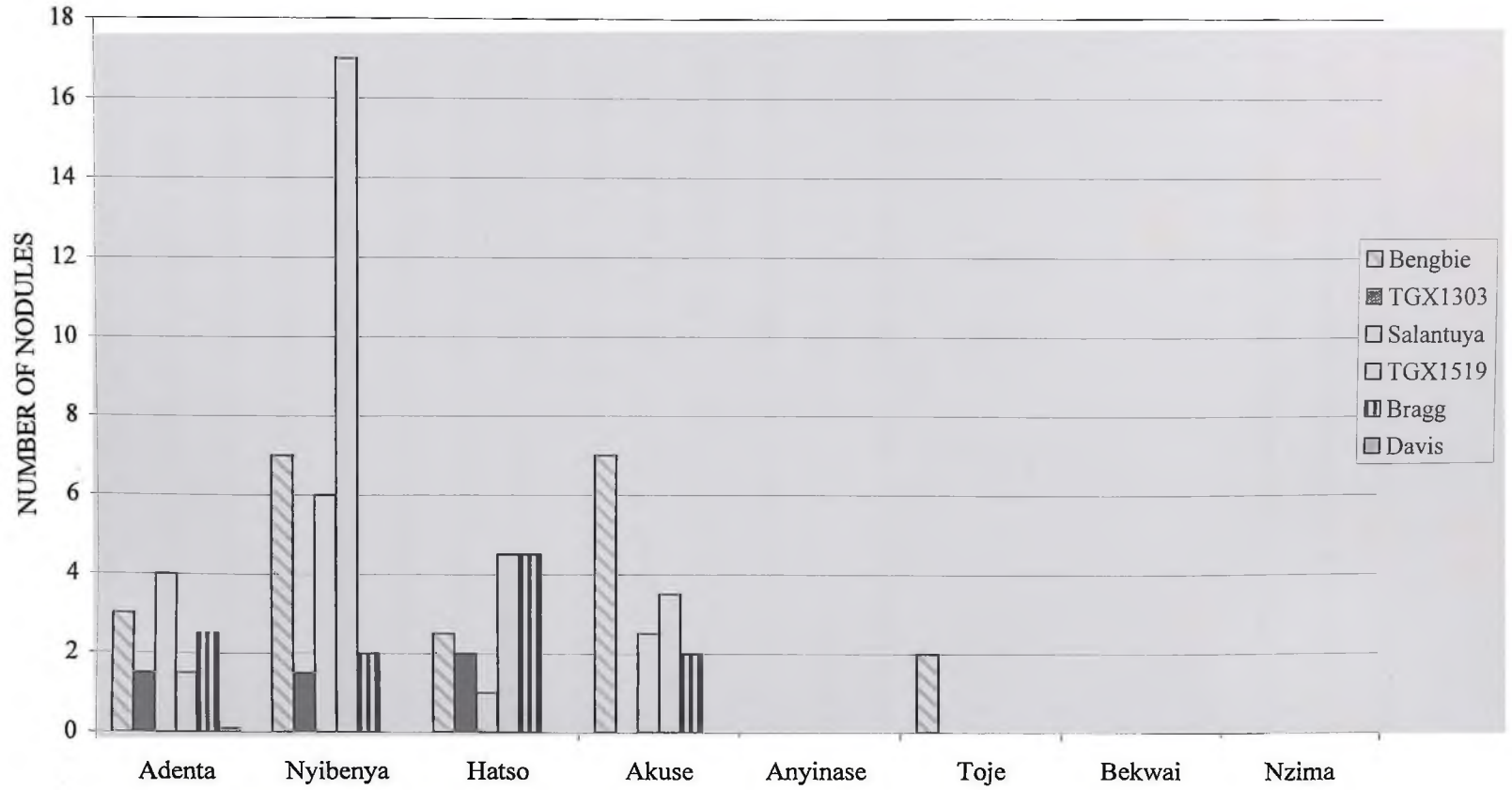
4.1 Nodulation potential of six soybean cultivars in eight Ghanaian soils.

. The results of studies carried out to assess the nodulation potential of six soybean cultivars in eight soils without inoculation showed variable nodulation potential of the cultivars in the soils studied (Fig 4.1). With the exception of Bengbie which nodulated with naturally occurring rhizobia in about 63% of the soils under study, the rest of the cultivars nodulated in 50% of the soils.

On the whole, TGx 1519 gave the highest average number of nodules per plant (Fig 4.1) which represented 36% of the total number formed by all the six cultivars, with Davis recording the lowest nodulation, as low as 0.009% per plant on average.

Considering soils, the highest nodulation occurred in Nyigbenya, with an average. of 11 nodules per plant. Nodulation in Nyigbenya soil series alone represented 44% of the total nodulation in the six soils under study. There was no nodulation in Anyinase, Bekwai and Nzima soil series, while only one cultivar, Bengbie, nodulated in the Toje soil series. The Adenta soil was the only one in which all the cultivars nodulated and thus also, the only one in which Davis formed nodules.

Fig. 4.1 NODULATION OF SOYBEAN IN EIGHT GHANAIAI SOIL SERIES



4.2 Estimation of population of indigenous soybean bradyrhizobia.

The number of naturally occurring rhizobia by the MPN count is presented in Table 4.1. The average population densities of indigenous bradyrhizobia capable of nodulating the six soybean cultivars in the eight soils indicated that soil had a great influence on the abundance of soybean bradyrhizobia. While extremely few bradyrhizobia were detected in Nzima, Bekwai, Toje and Anyinase soils, the remaining four soils contained more than 2×10^3 bradyrhizobia cells per gram soil with the highest bradyrhizobia count of 8×10^3 /g soil occurring in the Adenta soil and the lowest, 3.2×10^1 /g soil, in Nzima soil. With the exception of Toje, there appeared to be a relationship between the population of soybean bradyrhizobia and the ecozones under which the soils developed. This is based on the fact that the other soils which contained less than 1000 bradyrhizobia (cell/g soil) were all not capable of supporting nodulation in soybeans. A more interesting observation however was the fairly substantial bradyrhizobia count recorded in Anyinase, even though none of the soybean genotypes nodulated in that soil during the screening studies.

Table 4.1 Population of bradyrhizobia (cell/g soil) nodulating soybean.

<u>Soils</u>	<u>Bradyrhizobia Count</u>
Adenta	6000
Nyigbenya	5600
Hatso	4000
Akuse	4000
Anyinase	700
Toje	700
Bekwai	45
Nzima	32

4.3 Selecting Effective Strains of Soybean Bradyrhizobia for Nitrogen Fixation

Symbiotic effectiveness was determined using 60 isolates randomly selected from the screening experiment and the results presented in Tables 4.2 and 4.3. The results indicated variation

among the isolates in terms of nodule numbers formed on soybean as well as in symbiotic effectiveness. The estimated effectiveness values relative to the uninoculated but N fertilized control ranged from 0% to 144.6%. The isolates were categorised into effective, moderately effective and ineffective based on their effectiveness index with almost two-thirds (65%) of the isolates being classified as ineffective (Fig 4.2).

Table 4.2. Effectiveness grouping of 60 soybean isolates selected from the screening experiment.

Effectiveness Groups	Percentage of Isolates	Identification Nos. of Isolates
Highly Effective	15%	38,119,118,11,129,25,102,23, 39
Moderately Effective	20%	98,8,12,175,164,42,21,112,10 7,168,22,122, & 177
Ineffective	65%	57,174,79,47,75,40,10,153,10 5,128,41,169,114,143,1,5,167, 161,9,101,80,106,178,45,91,1 26,104,179,116,166,82,103,14 1,117,144,158,44, & 13

Table 4.3 Effectiveness indices of 60 soybean rhizobia isolated from some Ghanaian soils.

<u>Isolate</u>	<u>Effective index</u>	<u>Shoot dry wt. of soybean/plant(g)</u>	<u>Nodule dry wt./plant (mg.)</u>
38	144.6	3.14	252
119	97.8	2.28	172
118	96.7	2.26	220
11	93.5	2.2	122
129	91.3	2.16	84
25	89.1	2.12	225
102	84.8	2.04	241
23	83.7	2.02	152
39	81.5	1.98	210
98	77.2	1.9	85
8	75.0	1.86	151
12	75.0	1.86	103
177	73.9	1.84	206
175	73.9	1.84	81
164	72.3	1.8	136
42	64.1	1.66	156
21	58.7	1.56	105
112	57.6	1.54	90

<u>Isolate</u>	<u>Effective index</u>	<u>Shoot dry wt. of</u> <u>soybean/plant(g)</u>	<u>Nodule dry wt./plant</u> <u>(mg.)</u>
107	54.2	72	64
168	52.2	1.44	103
22	51.1	1.42	103
57	45.7	1.32	70
174	43.5	1.28	105
79	41.3	1.24	64
47	41.3	1.24	100
75	34.8	1.12	36
40	32.6	1.08	63
10	31.5	1.06	62
153	29.3	1.02	85
122	28.3	1.0	77
105	27.2	0.98	86
128	26.1	0.96	64
41	25.0	0.94	72
169	23.9	0.92	93
114	22.8	0.9	64
143	20.7	0.86	42
1	18.5	0.82	48
5	18.5	0.82	35
167	17.5	0.8	51

<u>Isolate</u>	<u>Effective index</u>	<u>Shoot dry wt. of</u> <u>soybean/plant(g)</u>	<u>Nodule dry wt./plant</u> <u>(mg.)64</u>
161	14.1	0.74	77
9	12.0	0.7	65
101	12.0	0.7	41
80	12.0	0.7	35
106	10.9	0.68	69
178	10.9	0.68	52
45	9.8	0.66	74
91	7.6	0.62	86
126	7.6	0.62	31
104	6.5	0.6	62
179	6.5	0.6	22
116	5.4	0.58	17
166	5.4	0.58	20
82	4.3	0.56	15
103	4.3	0.56	15
141	2.2	0.52	19
117	1.1	0.5	36
144	0.0	0.48	18
158	0.0	0.48	11
44	0.0	0.48	15
13	0.0	0.48	10

Fig 4.2 **Sample of soybean plants used for effectiveness studies**



I= Nitrogen control

II, III and IV = Inoculated plants

V = Uninoculated control

Generally, effectiveness index ranking seemed to correspond well with shoot dry matter yield, however the same could not be said of nodule dry matter. The most effective isolate (isolate 38) according to the ranking produced almost 10 times as much dry matter as the lowest ranked isolate (Isolate 13).

4.4 Cross Inoculation Groupings of Some Selected Legumes.

The abilities of indigenous soybean bradyrhizobia to form symbiotic relationships with two other commonly cultivated legumes in Ghana, cowpea and groundnut were examined to determine their level of compatibility and promiscuity. The proportion of the soybean isolates that nodulated these two legumes was high, with 88.3% nodulating cowpea and 80% groundnut (Table 4.4). This shows a relatively higher nodulation in cowpea compared with groundnut..

Table 4.4 Cross inoculation grouping of 60 soybean isolates with cowpea and groundnut.

	<u>Number</u>	<u>%</u>
<i>B. japonicum</i> isolates which nodulated soybean	60	100
<i>B. japonicum</i> isolates which nodulated Cowpea	53	88.3
<i>B. japonicum</i> isolates which nodulated Groundnut	48	80.0

4.5 Response of some selected soybean cultivars to inoculation.

Nodule number and dry weight.

With inoculation, all plants nodulated fairly well, with the highest number of nodules (44 nodules per plant) produced on Tropical Glycine cross (TGx-1303), inoculated with isolate J23 (Table 4.5). However, there was no nodulation by the uninoculated plants. Nodule numbers seemed to have correlated well with nodule dry matter ($r = 0.7$), but the same could not be said of isolates J2 and J23 which in most cases produced many but rather tiny nodules. Also nodule dry matter seemed to correlate fairly well with % N-fixed ($r = 0.7$) as well as total N-fixed ($r = 0.7$) (Tables 4.5 and 4.6).

Shoot dry matter:

The shoot dry matter formed by Bragg, Bengbie and TGx inoculated with the seven selected soybean bradyrhizobia isolates is shown in Fig 4.3. Cultivar \times isolate interaction was significant ($P=0.05$).

Yield of inoculated Bragg plants was significantly higher than Bengbie and TGx in all case except when inoculated with isolates J23 and 122. For Bengbie, yield was improved by inoculation with all the seven strains. Similarly, yield increased by inoculation of TGx in all cases.

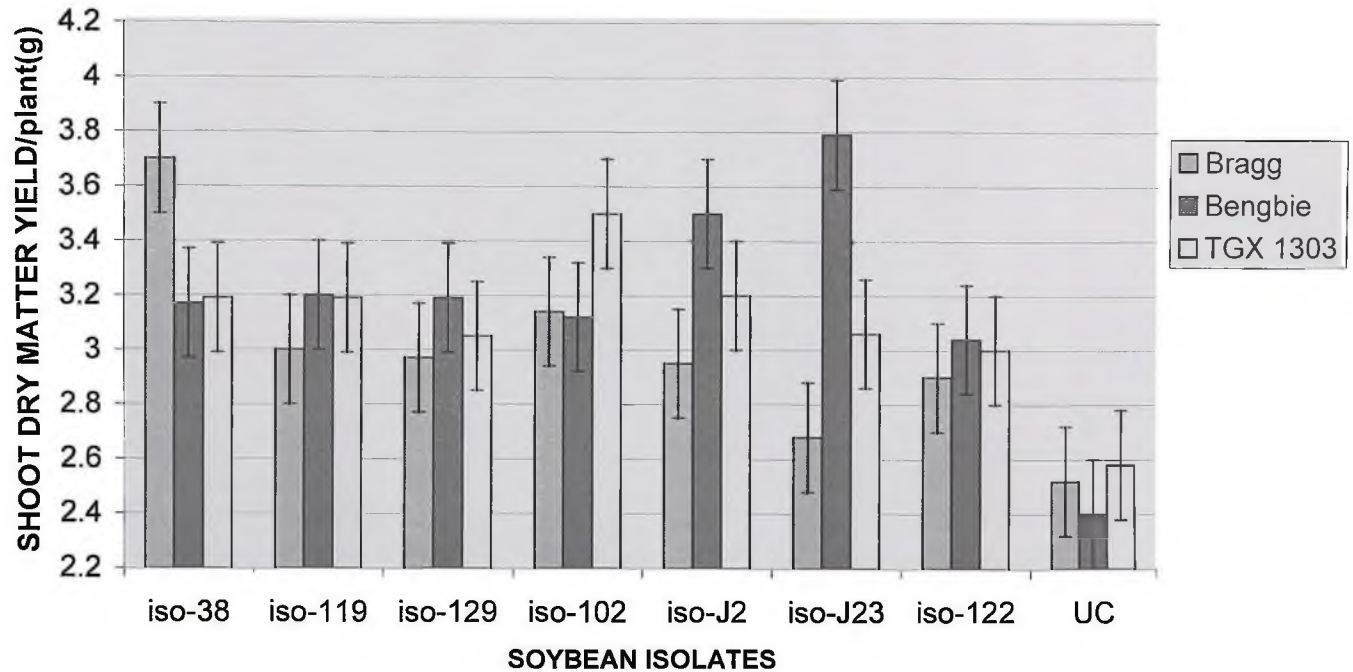
For Bragg, inoculation with iso-38 gave the highest yield, significantly different from the other isolates, between 21 and 42% higher. Differences between yields when inoculated with the remaining isolates were not significant, although inoculation with Iso-38 was the only one among them that gave higher yield than the uninoculated control.

Table 4.5 Number and dry matter of nodules formed by Bragg Bengbie and TGXInoculated with seven soybean bradyrhizobial isolates in Bekwai soil.

<u>INOCULUM/VARIETY</u>	<u>NODULE No/PLANT</u>	<u>NODULE DRY MATTER(mg/plant)</u>	
Isolate 38			
Bragg	33.0	86.7	e
Bengbie	17.5	48.8	
TGx	41.5	50.5	
\bar{X}	30.7	62.0	
Isolate 119			
Bragg	29.0	65.3	
Bengbie	14.5	32.0	
TGx	40.5	64.0	
\bar{X}	28.0	53.3	
Isolate 129			
Bragg	34.5	55.3	
Bengbie	18.5	35.5	
TGx	34.5	70.0	
\bar{X}	29.2	53.6	
Isolate 102			
Bragg	30.0	50.2	
Bengbie	12.5	26.5	
TGx	16.3	46.0	
\bar{X}	19.6	40.9	

<u>INOCULUM/VARIETY</u>	<u>NODULE No/PLANT</u>	<u>NODULE DRY MATTER(mg/plant)</u>
Isolate J2		
Bragg	39.0	32.0
Bengbie	26.5	49.8
TGx	35.5	77.0
\bar{X}	33.7	52.9
Isolate J23		
Bragg	36.5	33.0
Bengbie	36.5	40.5
TGx	44.0	55.0
\bar{X}	39.0	42.8
Isolate 122		
Bragg	17.5	41.5
Bengbie	15.0	40.5
TGx	16.0	55.0
\bar{X}	16.2	45.7
Uninoculated control		
Bragg	0.0	0.0
Bengbie	0.0	0.0
TGx	0.0	0.0
\bar{x}	0.0	0.0
LSD(0.05)	4.5	7.8

FIG 3 1NOCULATION EFFECT OF SELECTED SOYBEAN ISOLATES ON SHOOT DRY MATTER WT. OF THREE SOYBEAN CULTIVARS.



For Bengbie, Iso-J23 gave the highest shoot dry matter yield, which was significantly higher than plants inoculated with five strains, 38, 119, 129, 102, & 122, but not significantly different from that of Iso-J2.

For TGx, inoculation with Iso-102 gave the highest dry matter yield, and this was significantly higher than for plants inoculated with three of the strains, 129, J23 and 122, while the differences in yields were not significantly different when plants were inoculated with 38, 119 and J2.

On the whole, the best isolate for Bragg was 38, Bengbie J23, and for TGx 102, showing strain preference for each of the three soybean genotypes.

Total Nitrogen:

Generally the trend for total N per plant was similar to that of shoot dry matter (Table 4.6). With UC, Bengbie gave the lowest total N but this was not significantly different from TGx-1303. Total nitrogen produced by uninoculated Bragg was however significantly higher than that produced by Bengbie, but not TGx. There were no significant differences in total N among all varieties inoculated with 122 and 129, while with isolates J23, J2 and 102, Bengbie consistently produced highest and significantly different total N values from the other two cultivars. Bengbie responded greatly to inoculation, rising from the lowest when not inoculated to the highest

when inoculated with J23 J2 and 102. TGx on the other hand seemed to exhibit a reversal of that trend.

The total N results seemed to be consistent with the dry matter weight data. In both cases, all the isolates, except isolates 38 and 102, did not significantly improve the performance of Bragg (the non-promiscuous American genotype), while the same strains significantly improved the performance of the 2 promiscuous types.

Although Bragg produced the highest total N when uninoculated, with inoculation with seven strains, it produced significantly highest yield only with isolate 38, while the differences between Bengbie and TGx with this isolate were not significant. Comparing total N values of the various isolates with the UC, TGx and Bengbie gave significantly different amounts of total N with all isolates, the highest given by TGx inoculated with isolate 102, with higher total N value of 80% over the uninoculated control. TGx gave the highest total N with isolates 102 and 119.

Fixed Nitrogen:

Percentage N fixed was generally low and ranged from zero for the uninoculated treatments to 52.3%, with an overall average of 35.9% for the inoculated treatments (Table 4.6). Bragg appeared to be the best fixer; in four out of the seven inoculation treatments (with strains 38,119,102 & 122), it gave the highest % N fixed, besides giving the highest % N fixed value of 52.3% when inoculated with Iso-102. Percent nitrogen fixed by Bengbie was highest with strains J2 and J23, giving its highest % N fixed value of 43.1% when inoculated with Iso-J23. TGx on the other hand recorded the lowest %N fixed values, with values between 24.3 and 33.9%.

4.6 Effect of nitrogen fertilization on nodulation, nitrogen fixation and yield of Bragg, Bengbie and TGx in Bekwai and Adenta soils.

Nodulation:

Generally and in most cases plants grown in Adenta soil produced significantly more nodules than for the Bekwai soil (Table 4.7). All plants were fairly well nodulated with the overall nodulation being 15% better in Adenta than in Bekwai. Similarly, nodule dry matter, was higher in Adenta than Bekwai in all cases. The nodules in Adenta were generally bigger than in Bekwai (data not presented). Also, apart from Bragg and TGx fertilized at 10 kgN/ha whose nodule dry matter values were not significantly different, nodule dry matter values were significantly different among the cultivars in the rest of the cases.

At 10 kg N/ha rate of fertilizer application, nodule dry matter values were significantly higher than at 100 kg N/ha in both soils (Table 4.7).

Shoot dry matter:

In contrast to nodulation, shoot dry matter produced in Bekwai was significantly higher than in Adenta, with the highest produced by the non-nodulating (Non-nod) soybean plant at 100 kg N/ha fertilizer rate (Table 4.8). The increase in shoot dry matter of the non-nodulating soybean with the higher rate of fertilization was 185.7 % more than what pertained in Adenta. There were no significant differences in the shoot dry matter yield among the nodulating cultivars in Bekwai with the

Table 4.6. Inoculation effects of seven soybean Bradyrhizobium isolates on three soybean cultivars, Bragg, Bengbie and TGx in Bekwai soil.

<u>INOCULUM</u>	<u>/</u>	<u>TOTAL N/PLANT</u>	<u>%N FIXED/PLANT</u>	<u>N FIXED (mg)</u>
<u>VARIETY</u>				
Isolate 38				
Bragg		83.0	48.0	35.1
Bengbie		73.1	34.3	25.1
TGx		75.1	31.1	23.4
Mean		77.1	37.8	27.9
Isolate 119				
Bragg		66.4	36.8	24.6
Bengbie		61.5	33.5	19.0
TGx		74.6	28.2	21.1
Mean		67.5	32.8	21.6
Isolate 129				
Bragg		64.1	25.7	16.2
Bengbie		67.5	28.9	22.0
TGx		74.6	28.0	18.1
Mean		67.5	27.5	18.8

* Continued on next page

<u>INOCULUM</u>	<u>/</u>	<u>TOTAL N/PLANT</u>	<u>%N FIXED/PLANT</u>	<u>N FIXED (mg)</u>
<u>VARIETY</u>				
Isolate 102				
Bragg		67.3	52.3	35.2
Bengbie		63.7	36.8	23.3
TGx		87.3	33.9	29.7
Isolate J2				
Bragg		59.1	25.1	14.1
Bengbie		69.9	41.8	26.6
TGx		63.7	32.2	20.5
Mean		64.2	30.0	20.7
Isolate J23				
Bragg		61.3	31.4	19.3
Bengbie		74.3	43.1	30.3
TGx		63.8	25.9	19.0
Mean				
Isolate 122				
Bragg		60.2	34.3	20.6
Bengbie		59.6	26.4	13.6
TGx		62.3	24.3	15.1
Mean		60.7	28.3	16.4

* Continued on next page

<u>INOCULUM</u>	<u>/</u>	<u>TOTAL N/PLANT</u>	<u>%N FIXED/PLANT</u>	<u>N FIXED (mg)</u>
<u>VARIETY</u>				
Uninoc. control				
Bragg		59.8	0.0	0.0
Bengbie		48.4	0.0	0.0
TGx		55.9	0.0	0.0
Mean		54.7	0.0	0.0
LSD (0.05)		7.2	7.0	5.7

exception of Bragg at 100 kg N/ha rate, which was significant. The trend in Adenta was similar to that of Bekwai with only TGx producing significantly higher yield at the lower fertilizer rate over the higher rate. In both soils however the non-nods produced significantly higher yields than at 100 kg N/ha with more pronounced yield differences in Adenta.

Among the cultivars there were no significant differences observed in yield in both soils at both rates of fertilizer application except the non-nod, which was significantly lower than all the other cultivars in Adenta. Non-Nod gave significantly higher yield than TGX in Bekwai at 100 kg N/ha.

Nitrogen fixation:

Percent nitrogen fixed by plants in the Adenta soil (Table 4.6) was significantly higher than in Bekwai in all cases apart from Bragg fertilized at 100 kg N/ha rate. Percent N fixed was significantly higher at 10 kgN/ha rate than at 100 kg N/ha with the highest given by Bragg which registered over 2.5 times more %N fixation at 10 kg N/ha than at 100 kg N/ha in Adenta soil.

Table 4.7 Number and dry weight(mg)/plant of nodules formed by Bragg, Bengbie and TGx in Bekwai and Adenta Soils Fertilized with 10 and 100 kg N/ha.

Nitrogen Rate (kg/ha.)						
10 kg/ha.				100 kg/ha.		
	<u>Bekwai</u>	<u>Adenta</u>	\bar{x}	<u>Bekwai</u>	<u>Adenta</u>	\bar{x}
Bragg	25.5 (80)	33.0 (91)	29.0 (85.5)	7.5 (26.5)	7.5 (55)	7.5 (40.8)
Bengbie	22.0 (70)	34.5 (87)	28.0 (78.5)	8.5 (20.5)	12.5 (64)	10.5 (42.3)
TGx	34.5 (101)	25.0 (103)	30.0 (102)	12.5 (21.5)	15.0 (77)	14.0 (49.3)
\bar{x}	27.0 (83.7)	31.0 (93.7)		9.5 (22.8)	12.0 (65.3)	

LSD (nodule numbers) = 5.5

LSD (nodule dry wt.) = 7.8

Numbers in brackets represent nodule dry weight (mg/plant)

Table 4.8 Shoot Dry Matter Yield/plant (g) of Bragg, Bengbie and TGx in Bekwai and Adenta Soils Fertilized with 10 and 100 kg N/ha

	<u>10 kg/ha</u>			<u>100 kg/ha</u>		
	<u>Bekwai</u>	<u>Adenta</u>	\bar{x}	<u>Bekwai</u>	<u>Adenta</u>	\bar{x}
Bragg	3.07	1.92	2.5	3.45	2.1	2.8
Bengbie	3.27	2.0	2.7	3.42	1.89	2.7
TGX	2.99	1.8	2.4	3.2	2.14	2.7
Non-nod	3.00	1.05	2.0	3.58	2.14	2.9
\bar{x}	3.10	1.7		3.4	2.1	

LSD(5%): 0.31

Table 4.9 Percent Nitrogen Fixed by Bragg, Bengbie and TGx in Bekwai and Adenta Soils Fertilized with 10 and 100 kg /ha.

	Nitrogen Rate (kg/ha.)					
	<u>10 kg/ha.</u>			<u>100 kg/ha.</u>		
	<u>Bekwai</u>	<u>Adenta</u>	\bar{x}	<u>Bekwai</u>	<u>Adenta</u>	\bar{x}
Bragg	48.2	64	56.1	22.4	24.4	23.4
Bengbie	33.3	56.9	45.1	14.8	30.2	22.5
TGX	29.7	54.8	42.3	13.0	34.9	24.0
\bar{x}	37.1	58.6		16.7	29.8	
LSD (0.05)	5.07					

Among the cultivars Bragg gave significantly higher percent nitrogen fixed values than Bengbie and TGx; the differences in % N fixed between Bengbie and TGx were not significant. Soil \times cultivar \times fertilizer interaction for amount of N fixed was not significant. However the two-way interactions between fertilizer and soil, cultivar and soil as well as cultivar and fertilizer were all significant. Amount of N fixed by plants grown in both soils at 10 kgN/ha was significantly higher, being about two times more than the amount fixed at 100 kg N/ha (Table 4.9).

Percent nitrogen fixed in all the soybean cultivars was significantly higher in Adenta soil than in Bekwai

Differences in total N accumulated in the plants were not significant for soil \times fertilizer \times cultivar interaction as well as cultivar \times soil interaction. Cultivar \times fertilizer and fertilizer \times soil interactions were however significant. Total N accumulated in plants followed a similar trend as % N fixed (Table 4.10).

Table 4.10 Amount of fixed and total N accumulated by Bragg, Bengbie and TGx in Bekwai and Adenta Soils Fertilized with 10 and 100 kg N/ha .

	<u>Total Nitrogen/plant(mg)</u>		<u>Fixed Nitrogen/plant(mg)</u>	
	<u>Bekwai</u>	<u>Adenta</u>	<u>Bekwai</u>	<u>Adenta</u>
Bragg	75.1	40.0	26.4	17.4
Bengbie	76.6	36.3	18.5	15.8
TGx	75.8	40.7	16.1	17.8
Non-nod	66.8	29.3	0	0
LSD(5%)	6.2		LSD(5%) 3.05	
	$S \times C \times F = ns$	$S \times F = s$	$S \times C = s$	$C \times F = s$

Table 4.11 Total N accumulated by Bragg, Bengbie and TGx in Bekwai and Adenta soils fertilized with 10 and 100 kg N/ha.

	Bekwai		Adenta	
	<u>10 kg N/ha</u>	100 kg N/ha	<u>10 kg N/ha</u>	100 kg N/ha
Bragg	74.2	76.0	38.2	57.3
Bengbie	76.3	77.0	36.1	36.5
TGx	76.3	75.3	40.4	40.9
Non-Nod	63.1	70.6	20.5	38.1
X	74.5	74.7	33.8	43.2

LSD (0.05) = 8.7

$S \times C \times F = ns$

$S \times C = ns$

$S = \text{Soil}$ $C = \text{Cultivar}$ $F = \text{Fertilizer}$

$SF = s$

$C \times F = s$

$s = \text{Significant}$

$ns = \text{not significant}$

Total nitrogen in plants was generally higher in Bekwai than in Adenta, (86.4 to 128.2% higher) with the highest difference in total N in the plants recorded by non-nodulating plants and the lowest by TGx. In both soils there were no significant differences among Bragg, Bengbie and TGx, which were all significantly higher than values for the non-nodulating plants (Tables 4.10 and 4.11).

In Bekwai, total N values were not significantly different among the cultivars, which were significantly higher than the non-nodulating plants at 10 kg N/ha but not at 100 kg N/ha. In Adenta, total N values were also not significantly different among the cultivars which were all significantly higher (76.1-97% higher) than the total N values of the non-nodulating plants. At 100 kg N/ha however, Bragg gave significantly higher total N over the other cultivars, including the non-nod plants. There were no significant differences between Bengbie, TGx and Non-nod at the 100-kg N/ha rate. . Although soil \times fertilizer \times cultivar interaction was generally not significantly ($P < 0.05$), (Table 4.10) total N values in Bragg and Non-nod were significantly higher at 100 kg N/ha than at 10 kg.

Total N accumulated in the plants was significantly different in the two soils. Comparing total N at both fertilizer rates, there were significant significant differences between the soybean genotypes in Adenta but not in Bekwai. Nitrogen fixed was significantly different in the soils at 10 kg N/ha but not significant at 100 kg N/ha. In both soils N-fixed at 10 kg N/ha was significantly different and more than double the values obtained at 100 kg N/ha (Tables 4.10 and 4.11).

CHAPTER FIVE

5.0 DISCUSSION, SUMMARY AND RECOMMENDATION.

5.1 Introduction

Studies were carried out to assess the nodulation potential of six soybean cultivars in some uninoculated Ghanaian soil types, and to estimate the populations of naturally occurring bradyrhizobia present in the soils, and their efficiency in nitrogen fixation. Experiments were in addition performed to assess the response of soybean to bradyrhizobial inoculation and how this affects nitrogen fixation.

5.2. Nodulation potential of soybean in Ghanaian soils.

Nodule number and mass have often been employed for indirect assessment of nitrogen fixation (Weber, 1966; Westermann and Kolar, 1978).

The results obtained from the studies showed that of the eight soils, it was in only three of these that no nodules were formed by any of the soybean cultivars, and each cultivar was nodulated, at least in one of the soils. What was perhaps interesting was the fact that Bragg a non-promiscuous American soybean genotype, from which several supernodulators have been derived, nodulated considerably well in these soils, an observation contrary to some well established reports in the literature that non-promiscuous American soybean genotypes do not normally nodulate in tropical soils (Pulver *et al.*, 1978). However, nodulation of another non-promiscuous American genotype, Davis, was poor, which of course confirms the well-acclaimed view in the literature.

The most probable number (MPN) count was carried out to estimate the population of indigenous soybean bradyrhizobia present in the soils (Vincent, 1970), and also served as an indirect means of predicting whether or not soybean would respond to inoculation (Thies *et. al*, 1991).

The results obtained were indicative of variability of bradyrhizobia population in the soils. This is a confirmation of Abaidoo's (1997) observation that, bradyrhizobia population in African soils (by the MPN technique) was variable and ranged from 2×10^0 to 3.38×10^4 bradyrhizobia g^{-1} soil. Similar studies by Fening (1999) showed that average density of indigenous bradyrhizobia capable of nodulating cowpea varieties in Ghanaian soils varied within and between the localities for the study.

The fact that half of the soils, hitherto not cropped to soybeans, contained large enough population to enable them to infect soybean is not unusual since Vincent (1980) reported of the presence of rhizobia even in virgin soils. This, perhaps indicates that either bradyrhizobia occur naturally as part of the indigenous soil population or that other native legumes serve as hosts and thus inoculant sources of bradyrhizobia for soybean. However the fact that Bekwai and Nzima soils contained less than 50 rhizobia per gram of soil seems to confirm the view held by Cuttalan and Hungria (1994) that, where soybean has not been previously grown, there is generally a response to inoculation with bradyrhizobia especially for the non-promiscuous cultivars.

The results from the screening experiment and MPN studies seemed to suggest some relationship between nodulation and bradyrhizobial numbers, from the viewpoint that soils which supported nodulation generally had higher bradyrhizobia counts, while those in which nodulation did not occur had low bradyrhizobia count. This indicates that nodulation and nitrogen fixation of soybean can be improved significantly through inoculation with bradyrhizobia in different soils.

Counts of bradyrhizobia capable of nodulating soybeans depicted that half of the soils studied contained reasonably large enough populations to enable them nodulate soybean well. This view is arrived at based on Danso's (1992) assertion that population range of 10^3 to 10^4 rhizobia per gram of soil was by most standards adequate for nodulation to occur in food legumes. Interestingly, Anyinase which recorded zero nodulation during the screening studies had substantial bradyrhizobia count, giving the indication that in spite of the fair amount of bradyrhizobia population present in Anyinase, conditions prevailing in the soil may not have been conducive for symbiotic compatibility between the legume and the microsymbiont. It is suggested that factors such as low pH and aluminium toxicity might be partly responsible for the zero nodulation score in Anyinase. Agronomic practices such as liming may greatly improve the nodulation potential in Anyinase. Bekwai and Nzima soils had very low bradyrhizobia counts, which may explain why there was no nodulation in these two soils, giving the indication that a yield response to inoculation could be obtained in these soils.

5.3 **Cross inoculation.**

The cross inoculation group concept is based on the ability of rhizobia to specifically nodulate a limited group of legume host species (Fred *et. al.* 1932). It is based on this concept that *Rhizobium* species have been classified as promiscuous or specific. The concept was therefore applied in this study to determine the symbiotic specificity or otherwise of native *B. japonicum* obtained from the screening experiment.

The results showed that large number of indigenous soybean bradyrhizobia had the potential of nodulating cowpea and groundnut, two common legume genera cultivated in Ghana. The results suggest that these bradyrhizobia that nodulated soybean were not highly specific. The possibility that most of these bradyrhizobia belong to the *Bradyrhizobium* spp. rather than *B. japonicum* needs to be investigated. It would be interesting to do further work to establish, among other things, the relative effectiveness and competitive abilities of these isolates on these hosts.

5.4 **Symbiotic effectiveness.**

Symbiotic effectiveness is a primary factor for the determination of incidence and magnitude of legume response to inoculant production (Singleton and Travers, 1986, Thies *et. al.*, 1991)

The symbiotic effectiveness test was carried out to determine which of the indigenous isolates were efficient in nitrogen fixation. Symbiotic effectiveness of indigenous rhizobia is necessary for assessing need for inoculation, and also an important parameter for the selection of the strains for inoculant production (Singleton and Travers, 1986; Thies *et. al.* 1991).

The results indicated that only 15% of the isolates were highly effective relative to the uninoculated control, 20% were moderately effective and 65% ineffective. This was not a drastic departure from the normal distribution pattern reported in a similar study by Singleton and Stockinger (1982). Also the results indicated that soybeans were nodulated by bradyrhizobia strains which were largely ineffective and thus in nitrogen fixation. However, the fact that some considerable number of the isolates was effective means that the latter could, when selected, be of immense use for inoculation, nitrogen fixation enhancement and inoculant production. Even though this requires further work and information, if this goal is achieved, it stands to benefit from comparative advantage in the sense that once the isolates are of native origin, they are likely to adapt better to local conditions and may have a better competitive edge over imported inoculants. Further studies to assess what proportions of effective versus ineffective segments of the isolates are specific for soybean, or which of these belong to the cowpea miscellany and are only opportunistic nodulators of soybean would be interesting.

5.5 Response of soybean to inoculation

Nodulation is a primary requirement for nitrogen fixation in legumes, and particularly for a soil such as Bekwai where earlier screening studies had established no nodulation due to very low and rather inadequate bradyrhizobia count. The need to inoculate such soils cannot be over emphasized. Results obtained from the inoculation studies showed that all the inoculated plants nodulated and had fairly reasonably good nodule numbers while there was no nodulation in the uninoculated control, a first signal that perhaps inoculation had led to nitrogen fixation (Weber, 1966; Westermann and Kolar, 1978).

Although, generally, inoculation with any of the isolates produced significantly more shoot dry matter than the uninoculated control in all the three cultivars, the levels differed from cultivar to cultivar. In some instances, comparing the performance of the uninoculated control with the isolates in one cultivar there were no significant differences. The implication is that, even though on the whole inoculation led to improvement in shoot dry matter, the levels were not the same among the cultivars. Several reports have shown that nitrogen fixed by a *Rhizobium* strain is strongly influenced by the host plant (Graham and Rosas 1977; Hardarson *et. al.* 1984), and that nitrogen fixation supporting traits often vary among different hosts. For example, while Bengbie produced the highest dry matter with four isolates, the same could not be said of the rest of the isolates even with the same cultivar. The other cultivars had trends quite different from Bengbie. This is an indication of strain genotype interaction where the response for a strain was quite pronounced in some cultivars, others minimal, and yet others did not give significant responses. This suggests that, much as the bradyrhizobial isolate is considered to be very important in inoculation response, the plant genotype equally and significantly influences inoculation response, even though this consideration is often much overlooked (Pulver *et. al.* 1978; Awonaike *et. al.*, 1990). The three cultivars again showed differences in total percent nitrogen fixed as well as in amounts of nitrogen fixed. Again, the highest nodulation of each of the three cultivars was by a different strain, emphasising differences in strain preference to their host plants and specificity differences; a phenomenon which might be of prime importance in the screening and selection of bradyrhizobial strains for soybean yield improvement.

Another important consideration that emerged from the studies is that the two promiscuous genotypes seem to have responded to inoculation even better than the non-promiscuous

genotype (Bragg). This seems to be in contrast to well established opinion that non-promiscuous genotypes have more propensity for responding to inoculation in the tropics because native bradyrhizobia are not capable of infecting them; promiscuous genotypes in contrast are infected by native strains and hence may not readily respond to inoculation treatment (Rhodes and Nangju, 1979; Nangju, 1980). The results obtained are however in consonance with the observation made by Olufajo and Adu in 1992 that, significant responses could be obtained in promiscuous soybean genotypes on soils with low population of indigenous bradyrhizobia. . The fact that Bengbie, (a locally cultivated soybean genotype) responded greatly to inoculation, rising from the lowest when not inoculated to highest when inoculated with J23, J2 and 102; does not seem to conform to the view held by Pulver *et. al.* (1982) that, there is no significant response to inoculation by local soybean cultivars because of their incompatibility with *B. japonicum* strains and the presence of more compatible indigenous bradyrhizobia in African soils. However, inoculation response in TGx was very low, probably confirming the observation made by Kueneman *et.al.* (1984) that the marginal response of adapted soybean cultivars to *B. japonicum* inoculation was indicative of the fact that *Bradyrhizobium* spp were capable of maintaining high soybean yields without inoculation.

It has to be stated that the imported isolates from Thailand performed very well and were comparable to some of the local isolates, probably because, these Thai strains are also of tropical origin, and may thus easily adapt to the local conditions (Chowdhury, 1977; Awai, 1981). Perhaps this observation is a confirmation of the observation made by Olufajo and Adu (1992).

Finally, it might be worthy to mention here that, though there was some appreciable inoculation response which resulted in higher nitrogen fixation, the response levels seemed to be quite below expectation. Hence there might have been some environmental as well as unidentified factors, which may have hampered the realization of the full potential of the isolates. Temperature and antagonistic effects by some microorganisms might be culprit in this regard. Also according to Rennie (1982) N fixation of soybean globally is about 50%, therefore lower N fixed in the study may also be attributed probably to the high presence of ineffective rhizobia (65%) in the soils used for the studies, especially if the ineffective strains are highly competitive.

5.6 Response of soybean to inoculation and nitrogen fertility.

The advantage in using ^{15}N is in its ability to distinguish between sources of N, and thus makes it possible to distinguish the amount of fixed nitrogen in the plant from soil and fertilizer-derived N (Fried *et. al.*, 1983).

The response of soybean to nitrogen fertilizer as measured was the net effect of nitrogen uptake and nitrogen fixation over the growing period.

The fact that nodulation and percent nitrogen fixed were generally better in Adenta than in Bekwai at both rates of fertilizer application was not unexpected because of the presence of substantially more numbers of soybean bradyrhizobia in Adenta soil than in Bekwai. Nodulation was also better at 10 kg N/ha than at 100 kg N/ha of fertilizer application, which is in conformity with reports that high rates of inorganic N fertilizer inhibit or have a depressing effect on nodulation and subsequently nitrogen fixation (Hardarson *et. al.*, 1984; Rennie and

Kemp, 1983). In fact Carroll and Gresshoff (1983) explained that high external concentrations of nitrogen inhibit root infection by rhizobia.

N accumulation in Adenta was largely from fixation and not from soil, thus significant differences in total N and yield reflected variability in nitrogen fixing abilities of the soybean genotypes. However, for Bekwai, fixation was low, compared to uptake from soil and thus differences in nitrogen fixation had less variable effect.

At both rates of nitrogen application total nitrogen in Bekwai was higher than in the Adenta soil suggesting that perhaps soil chemical properties could have had a role to play in nitrogen accumulation and nitrogen fixation in the soybean plants. Adenta is known to be poor in terms of soil fertility (Table 3.2). Even though nodulation and percent nitrogen fixed were generally higher in Adenta, the actual amount of nitrogen fixed as well as total nitrogen accumulated in the soybean plants in Bekwai soils was higher than Adenta. This implies that BNF may not have been optimal enough to supply all the nitrogen needed in Adenta soil, in addition to the possibility of chemical fertilizer being more limiting in Adenta. On other hand, there could have been factors inherent in Bekwai that might have enhanced plant growth and hence total nitrogen fixed. Soil nitrogen, organic matter and phosphorus support dry matter yield, which are more favoured by Bekwai against Adenta (Table 3.2).

Observation of variability in cultivar response to inoculation, fertilization and perhaps soil effects is an indication that inoculation, fertilizer and soil response are influenced by plant genotype.

5.7

SUMMARY

Enumeration of bradyrhizobia capable of nodulating soybeans showed that half of the soils studied contained large enough populations to enable them nodulate soybeans well. This was confirmed by the screening test on soybean cultivars, where both promiscuous and non-promiscuous soybean genotypes were nodulated reasonably well by native soybean bradyrhizobial strains in these four soils.

Randomly selected native bradyrhizobial isolates from the screening studies used to cross nodulate groundnut and cowpea showed that large number of the native bradyrhizobia capable of nodulating soybean also had the potential for nodulating cowpea and groundnut, two commonly cultivated legumes in Ghana.

Effectiveness test performed on 60 randomly selected native bradyrhizobia isolates showed that 15% of the isolates were highly effective, 20% moderately effective and 65% ineffective. The inoculation studies gave an indication of improvement in nodulation, nitrogen fixation and improved shoot dry matter yield by the native and a few imported standard soybean bradyrhizobia strains from Thailand; with the native isolates performing as well as their imported counterparts. The promiscuous soybean genotypes seemed to have responded to inoculation even better than the non-promiscuous genotypes.

Nodulation and %N fixed were generally better in Adenta soil series than in Bekwai series at the two rates of fertilizer application, probably due to the substantially higher numbers of soybean

bradyrhizobia in Adenta soil than in Bekwai. Nodulation was also better at 10kg N/ha than at 100kg N/ha of fertilizer application in conformity with well documented reports (Hardarson *et al.*, 1984). Total N in plants grown in Bekwai was however significantly higher than in the Adenta soil, probably due to the fact that Bekwai is more fertile than Adenta.

5.8

RECOMMENDATION

The studies gave a general overview of the presence and behaviour of soybean bradyrhizobia present in some Ghanaian soils, and there is the need to do follow up studies to characterise bradyrhizobia into cowpea bradyrhizobia and soybean bradyrhizobia (*Bradyrhizobium japonicum*), and to study their performance in nodulation and nitrogen fixation in soybean, cowpea and other related legumes.

Further work needs to be done to ascertain among other things, the effectiveness and competitive ability of bradyrhizobia strains before they could be used for inoculant production. Also further studies to assess what proportions of effective versus ineffective segments of the isolates are specific for soybean or which of these belong to the cowpea miscellany and are only opportunistic nodulators of soybean would be worth looking at.

The levels of inoculation response and nitrogen fixation were quite lower than expected, due probably to environmental factors (such as temperature) and other soil factors including antagonistic effects of other soil microorganisms. There is also the possibility that the preponderance of ineffective strains in the soil may have impeded nodulation by the inoculant strains which calls for competition studies as mentioned earlier. The other factors also need to be investigated to further enhance the nitrogen fixing ability of soybean bradyrhizobia.

Finally, it is my expectation that the results and further work would provide the basis and impetus for inoculant production in Ghana and the West Africa sub-region as a whole to help alleviate the protein malnourishment prevalent in the aforementioned areas through the consumption of soybean and other soya-products.

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