EFFECTS OF *BRADYRHIZOBIUM JAPONICUM* INOCULATION AND NITROGEN FERTILIZER ON GROWTH, NODULATION AND YIELD OF SOYBEANS.

BY

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DECLARATION

I, Teresa Jane Kabura Mwangi, declare that this thesis is my original work and has not been submitted for a degree in any University.

Signed Mwangi

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Date April 22, 1994

This thesis has been submitted for examination with my approval as University Supervisor.

Signed JKeter

DR. J.K.A. KETER

Date April 22, 1994

This work is dedicated to my two lovely children, Stella and Tony.

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ABSTRACT

High crop production cost, which is a major constraint limiting agricultural production in Kenya, can be reduced by partial substitution of N fertilizers with biologically fixed nitrogen. This study was conducted to determine whether the transformation of atmospheric N_2 by Bradyrhizobium japonicum bacteria into forms readily available for use by soybean (Glycine max (L.) Merrill) can substitute for or supplement N fertilizer in soybean production. The effects of five levels of N (0, 20, 40, 80 and 160kgN/ha) and inoculation with NUM 504 and NUM 508 Bradyrhizobium japonicum strains on growth, nodulation and yield of Duiker and Sable varieties of soybeans were investigated under greenhouse and field conditions on Humic and Rhodic Ferralsols, respectively. The Humic and Rhodic Ferralsols had a mean N content of 0.17 and 0.2%, respectively at 0-20cm depth and were completely devoid of Bradyrhizobium japonicum bacteria.

Inoculation significantly ($P<0.001$) increased the shoot dry weights and plant heights of the plants grown in the greenhouse. Plants inoculated with NUM 508 in the field had significantly ($P=0.05$) higher percent N in the shoots and grains compared with the control. Nitrogen significantly ($P<0.01$) increased the percent N content of the grains. Interaction between N and inoculation with regard to grain yield was significant ($P<0.05$); NUM 508 produced the highest grain yield, i.e., 4413.67kg/ha at zero-N level, compared to 3846.20kg/ha for the uninoculated treatments at the same N level. Nitrogen at 20, 80, and 160kgN/ha increased the grain yields of non-inoculated plants compared to the zero-N level. The grain yields of zero, 20, 80 and 160kgN/ha of non-inoculated treatments were 3846.20, 4161.09, 4117.79 and 4066.33kg/ha, respectively. Increasing N levels decreased the grain yields of inoculated plants linearly. The grain yields of plants inoculated with NUM 504 and NUM 508 decreased from 4135.58 and 4413.67kg/ha at zero-N level to 3264.51 and 3238.42kg/ha at 160kgN/ha, respectively.

The results indicate that application of N decreases nodulation of soybeans. Inoculation is absolutely necessary for soybean nodulation. A significant ($P<0.05$) variety x strain interaction existed for nodule dry weights and grain size under field conditions. NUM 508 formed heavier nodules with Duiker variety than NUM 504 while the latter formed heavier nodules with Sable variety than the former strain. A similar trend was observed with grain size. NUM 508 produced bigger grains with Duiker variety than NUM 504, but the latter produced bigger grains with Sable variety than the former strain. Inoculation without N fertilizer increased the grain yields of soybeans but N fertilizer improved the grain yields of uninoculated soybeans only.

CHAPTER ONE

INTRODUCTION

Nitrogen constitutes about 78% of the atmosphere, yet it is one of the major elements limiting agricultural production in tropical soils which are generally highly weathered and deficient in nitrogen (Karanja and Kibunja, 1989). Consequently continuous nitrogen enrichment, either through addition of nitrogen fertilizer or by transformation of atmospheric N_2 into forms that are readily available to higher plants, is necessary for good crop yield on most of the soil types. Soybean (Glycine max (L.) Merrill) is one the crops whose grain yields can be improved either through addition of N fertilizer to the soil, atmospheric N_2 fixation or both. This crop contains considerably high nutritional quality protein and therefore assumes an important role as a potential source of low-cost, readily available protein in a developing country such as Kenya where animal protein is in short supply and quite expensive for low income people. Soybean is also an important oil crop and is considered an industrial crop but it can be equally well used directly as human food. Like other legumes, soybean roots develop nodules in the presence of an appropriate strain of Bradyrhizobium bacteria. The nodules contain millions of Bradyrhizobium bacteria which obtain their energy from the plant and inturn supply the plant with nitrogen which they fix from the atmosphere.

In Kenya small-scale soybean growing was started in the early sixties in Nyanza and western provinces (Njuguna, 1985). The crop was also grown for fodder by large-scale farmers on a limited scale in Trans Nzoia, Uasin Gishu, Nakuru and Laikipia districts. In 1982, the East African Industries had some interest in soybeans and commercial production by small-scale farmers, mainly for oil extraction, was successful. Variety evaluation has been going on in Kenya for quite sometime and the possibility of large-scale production has been demonstrated in Kisii district (Thairu and Shakoor, 1985). However, due to marketing problems, lack of processing and production technology, limited on-farm utilization, Kenya's high potential for soybean production has never been exploited. This is unfortunate because Kenya needs a lot of vegetable oil. In 1984 alone Kenya spent Ksh.250 million to import 65,000 tons of vegetable oil for the manufacture of kimbo and cowboy vegetable shortenings (Njuguna, 1985). As demand for vegetable oil and protein continues, Kenya farmers will have no alternative but to expand areas under soybean crop. In areas with bimodal rainfall, it is possible to produce two crops per year.

Soybean production in Kenya is slowly gaining momentum. The Ministry of Agriculture, Livestock Development and

Marketing has asked Kenya Seed Company to bulk seeds for farmers in order to alleviate shortage of animal feeds. Consequently, the Company has made a wide collection of germplasm mainly from other African countries and it is being screened at the Company's Elgon Down Farm in Kitale, Kenya. The East African Tanning and Extract company personnel are growing the crop on their farms in Uasin Gishu district. They use the grains to formulate animal feeds. Some farmers in Rift-Valley, Western and Nyanza provinces also undertake the growing of the crop and use the grains either for human consumption or for animal feeds.

In Kenya, research concerning nitrogen fertilization and inoculation of soybean has been minimal. Only two cases have been cited in the limited literature reviewed. Okalo and Zschernitz (1971) reported lack of significant responses of soybeans to nitrogen application while Keya et al. (1981) reported increases in soybean seed yield due to inoculation. There is need to further test and evaluate these findings by using different soil types under various ecological zones in Kenya before proper recommendations can be given to the farmers.

Currently, most of the farmers growing soybean apply nitrogen fertilizer to the crop. Kenya does not manufacture nitrogenous fertilizers or any other types of fertilizers.

Infact all fertilizers are imported at exorbitant prices, thus draining much needed foreign exchange. Therefore the need to carry out research geared towards efficient utilization of nitrogen by the crop and also consider biological nitrogen fixation as an alternative or supplementary means of supplying nitrogen cannot be overemphasized. Hence the importance of this study which has the following objectives.

(i) To determine the effect of nitrogen fertilizer on growth, nodulation and yield of soybeans.

(ii) To examine the effect of Bradyrhizobium japonicum inoculation on growth, nodulation and yield of soybeans.

(iii) To establish a starter dose of nitrogen that is compatible with Bradyrhizobium japonicum inoculation for soybean production.

(iv) To investigate the interaction between variety and commercially used Bradyrhizobium japonicum strains on soybean growth, nodulation and yield.

(v) To compare the effect of Bradyrhizobium japonicum inoculation and nitrogen fertilizer on soybean nitrogen content.

CHAPTER TWO

LITERATURE REVIEW

2.1. NITROGEN NUTRITION

Soybean derive N from the soil, from fertilizer and by fixation of atmospheric N through a symbiotic relationship with Bradyrhizobium bacteria. Presumably each of these three sources can supply the N required by the plant. Hughes and Herridge (1989) combined data on crop N, seed N and fixed N to calculate N balances. They found that potential gains of soil N were greatest under the non tilled Soybeans which was on average 80kgN/ha, while Soybean sown into a cultivated seed bed showed an average gain of 30kgN/ha. A yield of 1940kg/ha of grain obtained with non-nodulating Soybeans that received no N fertilizer proved that prairie soil in Iowa, USA can supply 116kgN/ha to a growing crop (Webber, 1966a).

In addition to the mineral N supplied by the soil, there is N_2-fixation by nodule bacteria. The influence of inoculum level on N_2-fixation showed that in 24 day-old plants, N percentage and total N were directly related to the numbers of rhizobia on the seed (Burton, 1976). Thus suggesting that these young plants were wholly dependent on N_2-fixation for their N needs. Nitrogen requirement of the plant increases towards flowering and pod setting stages, when N_2-fixation alone cannot be sufficient for crop development. This was demonstrated by Ryle et al. (1978) who grew Soybean from seed germination to seed maturation with two contrasting patterns of N metabolism. The plants were either wholly dependent on N_2-fixation or on abundant supply of NO_3-N but lacking root nodules. Plants fixing atmospheric N_2 assimilated less N than equivalent plants given NO_3-N. About 40 percent of the N was assimilated after N_2 fixation had ceased in nodulated plants. One of the conclusions drawn was that the symbiotic association fixed insufficient N for optimum growth.

Nitrogen fixation and soil N are not always adequate for maximum Soybean yields and addition of N fertilizer is sometimes worthwhile. Maximum yields and nodulation of Soybean grown in the field was obtained with application of 40kgN/ha (Shanidullah and Hossain, 1980). Although nitrogen is not generally considered a limiting factor with respect to Soybean growth, available evidence points to the fact that a small amount of applied N in association with effective symbiosis can result in the highest yields (Ohlrogge, 1960), but responses of Soybean to N fertilization have been very inconsistent. The large amounts of nitrogen required by Soybeans in the short growth period of 3-4 months places a high demand on the soil nitrogen and on nitrogen fixation. According to Kuwahara et al. (1986), about 8kg of N is required per 100kg of harvested material. This cannot be met simply through nitrogen fixation and soil nitrogen in a high yield situation. Data collected by Herridge and Brockwell (1988) suggested that soil nitrate and N_2 fixation were not always complementary in meeting the N requirements of the growing soybean crop. With the development of high yielding varieties, seed yield and protein content may be increased with nitrogen fertilizer (Ham et al., 1976). Thus the ability of nodulated soybean to fix atmospheric nitrogen does not make it independent of other sources of nitrogen even when symbiosis is fully effective.

In pot experiments, reduction of available soil-N by means of incorporation of straw reduced the dry matter production and N content of nodulated soybean plants (Norman and Krampitz, 1946; Pinck et al., 1946); however, nitrogen application increased their yield and N content. The concept involved here is one of competition for available soil N between soybean roots and micro-organisms decomposing the straw. However, no reduction in yield or N content of soybeans was detected by Englehorn et al. (1947), after incorporating 9t/ha of small grain straw under field conditions. This contradiction can be explained by the fact that root development is restricted in pots unlike in the field where plant roots explore greater soil volume.

Investigations of the fate of fertilizer nitrogen in the soil plant ecosystem were carried out under green house conditions using two soil types, namely, andosol and yellow soil (not classified) by Chiu and Yoshida (1986). Soybean plants grown on andosol derived 21 percent of the total plant N from fertilizer, 27 percent from soil and 50 percent from the air. The corresponding values in the plants grown on yellow soil were 29 percent from fertilizer, 48 percent from soil and 23 percent from the air. The amount of N_2-fixed was higher on the andosol than on the yellow soil due to the presence of efficient indigenous rhizobia populations on andosol soil. According to Zapata et al. (1987), the amount of N derived from N_2-fixation at physiological maturity accounted for 47 percent of the total N assimilated, while the contributions from soil and ^{15}N-labelled fertilizer accounted for 50 and 3 percent, respectively. The conclusion that easily emerges from the few quoted experiments is that the amount of N contributed from each of the three possible sources to a growing soybean plant is dependent on soil physical, chemical and biological properties.

2.1.1. Critical periods for nitrogen nutrition

The period just before flowering or onset of flowering is frequently named as the best time to apply N by those contending that the soybean crop needs more N than the soil and symbiosis commonly provide (deMooy, 1973). Iwata and Utada (1967) applied N at various stages of growth in outdoor sand cultures. Soybean yields were reduced most by withholding N during the period 2 to 3 weeks prior to flowering. They also observed that withholding N for one month prior to critical period or for any 2-week period after flowering did not lead to a large reduction on yield.

In field trials 150kgN/ha was applied to soybeans at various stages from flower bud appearance to pod set by Hoshi et al. (1978). Yields increased with delay in application of N and it was concluded that N should be applied just after flowering. Seed yield per plant of soybean cv. cutler 71 grown in the green house were increased with increase in N supply from 5 to 18meq of NO_3^-/liter in the nutrient solution during flowering (Brevedan et al., 1978), maintaining N supply at the higher level until maturity did not significantly increase numbers of pod and seed yield per plant. They obtained similar results in the field trials in which plants were given 168kgN/ha at the beginning or end of flowering, marked less effect was obtained with N application at the end of flowering. Top dressing of N at flowering stage enables increased but not maximum yields. To obtain maximum yields, a technique is needed for supplying combined nitrogen without causing a decrease in the activity of nitrogen fixation by the root nodules (Kuwahara et al., 1986).

2.1.2. Effect of nitrogen on growth and yield

Under tropical conditions, application of small amounts of N is needed for well nodulated soybean and cowpea in soils with low N status (Kang and Ayanaba, 1977). According to Pal and Nutman (1987), even promiscuous cultivars of soybeans are incapable of fixing enough atmospheric nitrogen symbiotically to achieve maximum yields and therefore require fertilizer N to be applied in splits at several growth stages. However, this was disproved later by Olufajo (1990) who found that seed yields were not influenced by N application in promiscuous soybean.

High N rates resulted in taller plants and higher root dry weight of soybean than low N levels (Essa and Dulaimi, 1985). But sometimes N fertilization increases only vegetative growth but not seed yield (Karlen and Hunt, 1985). Similar finding had been reported earlier by Ratner (1977) who even recorded a decrease in crude protein, delayed setting and ripening of pods due to mineral N fertilization.

Often, application of N increases seed yield and yield components. Seed yield, pod yield, number of pods and a 100 seed weight increased with increasing N supply (EL-Kady et al., 1982; Rabie et al., 1979). When 40kgN/ha was applied, grain yield increased by 27 percent compared with unfertilized treatments (Kang et al., 1985). Slightly higher N level (60kgN/ha) increased dry matter yields by 11-25 percent and seed yields by 37-40 percent in comparison with treatments receiving 20kgN/ha (Afza et al., 1987). Increasing nitrogen rates from zero to 80kgN/ha at planting continued to increase dry matter yields, leaf area and grain yield of soybean (Pasaribu et al., 1987). Haque et al. (1980) observed that uninoculated plants receiving no N showed N deficiency symptoms throughout the field trial, but application of 90kgN/ha corrected the N deficiency symptoms and significantly increased seed yield compared with the control treatments in which N was not applied.

The effects of 0, 30, 60 and 90kgN/ha on yield of soybean cv. Bossier were studied in the field by Jamro et al. (1990). They reported seed yield increases from 6.72-12.34g/plant as N rate increased from zero to 90kg/ha. Number of branches/plant, plant height, pods per plant and seeds per pod also increased with N application. N fertilizer applied at the rates of zero to 120kgN/ha increased seed yield from 0.92 to 2.969t/ha (Sunarlim et al., 1980), however, maturity was delayed by higher N fertilizer rates. This was in agreement with the finding of Watanabe et al. (1983), who reported yield increment of 13.2 percent using same N rates, the yields increase was attributed mainly to an increase in 100 seed weight and pod number. Sorensen and Penas (1978) applied zero to 274kgN/ha to soybeans annually at 13 sites over a period of 3 years. They observed increase in yield at 9 sites. Where N application gave response, seed yield and seed size generally increased linearly with level of applied N, an indication that responses with even higher N rates would have been expected. From the rates of N used in the reviewed experiments, it appears that there is a possibility of increasing the grain yield of non-inoculated soybeans by application of N fertilizer.

Sometimes inoculation can make soybean crop independent of fertilizer N, Semu and Hume (1979) reported no yield increases in nodulated soybeans due to N application, an indication that N_2-fixation could support maximum yields. In inoculated nodulating isolines, application of N had little effect on seed yield per plant, while on non-nodulating soybean lines, N fertilizer increased seed yields (Deibert et al., 1979; Pal and Saxena, 1976). Nodulating and non-nodulating lines grown in sand culture with nutrient solution containing 12.5 to 400ppmN indicated that growth and seed yield of non-nodulating lines increased with increasing N concentration until they reached similar levels with nodulating lines at 400ppmN (Matsunaga et al., 1983). When zero to 80kgN/ha was applied as urea to non-inoculated plants, there was no significant difference in yield between the inoculated and uninoculated treatments (Boonchae and Schiller, 1978). This observation is an implication that nitrogen levels of 80kg/ha and below increased yields only in the uninoculated treatments.

The possibility of obtaining high yields of soybean by using nitrogen in conjunction with Bradyrhizobium inoculation has been demonstrated. Mahmoud et al. (1979) reported that inoculation plus 75kgN/ha resulted in higher nodulation and seed yields than 100kgN/ha applied alone. With 120kgN/ha Bishnoi and Dutt (1983) reported higher dry matter, seed size and seed yields in inoculated treatments compared with non-inoculated treatments receiving same nitrogen rates. According to Dubetz and Rennie (1980), application of N to non-inoculated soybeans from zero to 160kgN/ha increased the mean yields from 1.23 to 1.72t/ha; however, when inoculation was used the yields were 2.1t/ha at all rates of N. The likelihood of a companion crop benefiting from nodulated soybeans cannot be ruled out. This was demonstrated by Vasilas and Ham (1985) who grew soybean lines either alone or in 1:1 mixture and applied 10 or 100kgN/ha labelled with ^{15}N. Increasing the fertilizer rate did not increase yields of the mixture but increased the yields of the non-nodulating lines grown alone.

Inverse relationship of fertilizer N and inoculation has also been documented. Application of 100kgN/ha decreased the yields of nodulating soybeans (Vasilas and Ham, 1985). Nitrogen fertilizer at 120kgN/ha in conjunction with inoculation increased the yield of green and dry matter but decreased the activity of symbiotic N_2-fixation and yield of soybeans (Karyagin, 1980). Tracer experiments with $^{15}NH_4$-N in pot trials showed that as fertilizer N was increased, absorption of N from the atmosphere over a period of 40 days decreased (Yoshida, (1979). Findings of Allos and Bartholomew (1955, 1959) clarified the extent to which N_2-fixation can be reduced by application of N fertilizer. Low rates of mineral N under N-deficient conditions increased growth and N absorption more than they diminished N_2-fixation in solution cultures. When N was applied at the rate of 10-15 percent of the total soybean requirement, N_2-fixation contributed 83 percent of the total N. This percentage decreased to 42 when more N was supplied than absorbed by the crop.

2.1.3. Effect of nitrogen on nodulation and N_2-fixation

Nitrogen had no consistent effect on nodulation of a promiscuous soybean but there were significant inoculation and nitrogen interactions for nodule number and nodule dry weight at flowering (Olufajo, 1990). The inoculation and nitrogen interactions can be exploited in identifying strains that can tolerate high N levels. Low levels of N increased the number of nodules on tap and lateral roots, total number of nodules and fresh weight of nodules per plant while high N levels reversed the effects (EL-Kady et al., 1982). The trend was the same when 25kgN/ha was applied, symbiotic N_2-fixation by Bradyrhizobium bacteria remained high in nodulating plants while with 50kgN/ha, the proportion of total N due to fixation was reduced (Ruschel et al., 1979). Increasing nitrogen level to 30kgN/ha did not inhibit N_2-fixation (Lin, 1983). Afza et al. (1987) observed that the proportion of N in the plant derived from N_2-fixation was not affected by the application of 60kgN/ha but was reduced when N application totalling 80kgN/ha was added. Soybeans cv. William treated with zero to 160kgN/ha as $(NH_4)_2SO_4$ showed that low N levels resulted in more nodules per plant than high N levels. Increasing applied N decreased nodule fresh weight and dry weight (Essa and AL-Dulaimi, 1985). During intensive N_2-fixation over about 50 days, N_2-fixing activity of plants receiving no N fertilizers was 124.14μmolC_2H_4/pot compared with 45.65μC_2H_4/pot for those treated with N (Zhang et al., 1984).

Most of the data in the literature support a reduction in nodulation from any application of combined N. Nodule weight per plant decreased with increasing N application (Jamro et al., 1990; Matsunaga et al., 1983; Papastylianou, 1986). The addition of 20kgN/ha as ammonium nitrate depressed nodulation and N_2-fixation in soybeans (Dean and Clark, 1980). Residual effects of 60kgN/ha from NH_4NO_3 were a 3-fold reduction in nodule number, a 4-fold reduction in nodule fresh weight and a non-significant reduction in average nodule size (Tran and Hinson, 1977). Nitrogen rates from zero to 120kgN/ha decreased nodules from 9.8 to 4.7 per plant (Sunarlim et al., 1980). This was in concurrence with the finding of Bishnoi and Dutt (1980). The nodule number and weight continued to decrease with increasing N application up to 250kgN/ha (Abdalla et al., 1985).

While comparing the effect of zero and 280kgN/ha as NH_4NO_3, Hatam (1977) found that the application of mineral N decreased nodule dry weight. In the control treatments, average nodule dry weight was 0.842g plant while that of N-fertilized treatments was 0.044g/plant. In the following year, the trend was the same but the difference in nodule dry weight was slight. The control treatments had nodule dry weight of 0.075g/plant while the N-fertilized treatments had 0.007g/plant. A highly significant positive correlation existed between nodule weight and nodule activity. From this particular experiment, it seems that some Bradyrhizobium bacteria strains can form effective nodules even at high levels of nitrogen corresponding to 280kgN/ha.

Host strain specificity also influences the extent to which N_2-fixation is depressed by applied nitrogen. This was highlighted by Danso et al. (1987) in Greece where the amounts of N_2-fixed by Chippewa, Williams and Amsoy 71 varieties grown in the field were similar at 20 and 100kgN/ha levels when a nitragin Bradyrhizobium japonicum strain was used for inoculation. However, when a local strain was used for inoculation N_2-fixation was substantially depressed at the higher N fertilizer level in Chippewa and Williams varieties. This is an interesting observation that can be manipulated to realize high yields in commercial soybean production where nitrogen in conjunction with Bradyrhizobium inoculation has been proved necessary in promoting soybean yields.

2.1.4. Effect of different sources of combined nitrogen

A significant negative correlation between nodule nitrate concentration and nodule fresh weight of soybeans was reported by Streeter (1985), but such a correlation between nodule nitrite concentration and nodule weight or acetylene reduction was not found. He concluded that nitrite did not play a role in the inhibition of nodule growth and nitrogenase activity. In an attempt to explain how combined nitrogen, mainly in form of nitrate affects the nodulation of legumes, Allison and Ludwig (1934) proposed that the amount of carbohydrate available for nodule formation is decreased because it is used during the assimilation of combined nitrogen. However, nitrogen as ammonium sulphate, asparagine or urea did not affect initial nodulation even though they were assimilated in approximately the same amounts as nitrate-nitrogen (Gibson and Nutman, 1960).

Addition of 1-20ppmN in two forms to the nutrient solution of 10 day-old soybean plant decreased nodulation, N content and were more pronounced with N supplied in form of NO_3^- than NH_4^+ (Rayer, 1984). This concurred with the work done by Yoshida (1979) which showed that as fertilizer N was increased absorption of N from the atmosphere over a period of 40 days decreased. NO_3-N had greater effect than NH_4-N, particularly at later stages of growth.

Nitrogen fixation was irreversibly inhibited by 4mM of NH_4NO_3 and 4mM of NH_4Cl but only mildly and reversibly inhibited by 4mM of KCl (Imsande and Ralston, 1981). This finding illustrated that nitrogen containing salts are the ones that suppress N_2-fixation. Apart from the inhibitory effect of nitrogenous compounds in the rhizosphere on root infection with Bradyrhizobium bacteria and on the formation of nodules as suggested by Tanner and Anderson (1964), the activity of the bacteria even after nodules have been actively operating is presumably reduced when combined N is applied. In order to obtain maximum yields from soybeans, a technique is needed for supplying combined nitrogen without causing a decrease in the activity of nitrogen fixation by the root nodules. In the present crisis relating to shortage of nitrogen fertilizers, it is necessary to judiciously define the optimum amounts of inorganic nitrogen required to improve the yields of soybean in conjunction with Bradyrhizobium inoculation in different soil types.

2.1.5. Effect of N fertilizer on N content

In inoculated nodulated isolines, application of N had little effect on concentration or rate of accumulation of N, on the amount of N in the plant parts or the whole plant while in non-nodulating isolines, N fertilizer increased all of them (Deibert et al., 1979; Pal and Saxena, 1976). The findings suggested that in effectively nodulated soybeans N content is not affected by fertilizer N. On the contrary more recent work by Matsunaga et al. (1983) have shown that N accumulation at early pod filling increased with increasing N concentration for all nodulated cultivars. In the absence of Bradyrhizobium inoculation and native rhizobia in the soil, N fertilizer has been shown to increase total N content of the plant (Dean and Clark, 1980; Hatam, 1976; Pal and Norman, 1987).

According to Afza et al. (1987), application of 60kgN/ha increased N uptake in pods by 43 percent in comparison with treatments receiving 20kgN/ha. Using N rates of zero to 274kgN/ha, Sorensen and Penas (1978) observed an increase in plant N content at 7 out of 13 sites over a period of three years, which were associated with increase in seed size but not always with increases in yield. Sunarlim et al. (1980) reported an increase in total N in plants with application of zero to 120kgN/ha, although protein in seeds was not affected. But on the contrary Bishnoi and Dutt (1980) reported increased crude protein content of the seed using similar rates of zero to 120kgN/ha. Other researchers also found increased seed protein content with increasing N supply (EL-Kady et al., 1982; Kang et al., 1977; Watanabe, 1985).

2.2. SYMBIOTIC NITROGEN FIXATION

The symbiotic association of *Bradyrhizobium japonicum* and soybean shows great complexity in the infection process, nodule development, and host-strain specificities that determine the N_2-fixing capacity in the nodules (Vest et al., 1973). They attributed the distinct feature of the symbiosis to the appearance of the N_2-fixing enzyme nitrogenase in the *Bradyrhizobium* bacteria which neither the soybean plant nor the rhizobia alone can produce, only the symbiosis can induce the synthesis of nitrogenase. Dilworth and Parker (1969) suggested that genetic information for the nitrogenase system resides partly in the plant and partly in the *Bradyrhizobium* bacterium. According to Vest et al. (1973) complementation between the protein parts synthesized by the plant and by the *Bradyrhizobium* bacterium could explain the degree of effectiveness found in symbiosis of one plant with several Bradyrhizobium japonicum strains or of one strain with several different plants.

In two separate experiments, Kucey et al. (1988) reported significant increases in measurements of N_2-fixation by soybeans when inoculated with effective Bradyrhizobium japonicum strains. Statistical analysis showed that the combined variety-strain effect was responsible for most of the variation observed in all the parameters while nitrogen leve had no effect (Douka et al., 1986). The observation suggested that adapted cultivars properly inoculated can fix most of th nitrogen they need for high yields. This is of great economi importance for crops with high nitrogen fixing efficiency lik the soybeans.

Symbiotic nitrogen fixation plays an important role i soybean production. This has been demonstrated by Rahman an Sanoria (1990) who reported positive correlation of growt rate with N_2-fixation at flowering and the mid-pod formatio stage. Grain yields were found to increase with N_2-fixatio by Nishimune et al. (1983) on brown andosols which were low i N, but yield was more dependent on soil N on alluvial ar black andosols. N_2-fixation was significantly correlated wit grain yield of soybean.

2.2.1. Accumulation of fixed nitrogen

Under favourable temperature and moisture conditions, soybean seedlings have visible nodules at about 9 days after planting (Bergersen, 1958). Soybean plants grown in sand culture showed that the number of nodules increased from 40 days after sowing onwards, reaching peak values at early pod filling, 100 days after sowing (EL-Kady et al., 1982). When temperatures and moisture conditions are favourable N_2-fixation in the nodules begins at about 14 days after sowing (Hard et al., 1971). According to Imsande and Ralston (1981) N_2-fixation increased 100 fold during 3 week growth reaching $50\mu molC_2H_4/hr$. Awai (1981) reported nitrogenase activity of $12.056\mu molC_2H_4$ plant/hr for soybean plants inoculated with SN2(S) strain of Bradyrhizobium japonicum at 50 days after sowing. On the contrary, Chiu et al. (1990) observed that the N_2-fixation up to 36 days after sowing was negligible. Two pot experiments conducted earlier by Ruschel et al. (1979) had shown that by 35 and 55 days after sowing, hardly any N_2-fixation had taken place. This contradiction on the time of onset of N_2-fixation is probably due to varietal, temperature and moisture differences prevailing during growth period.

In pot and field experiments, N_2-fixation activity of nodulating isolines of soybean was determined at 10-day intervals during 20-114 days after sowing (Zang et al., 1984).

According to them, acetylene reduction assays showed that N_2-fixation was very low, as it was between 30 and 40μmolC_2H_4/pot at budding and flowering stages, it increased thereafter and reached a peak of greater than 140μmolC_2H_4/pot at seed filling stage. Well nodulated plants acquire most of their N during reproductive growth, thus regardless of the age of plants at the time of inoculation with Bradyrhizobium japonicum the highest rate of N_2-fixation occurred during the pod filling stage (Imsande, 1988). A similar observation was recorded by Nelson and Weaver (1980). According to them, the accumulation of N in pods was greatest during the first 17 days of pod filling which was 7.5kgN/ha daily without a net loss of N from the leaflets, an indication that N_2-fixation could meet the N needs of soybeans without mobilization of leaf N. The nodule activity increases with development of the plant, reaching a peak at pod filling stage and then declining sharply to almost nil at the end of seed filling stage (Hatum, 1977).

The contribution of N derived from the atmosphere by soybeans differed significantly with stage of development (Zapata et al., 1987). They observed that during the vegetative and flower initiation stages of growth, N_2-fixation was low (21%) only, then a rapid increase in N_2-fixation occurred thereafter and became substantial during periods of active sink development when greater than 65% of the plant N was accrued from N_2-fixation. They also noticed a decline in

N_2-fixation towards physiological maturity when only 56% of the plant N was derived from N_2-fixation. Garner et al. (1985) also recorded a decline in nitrogenase activity at R6 stage (i.e., pod containing full size beans at one or more of the uppermost 4 nodes). He suggested prevention of the decline in the nitrogenase activity at this stage as a breeding objective to improve nitrogen supply during seed production. According to estimates made from acetylene reduction data by Weber et al. (1971) more than 80 percent of nitrogen is fixed between flower and approaching green beans stage. Sixty five (65) percent of the total seasonal N_2-fixation occurred after N began to accumulate in the reproductive tissue (Israel, 1981), during reproductive development N_2-fixation met 83 percent of the reproductive tissue N requirement. The N contents in leaf lamina, petiole, stem and husk decreased while it increased in seed with age of soybean plants (Singh and Saxena, 1978), accumulation of total N in leaf lamina, petiole and stem increased up to 65 days after sowing and thereafter it increased in seeds.

2.2.2. Quantity of nitrogen fixed

The amount of symbiotically fixed nitrogen has been estimated by the technique of comparing the yield of N of nodulating and non-nodulating genotypes on assumption that uptake by the two root systems and physiological behaviour are the same (Webber, 1966b), though the assumption is not completely valid. Other techniques commonly used include the measuring of nodular nitrogenase activity in the reduction of acetylene to ethylene (Sloger, 1969) and also by means of ^{15}N-labelled fertilizers (Allos and Bartholomew, 1955; 1959). Estimations based on both the N balance and ^{15}N dilution methods were not statistically different as the percentage of N derived from N_2-fixation was 41 and 33kgN/ha respectively (Chiu et al., 1990). N_2-fixed estimated by acetylene reduction was less than 50% of that determined from plant uptake, which reached 311kgN/ha in the highest yielding trial (Bezdicek et al., 1978).

Under general conditions, N_2-fixed by nodule bacteria on soybean was estimated at less than 100kgN/ha, sufficient to produce a yield of 1.3t/ha (Yoshida, 1979). The estimated amount of N_2-fixed over five-year period was between 4 and 127kgN/ha (Nishimune et al., 1983). Higher amounts of nitrogen fixed was estimated by Abdalla et al. (1985) who found that soybean cv. Clark derived 210kgN/ha from N_2-fixation, indicating that 260kg fertilizer N would be needed for comparable yield by uninoculated plants. Almost similar amounts of fixed nitrogen were reported by Tsuru (1979) of two soybean cultivars which fixed 226 and 250kgN/ha respectively. Symbiotically fixed nitrogen in soybean studied by an acetylene reduction method was found to be 568.6kgN/ha in 120 days (Sodyakov et al., 1983).

The N status of soil influences the quantity of N_2-fixed. An average of 317kgN/ha was fixed when soybeans were planted on soil with low N while about 123kgN/ha was fixed on soil with high N content (Herridge, 1981). For plants grown in a greenhouse on nitrogen deficient soil, N estimates showed a high level of N_2-fixation, from 58 to 89% by N-difference method and 51 to 95% by ^{15}N enrichment method (Kohl et al., 1980). Results of a field experiment conducted on a fertile soil showed a modest level of N_2-fixation by both methods, for example 7.3 to 51% was estimated by N-difference method and 5.4 to 46% by ^{15}N enrichment method.

Variety effect has also been shown to influence the quantity of N_2-fixed. N assimilation of soybean cv. Lee was compared with that of high yielding cultivars using non-nodulating isoline (N-N Lee) as reference plant at 10 locations over an 8-year period. Results showed that Lee soybean fixed an average of 172kgN/ha, while the highest yielding cultivar fixed 217kgN/ha (Thurlow and Hiltbold, 1985). ^{15}N dilution measurements of N_2-fixation showed levels ranging from 32 to 161kgN/ha, depending on host cultivar, bacterial strain and location (Kucey et al., 1988). This study showed that inoculation of soybeans with effective Bradyrhizobium japonicum strains can result in significant increases in yield and of N content through N_2-fixation.

2.2.3. Effect of inoculation on dry matter and yield

Soybean is a new crop in many areas of the tropics, and it is therefore necessary that the seeds be inoculated with *Bradyrhizobium* bacteria where no soybean crop has previously being grown in the field (Kang, 1975). This idea was supported by Semu and Hume (1979) who showed that inoculation increased seed yields only when soybeans were introduced into new areas in Ontario, Canada. Responses to inoculation are expected in soils in which the specific rhizobia are absent or sparse, where indigenous rhizobia are ineffective or partially effective in N_2-fixation (Vincent, 1965). This was recently supported by Volkora and Chernova (1988) who found that the effect of inoculation decreased as the population of indigenous rhizobia in soil gradually increased .

Increases in yield of soybean due to *Bradyrhizobium* inoculation has bean reported (Kucey et al., 1988; Chowdhury, 1977) while Olufajo (1990) even reported increased seed yield and number of pods per plant of inoculated promiscuous soybean in 1 out of 3 years. In field trials, dry matter accumulation at all growth stages were higher in the inoculated plants than in the control (Liang et al., 1991; Rao and Patil, 1977). According to Essa and Dulaimi (1985) inoculated plants were taller and produced higher root dry matter weight than uninoculated plants. Inoculation of soybeans in two field experiments increased plant dry matter weight, seed yield and 100 seed weight in all the six cultivars used (Ibrahim and Mahmoud, 1989). They further noted that inoculation did not affect K, Na, and Ca content of plant tops but decreased the P content, P content was increased and Ca content decreased in seeds by inoculation but did not affect K and Na contents of the seed. They also found that inoculated plants were capable of absorbing sufficient quantities of micronutrients.

While trying to ascertain the effectiveness of 28 strains of Bradyrhizobium in N_2-fixation for use in commercial inoculants Volkova and Chernova (1988) found that soybean grain yield increased by 6-10 percent. In Thailand, inoculation of soybean cv. 552 and SJ4 increased yields by 20-26 and 17-18 percent respectively (Chomchalow, 1981). In a field never previously cropped with soybeans inoculation increased seed yield by 65 percent over uninoculated treatments (Pasaribu et al., 1987). Inoculation of soybean seeds with 5 Bradyrhizobium strains increased seed yields by 26-77 percent in 1974 and by 10.5-28 percent in 1975 (Singh and Tilak, 1977). Higher percentage due to inoculation was reported by Nangju (1980) who recorded increased plant height of 36-47 percent and seed yield of 40-97 percent.

The selection of an effective strain of Bradyrhizobium is very important as yields may be doubled by inoculations (Leng and Whigham, 1974). In trials in India, seed yield averaged 3.48t/ha over 3 years after inoculation with nitragin strain of Bradyrhizobium japonicum compared with 1.76t/ha in the untreated control. Inoculated soybeans cv. Clark 63 yielded 1.86t/ha of seeds compared with 1.34t/ha in the uninoculated control, and cv. Williams on a different site yielded between 2.3-2.7t/ha when inoculated compared with 0.96t/ha in the control (Okon et al., 1979). Bezdicek et al. (1978) reported that the highest seed yields for inoculated treatments in two consecutive years were 3.5 and 2.5t/ha respectively compared with 1.0 and 1.2t/ha on uninoculated plots in Washington, USA. Seed yield of SJ1 and SJ2 cultivars of soybeans was increased by inoculation from 104 and 111kg/ha respectively to 732 and 417kg/ha on the Hangchat soil and from 1.17 and 1.67t/ha respectively to 1.52 and 2.13t/ha on the Yasothan soil in Northern Thailand (Boonchie and Schiller, 1978).

In Hokkaido, Japan previous use of Bradyrhizobium japonicum as an inoculant for soybeans had resulted in little beneficial response (Ishii, 1990). His study showed that survival of Bradyrhizobium japonicum was poor in most of the soils studied and therefore suggested improvement to the inoculation technique. The suggestions included use of high inoculum density to counteract antagonistic effects of rhizosphere micro-organisms. Addition of antibiotic to the inoculum to suppress competition from other micro-organisms and use of charcoal or powdered peat as an inoculant carrier to minimize contact of the inoculant with the soil. Researchers have already documented beneficial effects accrued from use of high inoculum density. According to Muldoom et al. (1980) seed yields increased linearly with rates of inoculant which were 0.25-1 times higher than the manufacturer recommended rates. Increasing rates of inoculant from zero to 15kg/ha hastened maturity and increased soybean yields from 3.294 to 3.529t/ha (Sunarlim et al.,1980). A similar trend was observed by Burton (1976) who reported that increasing the inoculum level from zero to 3000x10^3rhizobia/seed increased seed yield from 1.59 to 2.13t/ha.

Haque et al. (1980) found that uninoculated plants receiving no N showed N deficiency symptoms throughout the field trials and inoculated but unfertilized plants showed N deficiency during flowering owing to poor nodulation. Perhaps inoculation in conjunction with fertilizer N would have improved the situation. Yield increases due to inoculation have been amplified by use of fertilizer N. Abdalla et al. (1985) found that with inoculation, dry matter production of soybeans increased with increasing N applications up to 250kgN/ha from 4.89 to 5.57t/ha while dry matter yield of uninoculated plants was highest with 36kgN/ha at 4.82t/ha. In some cases inoculation alone has produced soybean yields equivalent to those produced by high rates of nitrogen. In field trials in Kenya, inoculation increased the seed yield of soybean from between 0.37 and 2.52 to 0.39 and 3.85t/ha depending on the site and was more effective than application of 90kgN/ha (Keya et al., 1981). In Cyprus, Papastylianou (1986) found that grain yield of inoculated plants was not statistically different from that of plants receiving 180kgN/ha, the yields for control, inoculated and fertilized plots were 3.7, 4.8 and 4.8t/ha respectively.

On the contrary,lack of yield improvement by inoculation has also been documented by some researchers (Dunigan et al., 1984; Howle et al., 1987; Nelson et al., 1978). The lack of response due to inoculation is attributed mainly to the presence of native effective strains of Bradyrhizobium japonicum in the soil (Abel and Erdman, 1964; Ham et al., 1971) and the cultivar and strain interaction (Caldwell, 1966; Caldwell and Vest, 1968). Tran and Hinson (1977) found no significant differences in seed yield of soybean while comparing non-inoculated control with inoculated treatments in a field where nodulated soybeans had not been grown for four years. These results show that sufficient Bradyrhizobium bacteria survived in the soil for a period of four years, during which reinoculation was not profitable. A significant decrease (3 percent) in seed yield due to inoculation has been reported by Dunigan et al. (1983), a scientific explanation of the decrease is not easy in such an instance. Presumably the soil N was high in the experimental site and therefore inoculation might have promoted excessive vegetative growth at the expense of the seeds.

2.2.4. Effect of inoculation on nodulation

In countries where nitrogen fertilizers are imported and the technology for manufacturing them is limited or too expensive to afford, a greater demand is being made on alternative and inexpensive sources of nitrogen. Some African countries such as Rwanda, Malawi, Egypt and Zimbabwe have turned to efficient exploitation of biological nitroger fixation by legumes in their farming systems in an attempt t cut down on fertilizer expenses. Nitrogen supply by the legumes is dependent on nodules being formed by a strain o *Bradyrhizobium* bacteria effective in N_2-fixation on that hos plant. Where such strains do not occur naturally in the soil they can be provided by inoculation of seed with selecte strains. Inoculation of soybean seeds with commercia inoculants of *Bradyrhizobium japonicum* in Iraq increased th number and dry weight of nodules (Essa and Dulaimi, 1985) This finding was supported by Papastylianou (1986) work don in Cyprus which showed that nodules were only formed c inoculated plants and their number varied with inoculant

Nodule leghaemoglobin content has also been found to increase with inoculation (Singh and Tilik, 1977).

Inoculation can also boost the nodulation of a promiscuous soybean, Olufajo (1990) reported that Bradyrhizobium inoculation consistently enhanced nodulation of a promiscuous nodulating soybean. Percentage nodule formation by strain 110 of Bradyrhizobium japonicum increased from between zero and 4 without inoculation to 14-40 with inoculation (Hunt et al., 1981). Inoculation rate was linearly related to the number of tap root nodules, total number of nodules and the weight of nodules per plant (Smith et al., 1981). Increasing the rate of inoculant from zero to 15kg/ha increased the number of nodules per plant from 1.0 to 13.5 respectively (Sunarlim et al., 1980)

Use of a commercial inoculant over a 4-year period in different locations gave no significant increases in nodule number, nodule dry weight and N_2-fixing activity (Dunigan et al., 1980). This concurred with the finding of Howle et al., (1987) who reported no differences due to inoculation in number and dry weight of nodules per plant in six soybean cultivars grown for 2 years. Conducting experiments on two sites, where soybean had not been grown for at least 15 years, Nelson et al. (1978) found no significant differences between nodule weight of inoculated and uninoculated plants.

Although soybean had never been grown previously at the two experimental sites, Chowdhury (1977) found nodule on uninoculated plants. Nodulation was improved by inoculation but in some cases nodule weight was decreased. While comparing uninoculated control with inoculated treatments Hinson (1977) found no significant differences in nodule number, size or position on roots of 14 day-old plants of soybean grown on a field where nodulated soybean had not been grown for 4 years. He hypothesized that sufficient *Bradyrhizobium* bacteria survived for four years, thus eliminating the need for re-inoculation.

2.2.5. Effect of inoculation on nitrogen content

Plant N content was increased by inoculation (Boonchee and Schiller, 1978). Inoculation with *Bradyrhizobium japonicum* produced a higher crude protein content than application of nitrogen (Ratner, 1977). In field trials inoculation increased the N content of roots, leaves and nodules of soybean at the flowering stage (Maskey and Bhanarai, 1981). In pot trials Liang et al. (1991) reported an increment in total N content by 380.6 and 318.6 percent in cv. Hefeng No.25 inoculated with 61A76 and E45 strains respectively. In field trials, N content at all growth stages were higher in the inoculated plants than in the control. .

Total plant N was 75kg/ha in non-nodulating and 7300 kg/ha in nodulating lines (Nelson and Weaver, 1980). However, Howle et al. (1987) reported no differences due to inoculation on nitrogen content of the plant. Contrary to what is expected Nelson et al. (1978) reported no significant difference on leaf N content due to inoculation of soybean on two locations where soybean had not been grown for 15 years.

2.3. HOST-STRAIN SPECIFICITIES

2.3.1. Soybean genotype responses

Bradyrhizobium japonicum is quite specific for soybean and while some strains of this species induce nodulation of other legume species, mainly cowpea, only a few of the Bradyrhizobium strains of the cowpea group induce nodule formation on selected soybean varieties (Leonard, 1923). Variation in nodulation responses between soybean genotypes and Bradyrhizobium japonicum strains are well documented. Data on average nodule per plant for 55 Glycine Max (l.) Merrill genotypes showed that all genotypes formed nodules with multi-strain composite inoculants but only 46 of the genotypes did so with strain SB16 (Khurana, 1981). Three soybean varieties showed differential response in nodulation and seed yield to inoculation with 4 Bradyrhimobium japonicum strains (Ganacharya and Nirmal, 1978). Without inoculation, Malayan and CES486 cultivars showed good nodulation, and inoculating them did not significantly increase nodulation or yield. In contrast, Bossier and Jupiter cultivars nodulated poorly without inoculation and showed large increases in nodule weight and numbers when inoculated (Nangju, 1980).

Out of 16 promiscuous soybean lines evaluated in a field experiment on virgin land, 6 gave higher yields in the absence than in the presence of inoculation with *Bradyrhizobium japonicum* (Joshi et al, 1985). Genotypes P1371607, P11377578 and Williams were inoculated with either strain USDA 123 or 110 . With USDA 123 nodulation and N accumulation were significantly less in the P1 genotypes than in Williams. In contrast, with USDA 110, nodulation and N accumulation of the P1 genotypes were similar to that of Williams (Gregan and Keyser, 1986). It was suggested that the trait identified in the P1 genotypes might be useful in developing soybeans able to exclude the indigenous USDA 123 in favour of more efficient inoculant strains.

Variations in nodulation of Mammoth, Yellow and Laredo soybean cultivars inoculated with certain *Bradyrhizobium* strains were found by Briscoe and Andrews (1938) to be as great as the differences between soybean and cowpea cross inoculation groups. These workers and Erdman (1944), working with different strains and varieties, reported that locally isolated Bradyrhizobium japonicum strains usually induced better nodulation on varieties adapted to the location than did strains introduced from a different location. This was recently supported in Tanzania by Singh and Mligo (1982) where 14 varieties from different sources were evaluated for their ability to form nodules with and without inoculation. Nine varieties formed good nodules only when inoculated while 5 tropical varieties showed moderate to good nodulation irrespective of inoculation.

Although a symbiotic relationship was established between most of the 10 varieties and 5 Bradyrhizobium japonicum strains, nodules were most numerous with only 2 varieties, but the number of nodules on individual varieties depended on the strain used (Saric and Fawzia, 1983). Soybean genotypes P1377578 and P1417566, along with Williams, were inoculated with 4 single strain treatments and other 6 dual strain treatments by Cregan et al. (1989). Genotype P1377578 severely reduced the nodulation and competitiveness of USDA 123 and USDA 127, while genotype P1417566 similarly affected the nodulation and competitiveness of USDA 129. The results indicated that host control of restricted nodulation and reduced competitiveness is quite specific and effectively discriminated between Bradyrhizobium japonicum strains which are serologically related. Cultivar Bragg consistently showed greater nodule numbers and nodule dry weight averaging 280 nodules and 0.69g/plant compared with 193 nodules and 0.54g/plant for cultivar Contennial (Zimet et al, 1985). Nitrogenase activity on whole plant basis was also greater for Bragg with 10.97μMol ethylene formed per hour in each plant compared with 7.37μMol C_2H_4/hr for Contennial.

Variation in N_2-fixation responses between soybean genotypes and Bradyrhizobium japonicum has also been documented. A significant variation was observed in the amount and proportion of nitrogen derived from fixation amount 3 cultivars that were inoculated with the same strain (Danso et al., 1987). In 7 soybean cultivars, the highest N_2-fixation rates were generally in cv. Shih-Shih and lowest in cv. Training No.4 and Kaohsiung No.3 despite the fact that they were inoculated with the same strain; no relationship was found between N_2-fixation rate and yield (Lin, 1983). Cultivars Lee, Bay and Essex were the best nitrogen fixers on the basis of nodule number and high acetylene reduction out of 20 commercial cultivars screened for N_2-fixation by Sapra et al. (1984). Large differences in the amount of N_2-fixed occurred between the 10 varieties and 5 Bradyrhizobium japonicum used as well as within varieties depending on the strain used (Saric and Fawzia, 1983).

The local N_2-fixing soybean cultivar Magoye rated highest among three cultivars tested for symbiotic N_2-fixation ability with *Bradyrhizobium japonicum*, and it derived 65-70 percent of its N uptake from the atmosphere (Munyinda et al., 1988). Eighteen genotypes were screened with 2 strains in a green house to determine N_2-fixation rates by Reddy and Faza (1990). They found that nitrogenase activity was consistently greatest for both strains in cv. Hoberland, 10.26 and $17.43\mu MC_2H_4$/hr respectively, whereas little activity was detected in genotype Ogden with USDA 110 and genotype P1230978 with tal 378. With strain 3Ilb110, seed dry weight yields were 185g/plant in cv. Ramsom while that of cv. Davis was 164g/plant (Israel, 1981), the high yield was associated with high N_2-fixation before reproductive development in cv.Davis and with high N_2-fixation during reproductive growth in cv. Ramsom.

Total amounts of N_2-fixed were strongly influenced by the specific combination of host genotype and rhizobial strains, the sensitivity of nitrogen fixation to nitrogen fertilizer was also dependent upon the host genotype and *Bradyrhizobium* strain association (Senaratne et al., 1987). They suggested that it is inadvisable to describe a certain bacterium as being fully effective/ineffective, sensitive/tolerant to nitrogen except in relation to a particular host genotype. There is scope in obtaining promising host-*Bradyrhizobium* associations tolerant of nitrogen through exploiting the interactive effects between host-genotypes and strains of *Bradyrhizobium japonicum*. It is evident from these reports that the soybean genotype plays an important role in the establishment of the symbiotic association with *Bradyrhizobium japonicum.*

2.3.2. Variability in *Bradyrhizobium japonicum* strains

Not only do soybean genotypes differ in their susceptibility to nodulation but *Bradyrhizobium japonicum* strains also differ in their ability to induce nodulation and to fix nitrogen. Inoculation with single strains or mixtures of *Bradyrhizobium japonicum* led to genotype x culture interactions for different characteristics, indicating that *Bradyrhizobium* strains have specificities for different varieties (Khurana, 1986). In pot and field trials conducted by Liang et al. (1991) strain 61A71 was found to have a wide adaptability to cultivars and environments while strain RB-9 was suited to early maturity cultivars and strain E_{45} to medium maturity cultivars. Strain 123 was infective and effective at all locations tested using soybean cv. Lee while strain 135 was very poor and did not form nodules at one of the locations (AL-Rashidi and Damirgi, 1978).

Significant differences in the symbiotic potential of the indigenous *Bradyrhizobium japonicum* isolates were demonstrated by Kang et al. (1991), the 30 isolates from the soil showed wide differences in effectiveness (measured as milligrams of shoot N per plant) and several were of equal or greater effectiveness than the reference strain. The mineral N status of the soil was a significant factor in affecting the competition pattern of Bradyrhizobium japonicum strains (Somasegaran and Bohlool, 1990). Differences between the effectiveness of the strains were masked under conditions of soil N availability. However, when soil N was immobilized with sugarcane bagasse, differences between the effectiveness of the strains became significant, Brandyrhizobium japonicum strain Tal 102(USDA110) was consistently the most effective and more often the most competitive of the strains used. Inoculation with strain SN2(S) produced more nodules and higher nodule weight than inoculation with strain 3f1b38 (Awai, 1981). Rhizobial strain effects were observed among soybean cultivars of the same maturity groups for nodule weight and nitrogenase activity (Reddy and Faza, 1990).

Strains Sm31 and Sm35 nodulated soybean roots earlier than the wild type strain (Maier and Brill, 1978), the Sm35 strain formed significantly more nodules per plant than the wild type strain. In addition, dry weight and N contents of plants inoculated with strain Sm31 and Sm35 were significantly higher than those inoculated with wild type strain. Inoculation of soybean seeds with different Bradyrhizobium japonicum cultures showed that IARI-II culture was the most effective in increasing nodulation, although it ranked third in seed yield among the 4 cultures used, the differences in yield due to strains were not significant (Ganacharya and Nimal, 1975). Chowdhury (1977) found significant cultivar x strain interaction with imported strains, indicating the need for selection of appropriate strains for particular cultivars but such an interaction was not significant with local strains.

In a field experiment conducted to study the performance of three Bradyrhizobium japonicum strains (USDA110, USDA138 and Tal379) in relation to their N_2-fixing potential on Clark and Colland varieties, Moawad et al. (1988) found strain USDA110 most effective in N_2-fixation and in increasing seed yield on both cultivars followed by USDA138 while Tal379 was the least effective. Rahman and Sanoria (1990) found that out of 9 Bradyrhizobium japonicum strains tested with one soybean variety, strains Bau-107, S-646 and TSH-501 were the most effective in increasing N_2-fixation, number of branches/plant and growth rate at different growth stages.

When comparing the effects of USDA31 and USDA110 strains on dry matter accumulation and N_2-fixation of soybeans, Schubert et al. (1978) reported that USDA110 strain produced more total dry matter weight, percent nitrogen and total N_2-fixed by 24, 7 and 31 percent respectively. Soybean cultivars Davis and Ramson fixed significantly higher N during the growing season when inoculated with strain 3F1b110 than with strain 3F1b31 (Israel,1981). Strain S140 (USA) gave a maximum increase in soybean yield over control (18.42 percent) at one experimental site while strain S_{18-8} (USA) gave maximum increase over control (24.45 percent) at the second site (Mohammad, 1979). All local strains proved better than the inoculant strain USDA35 for nodulation of soybean while only a few local strains were better than inoculant strain USDA117 (Rao and Patil, 1981), local strains increased yield by 158-321 percent above the control and exotic strains by 170-315 percent . It was concluded that some of the local and exotic strains were better than commercial inoculant strains in increasing nodulation, N_2-fixation and seed yield.

Among the inoculants tested by Hume and Shelp (1990), strain 532C supported the highest yields in 4 out of the 5 years,its treatments averaged 3.08t/ha compared with 2.76, 2.84, 2.83 and 1.96t/ha for the other three strains and uninoculated control, respectively. The consistently good performance of 532C across years and locations, suggested major advantages for this strain; it is now being used as a single strain inoculant in Ontario, Canada. Inoculation of soybean with Bradyrhizobium japonicum strain 110 increased yield by 0.3Mg/ha compared to strain 587, the yield increase was similar with or without fertilizer N application, indicating that strain responses were not totally caused by improved N efficiency (Karlen and Hunt, 1985). However, no specificity was found between strain 110 and any of the 6 cultivars grown for two years (Howle et al., 1987). From the reports cited, it can be concluded that some strains produce good nodulation and N_2-fixation for certain soybean varieties, compatible strains should be used with commercially grown varieties.

CHAPTER THREE

MATERIALS AND METHONDS

3.1. SOILS INVESTIGATED

The soils of National Agricultural Research center at Kitale, Trans Nzoia District, Rift-Valley Province (Latitude 1°N; Longititude 35°E) were utilized in this study. The soils are derived from basement system gneisses and schists rich in field spar, biotite, hornblende and garnet. There are also minor exposures of granites and pegmatitic dykes (Siderius and Muchena, 1977). The center is situated in an area with slightly undulating uplands at an altitude of 1860m above sea level. The soil moisture and temperature regimes are ustic and isothermic, respectively. The area receives mean annual rainfall of 1193mm which is fairly evenly distributed throughout the year with two peak rainfall periods in May and August. The mean annual temperature is 18.2°C with a relatively hot season usually from January to March (EAMD, 1974). The vegetation was originally moist combretum woodland to bushland but is now mainly used for rainfed maize and pasture research.

3.1.1. Soil samples for greenhouse experiment

The soil samples intended for the greenhouse experiment with soybean were collected over an area of approximately 0.5ha with a slope of 2-3%. The field was last cropped with soybeans in 1963 and was under maize for about five years up to 1989 when it was left fallow. The grass was first removed from various spots of the field using hoes. The top-soil (0-20cm) was then sampled using the hoes from the cleared portions and packed in gunny bags for transportation to Kabete Campus, University of Nairobi. About 1-kg sample composites from each gunny bag was placed in a polythene bag for subsequent chemical analyses. Another composite top soil sample was placed in a polythene bag and securely tied at the top in order to retain the field soil moisture necessary for the survival of soil micro-organisms. The latter sample was stored at 4°C to prevent further multiplication of native soil rhizobia until it was first used in the laboratory for the Most Probable Number (MPN) counts of Bradyrhizobium bacteria.

For the purpose of classifying the soil used, a soil profile pit measuring 1m x 2m and 1.32m deep was dug in the middle of the field where the soil samples for the greenhouse experiment were collected. The soil profile was described fully in terms of the various horizons identified, their colour, soil structure, pores, texture and consistency. The soil was tentatively classified using the FAO/UNESCO classification system while awaiting the analytical results from the laboratory; the physical and chemical data of the soil sampled from each horizon would then be used to confirm the classification.

3.1.2. Soil samples for the field experiment

The field experimental site was situated about 2km from the area where the soil for the greenhouse experiment was collected. The site was in a gently undulating field of 2-3% slope. The field was under lupins up to 1986 when it was left fallow and gave way to natural pasture for a period of about five years before it was ploughed in 1991. This site was ploughed, reploughed, harrowed and marked before the soil samples were taken. Three composite top-soil (0-20cm) and sub-soil (20-40cm) samples from the same hole of approximately 1kg each were taken using augers from each of the three replicates and placed in polythene bags. The samples were used for subsequent chemical analyses to evaluate the soil fertility status of the site. One Top-soil composite sample was placed in a polythene bag and tied at the top in order to retain the field soil moisture and hence facilitate the survival of soil microbes. This sample was stored at 4°C to prevent the multiplication of the native Bradyrhizobium bacteria until it was used for rhizobia counts in the soil.

For the purpose of classifying the soil used, a soil profile pit measuring 1m x 2m and 1.50m deep was dug about 2m above the third replicate but almost at the center of the experimental site lengthwise. The profile was described fully in terms of the various horizons identified, their colour, soil structure, pores, texture and consistency. The soil was tentatively classified using the FAO/UNESCO classification system while awaiting the analytical results from the laboratory; the physical and chemical data of the soil sampled from each horizon would then be used to confirm the classification.

3.1.3. Sample preparation

The soil for the greenhouse experiment was spread on thick plastic sheets for air drying. It was thoroughly mixed, debris removed and lumps broken using a wooden mallet before it was weighed into 2-kg portions for each pot. The samples for analyses were thoroughly mixed, debris removed and ground in a mortar with a pestle before sieving through 2mm and where necessary through 0.5mm. The soils were then used for measuring soil pH, and the determination of percent organic carbon, percent nitrogen, phosphorus, cation exchange capacity (CEC), exchangeable bases (K, Na, Ca, Mg) and texture.

3.2. SOIL ANALYSES

3.2.1. Soil reaction (pH)

Following the procedure outlined by Page (1982), ten grams of soil sieved through 2mm was mixed with 25ml of water and 0.01M $CaCl_2$ solution, respectively using a ratio of 1:2.5. In both cases, the suspension was shaken for 30 minutes using a mechanical shaker. It was left to stand for 10 minutes. Soil pH was then determined using a pH meter.

3.2.2. Organic carbon

Walkley-Black method outlined by Page (1982) was used to determine organic carbon. The carbon was oxidized at a temperature of approximately 120°C by adding potassium dichromate solution and concentrated sulphuric acid to the soil sample. The excess potassium dichromate not reduced by organic matter of the soil was determined by titration with ferrous sulphate using diphenylamine as indicator.

3.2.3. Total nitrogen

Total Nitrogen was determined using Kjeldahl method following the procedure outlined by Page (1982). Nitrogen compounds in the soil samples were digested with concentrated sulphuric acid together with potassium sulphate, copper sulphate and selenium powder catalyst mixture. During digestion nitrogen was converted to ammonia that was fixed by the excess acid as ammonium sulphate. The digest was made alkaline with sodium hydroxide and ammonia released. The ammonia was distilled and collected in flasks containing boric acid-indicator solution and the ammonia was then determined titrimetrically.

3.2.4. Phosphorus

Phosphorus was analyzed by double acid (0.05N HCl + 0.025N H_2SO_4) following the procedure described by Page (1982). A 5-g sample of each soil was placed in a 100-ml centrifuge tube and 50ml of double acid added. The tubes were shaken horizontally for 15 minutes using a mechanical reciprocal shaker. The soil was filtered using whatman No.42 filter paper and the filtrate collected in specimen bottles. The colour of the filtrate was developed using ascorbic acid dissolved in ammonium molybdate, potassium antimony tartrate and sulphuric acid solution. The intensity of the blue colour which keeps stable for at least 24 hours was dependent on the phosphate content and was measured by reading the absorbance on an sp 500 spectrophotometer after 15 minutes.

3.2.5. Cation exchange capacity and exchangeable cations

Cation exchange capacity (CEC) was determined by displacing the bases using 1N ammonium acetate solution at pH 7. The adsorbed ammonium cations (NH_4^+) were washed off by leaching with potassium chloride solution and the CEC determined from the quantity of ammonia distilled from the leachate following the procedure described in the Tropical Soils Analysis Unit Manual. Exchangeable potassium and sodium were determined using flame photometer while exchangeable calcium and magnesium were obtained by titration as outlined by Page (1982).

3.2.6. Soil texture

Soil texture was determined by hydrometer method for particle size distribution following the procedure outlined by Bouyoucos (1951). Hydrogen peroxide (H_2O_2) was used to destroy the organic matter present in 51-g sample of air-dried soil which was then dispersed by both mechanical and chemical agent (Calgon). Dispersion was done for 30 minutes and the suspension transferred to a glass cylinder. The first reading of the hydrometer and thermometer was taken at 40 seconds and the second one after 3 hours.

3.3. INOCULANT PRODUCTION

3.3.1. Source of cultures

Two strains of Bradyrhizobium japonicum from Nairobi Microbiological Resource Center (MIRCEN) were used in carrying out the experiments. The culture of NUM 504 originally from Niftal, where it is known as TAL 102, was procured from Chiromo Campus of the University of Nairobi where it had been lyophilized and ampouled. NUM 508, also from Niftal where it is referred to as TAL 379, had been maintained on agar slants in screw-capped test tubes stored at 4°C in Nairobi MIRCEN laboratory at Kabete Campus of the University of Nairobi.

3.3.2. Preparation of the media

3.3.2.1. Yeast extract mannitol agar (YEMA)

The routinely used medium (YEMA) composed of the following constituents, Mannitol, 10.0g; K_2HPO_4, 0.5g; $MgSO_4.7H_2O$, 0.2g; NaCl, 0.1g; Yeast extract, 1.0g and agar 16.0g. The first five chemicals were dissolved in distilled water, the volume made into a litre and pH adjusted to 6.8 by using either 0.1N HCl or 0.1N NaOH. Agar was then added and melted in the autoclave. This formula was adopted from Somasegaran and Hoben (1985). Some of the medium was dispensed in 95ml quantities into 250ml Erlenmeyer flasks which were plugged with cotton wool and covered with alumimium foil. The rest of the medium was dispensed in 5ml aliquots in test tubes and capped.

The medium in the flasks and test tubes was sterilized by autoclaving for 30 minutes at 121°C. The agar slants were prepared by cooling the medium whilst placing the test tubes at 45 degrees, YEMA from Erlenmeyer flasks was cooled to 40°C, poured into the sterile petri dishes and left to set. The agar slants and plain agar plates were incubated at 28°C for 1 to 3 days in order to check fast-growing contaminants. Contaminated plates and slants were discarded and the clean ones were stored for further use.

3.3.2.2. Dyes incorporated in YEMA medium

3.3.2.2.1. Bromothymol blue (BTB)

stock solution of BTB was made by dissolving 0.5g of the dye in 100ml ethyl alcohol. 5.0ml of the stock solution was then added into one litre of Yeast Extract Mannitol (YEM) broth. The YEM broth had similar chemical composition to YEMA except that the former lacked agar. The pH was adjusted to 6.8, agar was added and the medium melted in the autoclave. Following the same procedure as described for plain agar plates, BTB plates were prepared.

3.3.2.2.2. Congo red

Stock solution of congo red was made by dissolving 1g of the dye in 400ml of distilled water. 10ml of the stock solution was added into one litre of YEM broth and pH adjusted to 6.8. Agar was then added and the medium melted in the autoclave. Congo red plates were prepared following the same procedure as the one described for BTB plates.

3.3.2.3. Nutrient solution

The nutrient solution (hydroponic) used to grow soybean plants in the Leonard jars for MPN counts of rhizobia in the soil consisted of 1000μM Ca ($CaCl_2.2H_2O$); 500μM P (KH_2PO_4); 10μM Fe (Fe-citrate); 250μM Mg ($MgSO_4.7H_2O$); 250μM K (K_2SO_4); 1μM Mn ($MnSO_4.H_2O$); 2μM B (H_3BO_3); 0.5μM Zn ($ZnSO_4.7H_2O$); 0.2μM Cu ($CuSO_4.5H_2O$); 0.1μM Co ($CoSO_4.7H_2O$); 0.1μM Mo ($Na_2MoO_4.2H_2O$); and 70ppm N (KNO_3) as outlined by Somasegaran and Hoben (1985).

3.3.3. Processing of the carrier

3.3.3.1. Packaging of the carrier

Finely-ground filtermud was passed through 0.25μM sieve and 50-g portions weighed into autoclavable high density polythene bags. 10mls of YEM broth was added to the filtermud to facilitate steam sterilization and the bags were sealed using an audion electro sealer. The filtermund was thoroughly mixed with the broth and autoclaved for two hours at 121°C.

3.3.3.2. Carrier check for sterility

Ten grams of filtermud from one packet was weighed and transferred aseptically to 90ml sterile quarter strength of YEM broth to acquire 10^{-1} dilution. Further serial dilutions of up to 10^{-8} were made by aseptically transferring 1ml to 9ml after hand-shaking the covered bottles for 2 minutes. From each dilution 0.1ml suspension was plated in duplicate on congo red and plain agar plates using the spread plate method as described by Somasegaran and Hoben (1985). The plates were inverted and incubated at 28°C for 7 days.

3.3.4. Culturing of rhizobia

The stock culture slant containing NUM 508 was washed with 2ml sterile YEM broth and a loopful of the resulting broth suspension was transferred aseptically to 5ml sterile YEM broth in screw-capped test tubes. The ampoule carrying NUM 504 was opened following the procedure outlined by Somasegaran and Hoben (1985) and the bacteria similarly transferred to 5ml sterile YEM broth. The test tubes inoculated with NUM 508 and NUM 504 strains were incubated at 28°C for 7 days.

Pure cultures of both strains from plain agar plates were maintained on agar slants in screw-capped test tubes and stored at 4°C. These cultures were subcultured on freshly prepared slants monthly until the field experiment was planted. Starter cultures were transferred aseptically to the 95-ml sterile YEM broth medium in the Erlenmeyer flasks. The flasks were placed on a Gallenkamp orbital shaker at 100 revolutions per minute to facilitate aeration and hence faster multiplication for a period of one week.

3.3.5. Determination of quality for broth cultures

3.3.5.1. Screening broth cultures for contaminants

Cultures for both strains, NUM 504 and NUM 508, were screened for contamination by fast growing micro-organisms by streaking a loopful of each culture on the congo red and plain agar plates. The petri dishes were inverted and incubated at 28°C for 24 hours. This helped to detect contaminants with growth rate faster than rhizobia. Other tests to eliminate possible contamination of broth cultures included plating on BTB, measuring the pH and gram-staining broth samples.

3.3.5.2. Enumeration of rhizobia in broth cultures

Counts for viable rhizobia were carried out for both strains by aseptically transferring 1ml of broth into 9ml quarter strength YEM medium to achieve a dilution of 10^{-1}.

Similarly, serial dilutions of 10^{-2} up to 10^{-9} of broth cultures were made. 0.1ml of the dilutions 10^{-5} up to 10^{-9} were plated in duplicate on congo red plates, inverted and incubated at 28^{0}C for 5 days. The dilution of 10^{-6} was chosen for counting as it had more than 30 and less than 300 colonies as recommended. The number of rhizobia per ml of broth was calculated using the following formula.

No. of rhizobia/ml = dilution factor x mean plate count x 10.

3.3.6. Impregnation of broth cultures into the carrier

Sterile sealed packets of carrier were counter-checked for leakages and the good ones selected. The point of injection of the broth culture into the carrier was surface-sterilized with 95 percent alcohol to prevent contamination of the carrier and 40ml of the broth culture aseptically injected into the carriers using 100-ml disposable sterile syringe. The punctured area was sterilized again with 95 percent alcohol, left to dry and sealed with adhesive tape. The injected packets were carefully massaged and incubated at 28^{0}C for two weeks. The inoculant packets were then double-packed and stored at 4^{0}C until they were used.

3.3.7. Viable counts of rhizobia in the inoculants

dilution factor

Mean

One gram of each of the two inoculants prepared was weighed in duplicate and aseptically transferred into 9ml of sterilized quarter strength YEM broth contained in universal bottles. The bottles were covered and hand-shaken for 2 minutes. Tenfold serial dilution was carried out from 10^{-1} up to 10^{-8}, where 1ml was pipetted from the 10ml suspension to 9ml quarter strength YEM broth, shaken and the procedure repeated up to 10^{-8} dilution level. Plating was done at dilution intervals from 10^{-4} to 10^{-8}. From each dilution, 0.1ml suspension was plated on YEMA congo red plates in duplicates. The plates were inverted and incubated at 28°C for 5 days. The colonies were counted and the number of rhizobia per gram of inoculant was calculated as follows.

No. of rhizobia/g = dilution factor x mean plate count x 10.

3.4. MOST PROBABLE NUMBER (MPN) COUNTS OF RHIZOBIA

one hundred and fifty soybean seeds of uniform size and high viability were surface-sterilized in 3 percent sodium hypochlorite for 10 minutes in order to kill any Bradyrhizobium bacteria that might have adhered to the seeds. They were then washed in six changes of sterile distilled water and transferred onto sterile 0.8 percent water agar using sterile forceps. The seeds were incubated at 28°C and germination checked after 7 days. Three well germinated seeds were transferred aseptically from the water agar plate to each of the sterile Leonard jars.

About 30g of each soil was weighed into an Erlenmeyer flask containing 100ml of sterile water and mixed for 15 minutes. 10^{-1} up to 10^{-6} dilutions were made using universal bottles. For every dilution, three Leonard jars containing pre-germinated seeds were inoculated with 1.0ml of soil suspension per jar. Thus making a total of 18 jars for use with the Brockwell (1963) Tables. Three jars were left uninoculated as controls. The jars were transferred to the greenhouse and the plants allowed to grow for a period of four weeks before examining them for nodulation.

3.5. GREENHOUSE POT EXPERIMENT

3.5.1. Planting material

Two varieties of soybeans, namely, Duiker and Sable, which were supplied by the Kenya Seed Company were used. The Company is maintaining and bulking the seeds for sale. Both varieties were developed and released in Zimbabwe, where they have been identified as important varieties for the high altitude areas (Tichagwa, 1986). They perform well in areas above 800m, have satisfactory resistance to shattering and same maturity period. The cultivars have some similar morphological characteristics, such as having an indeterminate growth habit, purple flowers and yellow seed with a yellow hilum.

3.5.2. Experimental design and treatments

A 5 x 3 x 2 factorial experiment in a randomized complete block design with three replicates was used. The main factors were;

(a) 5 nitrogen levels:

N_1 ________________ 0kgN/ha
N_2 ________________ 20kgN/ha
N_3 ________________ 40kgN/ha
N_4 ________________ 80kgN/ha
N_5 ________________ 160kgN/ha

(b) 3 inoculation treatments:

S_0 ________________ Non-inoculated
S_1 ________________ Inoculation with NUM 504 strain
S_2 ________________ Inoculation with NUM 508 strain

(c) 2 soybean varieties:

V_1 ________________ Duiker
V_2 ________________ Sable

In all there were 30 treatments combinations which are shown in Table 1.

Table 1. Treatment Combinations.

1 $N_1S_oV_1$	11 $N_1S_2V_1$	21 $N_1S_1V_2$
2 $N_2S_oV_1$	12 $N_2S_2V_1$	22 $N_2S_1V_2$
3 $N_3S_oV_1$	13 $N_3S_2V_1$	23 $N_3S_1V_2$
4 $N_4S_oV_1$	14 $N_4S_2V_1$	24 $N_4S_1V_2$
5 $N_5S_oV_1$	15 $N_5S_2V_1$	25 $N_5S_1V_2$
6 $N_1S_1V_1$	16 $N_1S_oV_2$	26 $N_1S_2V_2$
7 $N_2S_1V_1$	17 $N_2S_oV_2$	27 $N_2S_2V_2$
8 $N_3S_1V_1$	18 $N_3S_oV_2$	28 $N_3S_2V_2$
9 $N_4S_1V_1$	19 $N_4S_oV_2$	29 $N_4S_2V_2$
10 $N_5S_1V_1$	20 $N_5S_oV_2$	30 $N_5S_2V_2$

3.5.3. Seed inoculation

The inoculation rates employed in the Microbiological Resource Center (MIRCEN) laboratory of the University of Nairobi were adopted. 100g inoculant packet inoculates 15kg of large legume seeds already wetted with 290-300mls of adhesive. These seeds are enough for planting an area of approximately one acre. Hence the rate of 0.25kg inoculant per hectare was used. The moist seeds were thoroughly mixed with the inoculants in the shade, sown immediately and covered with soil in order to minimize rhizobia exposure to ultra-violet (uv) light from the sun which kills the bacteria.

3.5.4. Planting

Two kilograms of soil (sieved through 2mm) was placed in each of 90 plastic bags and mixed thoroughly with 0.5kg of acid-washed sand, the latter being included to prevent soil compaction. Each mixture was poured into a plastic pot of about 16cm in diameter and 18cm high; each pot had three holes at the bottom to allow for drainage. Every pot had been separately placed on a watering pan and covered with a thin layer of course sand at the bottom before pouring the soil-sand mixture. A basal dressing of 50kgP/ha as single super phosphate and different rates of nitrogen as urea were added and hand-mixed into the medium. The pots were arranged in a randomized complete block design in the greenhouse.

The appropriate amount of distilled water to adjust the soil-sand mixture on each pot to field capacity was poured on the watering pan below the pot. Five soybean seeds of either Duiker or Sable variety, with the appropriate inoculation treatment, were planted in each pot in the greenhouse on 10th April 1991. The plants were thinned to three per pot, three weeks after seeding. Distilled water was added regularly from the bottom while a nitrogen-free nutrient solution which was also lacking in phosphorus and calcium was added weekly at the rate of 250ml/pot after thinning. The pots were kept free of weeds by uprooting them regularly, taking care to avoid transferring rhizobia from one pot to another. Fungal diseases and insects were controlled by spraying the plants with Benlate/dimethoate solution mixture 35 days after planting. Plant height was recorded by averaging the heights of the three plants in each pot at early podding stage, 60 days after planting.

3.5.5. Sampling

The experiment was concluded when the plants were at early podding stage, 63 days after planting. Three plants in each pot were cut off from the first cotyledon node and the shoots and roots separated. The root systems from each pot were washed free of soil by gently soaking them in bucke water, taking care to avoid removing the nodules durin process. The nodules from the three plants were picked the roots and their position in relation to the ta lateral roots, together with size and colour, were before recording their numbers. The shoots , root nodules were placed in separate paper bags and oven-dri 70°C for 48 hours for dry weight determination and subse nitrogen analyses of the shoots.

3.6. FIELD EXPERIMENT

A field experiment was carried out to corroborat results obtained from the greenhouse pot experiment, usi same experimental design and treatments. The pl material and seed inoculation method were similar to used in the greenhouse.

3.6.1. Planting

The field was reploughed and harrowed on June 27, 1 facilitate the layout of the trial and subsequent pla The layout was done on June 28, 1991 and the expe planted on July 1, 1991. The plot size was 2m x 2m 0.5-m path between the plots and a 1-m path betwe replicates. The spacing between and within rows was

20cm, respectively. This spacing gave a total of 6 row plot with 11 hills on each row. Planting holes were dug small hoes and 50kgP/ha applied in form of t superphosphate together with different rates of nitrog form of urea. The fertilizers were mixed with the soil b planting three soybean seeds, per hole, of either Duik Sable variety, with the appropriate inoculation treat The trial was kept weed-free by manual weeding which was twice during the experimental period.

3.6 2. Sampling

Three plants were selected at random from the cen each plot and dug out with hay forks at early podding 111 days after planting. The shoots were cut off fr first cotyledon node using secateurs and the roots dip buckets of water to soak the soil. The roots with undis nodules were placed in plastic bags which were then se tied at the top and taken to the laboratory. The nodule picked from the roots and their position in relation tap and lateral roots, together with size and colour noted before recording their numbers. The shoots, roo nodules which were in separate paper bags were oven-dr 70°C for 48 hours for dry weight determination and subs nitrogen analyses of the shoots.

Plant height was recorded by averaging the heights of three randomly selected plants in each plot when the crop reached physiological maturity, 128 days after planting.

3.6.3. Harvesting

Harvesting was done during the first week of December 1991, about 158 days after planting. The plants were uprooted manually after taking their stand count and a subsample of three plants taken from each plot. The subsample was used to determine the number of pods per plant, beans per plant and hence the number of beans per pod. The harvested plants from each plot were dried in gunny bags for five days before threshing. The threshing and winnowing was done for over a period of two weeks. The grains were weighed and moisture determined using Dickey-john moisture tester. The yield in kg/ha was calculated and adjusted to 13% moisture. Weights of 1000 oven-dried seeds taken from each plot were recorded as estimates of seed size. These grains were ground and stored in sealed plastic bags for nitrogen determination.

158 days

3.6.4. Nitrogen analyses of the shoots and grains

The percent nitrogen in the shoots and grains was determined by the improved micro-kjeldahl nitrogen determination method following the procedure outlined by

Horwitz (1975).

3.6.5. Statistical analysis

The model for the analysis of variance for any observation is given by:

$$Y_{ijkl} = \mu + \int_i + N_j + I_k + V_l + (NI)_{jk} + (IV)_{kl} + (NV)_{jl} + (NIV)_{jkl} + \Sigma_{ijkl}$$

Y_{ijkl} = Observation per plot of grain yield etc.

μ = The general mean

$\int_i$ = The effect of the i^{th} block

N_j = The effect of the j^{th} N level

I_k = The effect of the k^{th} inoculation

V_l = The effect of the l^{th} variety

$(NI)_{jk}$ = The interaction between the j^{th} N level and the k^{th} inoculation.

$(IV)_{kl}$ = The interaction between the k^{th} inoculation and the l^{th} variety

$(NV)_{jl}$ = The interaction between the j^{th} N level and the l^{th} variety

$(NIV)_{jkl}$ = The interaction between the j^{th} N level, k^{th} inoculation and the l^{th} variety

Σ_{ijkl} = The error term

The most recent statistical Analysis System (SAS) packages (1988) were used to carry out the statisti analysis of the data. Mean differences were tested by Dunc Multiple Range Test (DMRT) (Steel and Torrie, 1980). T test offers more protection against type 1 errors than least significant difference. Adjustment of grain yield plant population was done using covariance analysis significant differences between pairs of grain yield me detected by Scheffe's test which is more powerful than I (Steel and Torrie, 1980). The use of values of orthogo coefficients for orthogonal comparisons in regression employed to estimate the relationships between var parameters studied and nitrogen levels (Snedecor and Coch 1967).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1. SOIL CHARACTERISTICS

The soil had been characterised before planting in order to assess its suitability for soybean growth and survival of Bradyrhizobium japonicum rhizobia which had been found to be completely lacking as determined by plant infection technique. Apart from nitrogen, which was one of the treatments, nutrient deficiencies were supplied in quantities calculated to fall within the ranges generally recommended by the National Agricultural Research Laboratories (NARL) in Nairobi.

4.1.1. Physical properties

Appendix Tables I and II show some physical properties of the soil profile pits at the sites where the soil samples for the greenhouse experiment was collected and the field experiment conducted, respectively. The greenhouse soil had a thin Ap horizon (0-9cm) with a Munsell notation of 7.5 YR2/3 when moist and a colour that was very dark brown. This was followed by a transitional AB horizon (9-30cm) with a Munsell notation of 5 YR2/3 when moist, having a very dark reddish brown colour. Further down the profile, there was a Bw_1

horizon (30-61cm) and a Bw_2 horizon (61-132+cm) with almost similar Munsell notations of 5 YR3/4 and 5 YR3/6 when moist, respectively and dark-reddish brown colour. The soil for the field experiment had a slightly thicker Ap horizon (0-15cm) with a Munsell notation of 5 YR2/3 when moist and very dark reddish brown colour. This was followed by a BA transitional horizon (15-36cm) with a Munsell notation of 2.5 YR3/6, hence dark reddish brown in colour. The third horizon, Bw_1(36-80cm), together with the last horizon Bw_2(80-150+cm), had moist colours similar to that of BA horizon referred to above.

A comparison of the depths and colours shown in Appendix Tables 1 and 11 and chemical characteristics shown in Tables 2a and 2b in regard to the base saturation and organic carbon contents of the Ap and AB horizons of the soil used in the greenhouse with that of the Ap horizon of the soil used in the field experiment shows that the greenhouse soil had an umbric A horizon which was lacking in the field soil; the soil used in the greenhouse experiment was classified as Humic Ferralsol while the one for the field experiment was classified as Rhodic Ferralsol according to FAO/UNESCO soil classification. The two soils were deep (over 1.32 and 1.50m) due to relatively rapid weathering resulting from high rainfall and associated climatic conditions.

Mechanical analysis of the two soils showed that sand was the dominant fraction, ranging from 56-66% (Appendix Tables 1 and 11). This was followed by clay which ranged from 30-40%, while the silt fraction was very small, i.e. 2-4%. In both soils the texture was either sandy clay loam or sandy clay at different depths. The soils had a medium to strong sub-angular blocky structure which breaks to fine and medium crumbs under pressure. Both soils were porous with good aeration, friable when moist but sticky and plastic when wet.

4.1.2. Soil reaction

Soil pH had no consistent trend down the profiles (Tables 2a and 2b). The pH in water of the greenhouse soil samples ranged from 5.3 to 5.6 while the corresponding pH in 0.01M$CaCl_2$ ranged from 4.7 to 5.1. The pH of the field experiment soil was slightly higher as it ranged from 5.5 to 6.0 in water and 4.9 to 5.7 in 0.01M$CaCl_2$. The higher pH of the field experiment can be attributed to its high base saturation, which ranged from 56 to 90%, compared with the base saturation of the soil used in the greenhouse, which ranged from 30 to 53%.

The pH values of composite topsoil (0-20cm) samples taken at various places from the site where the greenhouse soil samples were collected are shown in Table 2c while tables 2d and 2e show the pH values of composite topsoil and subsoil

(20-40cm) samples taken at various places of the field experiment site, respectively. Both soils exhibit a moderately acidic reaction despite differences in their base saturation.

The difference between the pH in water and pH in $0.01MCaCl_2$ is approximately one unit. This difference is expected because the measurement of pH in soil-water suspension is influenced by the presence of soluble salts. The use of a salt, such as $0.01MCaCl_2$ tends to mask the variability of the pH caused by differences in the salt concentration of the soil solution and therefore give a more precise estimate of the soil pH than that measured in soil-water suspension. The concentration of soluble salts in soil-water suspension is assumed to be negligible with respect to the amount of salt added in the solution.

4.1.3. Organic carbon and total nitrogen

Table 2a shows the distribution of organic carbon and total nitrogen down the profile of the soil used for the greenhouse experiment. The top horizon Ap(0-9cm) shows the highest percentage of organic carbon and total nitrogen which were 2.3 and 0.16, respectively. Down the profile, both organic carbon and total nitrogen decreased consistently, as expected, with AB horizon (9-30cm) having organic carbon content of 1.4% and total nitrogen of 0.09%. Further down the profile, there was a Bw_1 horizon (30-61cm) which showed a lower organic carbon content, viz., 1.0% and total nitrogen content of 0.07% while Bw_2 horizon (61-132+cm) showed the least amounts of both organic carbon, that is, 0.6% and a total nitrogen content of 0.06%.

Table 2b shows that a trend similar to the above was repeated on the field experiment samples which had organic carbon content of 1.7% and total nitrogen content of 0.18% on the Ap horizon (0-15cm). The BA horizon (15-36cm) followed with 1.4% organic carbon and 0.09% total nitrogen. The lower horizons, Bw_1(36-80cm) and Bw_2(80-150+cm), had less organic carbon, viz., 0.9 and 0.6 to 0.4%, respectively. Total nitrogen in these two horizons also decreased, with Bw_1 having a total nitrogen content of 0.07% while the total nitrogen content of the Bw_2 horizon ranged from 0.07 to 0.06%.

Table 2c shows that the topsoil used in the greenhouse experiment had a mean organic carbon content of 2.4% and a mean nitrogen content of 0.17%. The soil had a favourable mean C:N ratio of 14, which ruled out the possibility of microbial immobilisation of applied nitrogen. Tables 2d and 2e show that the topsoil from the site used for the field experiment had a mean organic carbon content of 2.4% while the subsoil had a mean organic carbon content of 1.4%. The topsoil had a mean nitrogen content of 0.20% and the subsoil a mean nitrogen content of 0.13%. The topsoil and subsoil of the field experimental site had favourable mean C:N ratios of 12 and 11 respectively, hence there was no possibility of microbial immobilisation of applied nitrogen.

Constant enrichment of organic matter from plant and animal origin almost exclusively on the surface horizons explains the observed trend of its decrease down the profiles as well as the difference between the topsoil and subsoil. As the percent organic matter in the soil decreases, the percent of total nitrogen also decreases because the amount of nitrogen present in the soil depends on the mineralization of organic matter in soils with C:N ratios of less than 20 (Tisdale and Nelson, 1975). Therefore the higher the amount of organic matter in the soil, the higher the amount of nitrogen present in that soil.

According to NARL standards for deficiency and sufficiency ranges presented in Appendix Table 111, the total nitrogen data obtained in this study show that the soil used for the greenhouse experiment was deficient in nitrogen. The results show, however, that the field experiment was conducted on a site with just enough nitrogen for plant growth in the topsoil while the subsoil was deficient in this nutrient. Similarly, organic carbon data show that the soil used for the greenhouse experiment had moderate organic matter while the field experiment was conducted on a site whose topsoil and subsoil had moderate and low supply of organic matter, respectively.

4.1.4. Phosphorus

Table 2a shows the distribution of phsphorus down the profile of the soil used for the greenhouse experiment. The surface horizon Ap(0-9cm) had a phosphorus content of 6.3ppm while the AB(9-30cm), Bw_1(30-61cm) and Bw_2(61-132+cm) horizons had 2.0ppm phosphorus each. The phosphorus content down the field experimental pit also decreased with depth as shown in Table 2b. For the latter experiment, the surface horizon Ap(0-15cm) had the highest phosphorus content, i.e., 7.4ppm, followed by BA(15-36cm) horizon whose phosphorus content was 2.0ppm. The third horizon, i.e., Bw_1(36-80cm), had a phosphorus content of 1.0ppm while Bw_2 horizon (80-150+cm) had the least phosphorus content of 0.8 to 0.7ppm.

Table 2c shows that the phosphorus content of the topsoil used in the greenhouse pot experiment ranged from 5.8 to 17.5ppm, with a mean of 10.9ppm. Tables 2d and 2e show the phosphorus content of the topsoil and subsoil of the field experimental site, respectively. The amount ranged from 7.2 to 20.2ppm in the topsoil and 4.2 to 16.1ppm in the subsoil.

The mean phosphorus content of topsoil and subsoil was 13.0 and 6.2ppm, respectively. The decrease in phosphorus content with profile depth could be due to the decrease in organic matter content. According to Tisdale et al (1985) there is a positive correlation between the organic matter content and the amount of phosphorus present in the soil.

From the results presented, it is evident that the soil used for the greenhouse experiment was deficient in phosphorus by NARL standards which regard phosphorus content of less than 20ppm (Mehlich method) as low. Similarly, both the topsoil and subsoil of the field experimental site were deficient in phosphorus. Considering the limited mobility of this element in the soil, the variation in its concentration in the surface horizon might have been caused by haphazard application of phosphate fertilizer at one time or another during the cropping history of the land before the experiment was done. Phosphorus deficiency had been corrected by adding the fertilizer calculated to make the nutrient fall within the sufficient range.

Table 2a. Some chemical characteristics of the soil used in the greenhouse experiment.

Profile pit		pH		Extr.* P(ppm)	Total N %	Organic C %	CEC (me/100g)	Exchangeable bases (me/100g)				BS %
Horizon	Depth (cm)	1:2.5 (H_2O)	1:2.5 ($CaCl_2$)					Ca	Mg	K	Na	
Ap	0-9	5.5	4.9	6.3	0.16	2.3	11.0	2.50	1.50	0.40	0.08	41
AB	9-30	5.3	4.7	2.0	0.09	1.4	9.0	2.00	0.50	0.12	0.08	30
Bw_1	30-61	5.6	5.1	2.0	0.07	1.0	8.3	3.50	0.68	0.16	0.08	53
Bw_2	61-132+	5.5	4.7	2.0	0.06	0.6	7.0	1.25	1.00	0.08	0.08	34

Extr.* = Extractable P by double acid (0.05N HCL + 0.025N H_2SO_4) method

BS = Base Saturation

Table 2b. Some Chemical Characteristics of the soil of the field experiment.

Profile pit		pH		Extr.* P(ppm)	Total N (%)	Organic C (%)	CEC (me/100g)	Exchangeable bases (me/100g)				BS (%)
Horizon	Depth (cm)	1:2.5 (H_2O)	1:2.5 ($CaCl_2$)					Ca	Mg	K	Na	
Ap	0-15	5.9	5.0	7.4	0.18	1.7	14.2	4.50	1.50	1.75	0.15	56
BA	15-36	6.0	5.1	2.0	0.09	1.4	12.0	3.50	2.25	1.25	0.10	59
Bw_1	36-80	5.9	5.3	1.0	0.07	0.9	9.8	4.30	3.50	1.00	0.02	90
Bw_2	80-115	6.0	5.7	0.8	0.07	0.6	12.0	4.13	2.75	1.00	0.02	66
Bw_2	115-150+	5.5	4.9	0.7	0.06	0.4	10.0	4.75	2.25	0.50	0.02	75

Extr.* = Extractable P by double acid (0.05N HCL + 0.025N H_2SO_4) method

BS = Base Saturation

Table 2c. Some chemical characteristics of the topsoil (0-20cm) used in the greenhouse experiment.

No.**	pH		Extr.* P(ppm)	Total N %	Organic C %	C/N Ratio	CEC (me/100gm)	Exchangeable bases (me/100g)			
	1:2.5 (H_2O)	1:2.5 ($CaCl_2$)						Ca	Mg	K	Na
1	5.8	5.1	9.8	0.17	2.5	15	11.2	3.00	1.50	0.44	0.08
2	5.7	5.2	9.8	0.16	2.2	14	11.3	4.25	3.00	0.32	0.08
3	5.6	5.2	17.5	0.19	2.7	14	11.1	4.75	1.50	0.32	0.16
4	5.6	5.0	5.8	0.16	2.2	14	10.9	2.75	0.96	0.40	0.16
5	5.5	5.3	13.3	0.18	2.4	13	12.8	5.00	0.96	0.40	0.08
6	5.7	5.3	9.3	0.18	2.5	14	10.6	2.75	1.00	0.48	0.16
Range	5.5 – 5.8	5.0 – 5.3	5.8 – 17.5	.16 – .19	2.2 – 2.7	13 – 15	10.6 – 12.8	2.75 – 5	.96 – 3.00	.32 – .48	.08 – .16
Mean	–	–	10.9	0.17	2.4	14	11.3	3.75	1.49	0.39	0.12

Extr.* = Extractable P by double acid (0.05N HCL +0.025N H_2SO_4) method

No.** = Soil composite No.

Table 2d. Some chemical characteristics of the topsoil (0-20cm) of the field experiment.

No.**	pH		Extr.* P(ppm)	Total N (%)	Organic C (%)	C/N Ratio	CEC (me/100gm)	Exchangeable bases (me/100g)			
	1:2.5 (H_2O)	1:2.5 ($CaCl_2$)						Ca	Mg	K	Na
1	5.4	4.7	7.2	0.19	2.1	11	14.0	5.25	3.50	1.25	0.25
2	5.7	4.8	10.7	0.18	2.6	14	12.6	6.25	3.25	1.50	0.25
3	5.4	4.6	20.2	0.17	2.1	12	13.0	5.50	2.75	1.50	0.20
4	5.6	4.8	12.8	0.19	2.6	14	14.0	5.75	3.75	2.25	0.25
5	5.6	4.8	13.6	0.21	2.5	12	15.2	5.25	4.75	2.00	0.25
6	5.4	4.6	7.9	0.20	2.0	10	13.2	6.00	2.75	1.50	0.25
7	5.6	4.9	14.3	0.20	2.5	13	15.0	13.50	2.25	1.50	0.25
8	5.7	4.8	13.5	0.20	2.4	12	18.4	7.00	5.25	1.50	0.25
9	5.5	4.9	17.4	0.22	2.8	13	32.6	6.50	3.50	2.00	0.25
Range	5.4 – 5.7	4.5 – 4.9	7.2 – 20.2	.17 – .22	2.0 – 2.8	10 – 14	12.6 – 32.6	5.25 – 13.5	2.25 – 5.25	1.25 – 2.25	.20 – .25
Mean	–	–	13.0	0.20	2.4	12	16.4	6.80	3.50	1.70	0.20

Extr.* = Extractable P by double acid (0.05N HCL + 0.025N H_2SO_4) Method

No.** = Soil composite No.

Table 2e. Some chemical characteristics of the subsoil (20-40cm) of the field experiment.

No.**	pH		Extr.* P(ppm)	Total N (%)	Organic C (%)	C/N Ratio	CEC (me/100gm)	Exchangeable bases (me/100g)			
	1:2.5 (H_2O)	1:2.5 ($CaCl_2$)						Ca	Mg	K	Na
1	5.3	4.5	4.4	0.10	1.4	14	14.6	5.00	5.00	1.00	0.20
2	5.4	4.6	4.9	0.12	1.5	13	11.4	4.50	3.25	1.00	0.15
3	5.3	4.5	16.1	0.15	1.4	9	14.4	3.50	3.00	1.00	0.10
4	5.6	4.8	4.6	0.12	1.4	12	12.8	4.50	3.00	1.50	0.15
5	5.6	4.8	5.6	0.12	1.5	13	15.4	5.00	3.00	1.50	0.15
6	5.4	4.6	4.2	0.13	1.3	10	14.8	5.00	2.75	1.00	0.15
7	5.6	4.8	5.7	0.12	1.4	12	12.2	5.00	2.25	1.50	0.15
8	5.7	4.9	4.6	0.13	1.2	9	15.0	6.00	3.25	1.25	0.15
9	5.5	4.9	5.5	0.14	1.4	10	10.8	5.50	2.00	1.50	0.15
Range	5.3 – 5.7	4.5 – 4.9	4.2 – 16.1	0.10 – 0.15	1.2 – 1.5	9 – 14	10.8 – 15.4	3.50 – 6.00	2.00 – 5.00	1.00 – 1.50	0.10 – 0.15
Mean	–	–	6.2	0.13	1.4	11	13.5	4.90	3.10	1.30	0.15

Extra.* = Extractable P by double acid (0.05N HCL + 0.025N H_2SO_4) Method

No.** = Soil composite No.

4.1.5. Cation exchange capacity and exchangeable cations

Tables 2a and 2b show the distribution of cation exchange capacity (CEC) and exchangeable cations down the profiles of the soil used for greenhouse and field experiments. There was a consistent decrease down the greenhouse soil profile pit with Ap(0-9cm) horizon having a CEC of 11.0me/100g while AB(9-30cm), Bw_1(30-61cm) and Bw_2(61-132+cm) horizons had CECs of 9.0, 8.3 and 7.0me/100g, respectively. There was a decrease in CEC with depth in the field soil profile pit but it was not consistent. The Ap(0-15cm) horizon had the highest CEC, which was 14.2me/100g, compared with the lower horizons. The BA(15-36cm) horizon had a CEC of 12.0me/100g and Bw_1(36-80cm) horizon 9.8me/100g. The last horizon, i.e., Bw_2(80-150+cm), had a CEC ranging between 10.0 and 12.0me/100g.

Table 2c shows the CEC and exchangeable cations of the topsoil used for the greenhouse experiment while tables 2d and 2e show the CEC and exchangeable cations of the field experiment site topsoil and subsoil, respectively. The topsoil used for greenhouse experiment had a CEC ranging between 10.6 and 12.8me/100g with a mean of 11.3me/100g. The field experiment was conducted on a site with topsoil that had a CEC range of 12.6 to 32.6me/100g and a mean of 16.4me/100g. However, its subsoil had a CEC range of 10.8 to 15.4me/100g with a mean of 13.5me/100g.

The decrease in CEC with profile depth is most likely a reflection of the decrease in the amount of negative charges contributed by organic matter which also decreases with depth (Tables 2a and 2b). It is interesting to note that the highest CEC, viz., 32.6me/100g, is associated with the highest content of organic carbon which is 2.8% (Table 2d). The CEC which is on average lower than 16me/100g compares well with that of Owino-Gerroh (1988) for similar soil for which he obtained 14 and 11me/100g for depths of 0-15 and 15-30cm, respectively.

Table 2a shows that apart from sodium which remained constant throughout the depth of the profile of soil used for the greenhouse study, which was 0.08me/100g, other exchangeable cations decreased with depth. The exchangeable potassium, calcium and magnesium in the Ap(0-9cm) horizon were 0.40, 2.50 and 1.50me/100g, respectively, and the base saturation was 41%. The AB(9-30cm) horizon had lower exchangeable potassium, calcium and magnesium contents, namely, 0.12, 2.00 and 0.50me/100g, respectively, and also a lower base saturation, that is 30%. The Bw_1(30-61cm) horizon had exchangeable potassium content of 0.16me/100g, exchangeable calcium content of 3.50me/100g and exchangeable magnesium content of 0.68me/100g with a base saturation of 53%. The Bw_2(61-132+cm) had the least exchangeable potassium and calcium contents, namely, 0.08 and 1.25me/100g, respectively. The horizon had exchangeable magnesium content of 1.00me/100g and a base saturation of 34%. The general decrease of exchangeable cations and base saturation with profile depth was not surprising in this highly weathered soil used for greenhouse experiment. Consequently it can hardly replenish plant nutients from further weathering but rather derives them from mineralization of organic matter and/or additions of inorganic fertilizers to the soil.

The low base saturation was an indication of the poor fertility status of the soil used for the greenhouse experiment as supported by the data in Table 2c which show that the topsoil had exchangeable calcium ranging from 2.75 to 5me/100g with a mean of 3.75me/100g. Exchangeable magnesium ranged between .96 and 3.00me/100g with a mean of 1.49me/100g. However, mean exchangeable potassium and sodium amounts of 0.39 and 0.12me/100g, respectively were moderate for plant growth. The generally low fertility status of the soil used for greenhouse experiment was probably due to the fact that the soil had been under maize crops for several seasons before it was used for the experiment.

Table 2b shows that exchangeable potassium in the profile of soil used for the field experiment decreased with depth with Ap(0-15cm) horizon having 1.75me/100g while the other horizons, BA(15-36cm), Bw_1(36-80cm) and Bw_2(80-150cm+), had

1.25, 1.00 and 0.50 to 1.00me/100g, respectively. The exchangeable sodium content of 0.15me/100g in the Ap horizon was slighly higher than that in the BA horizon which was 0.10me/100g. However, the other two horizons, Bw_1 and Bw_2 had exchangeable sodium content of 0.02me/100g each. Exchangeable calcium, magnesium and base saturation showed no consistent trend down the field experiment profile pit. The Ap horizon had exchangeable calcium content of 4.50me/100g which was higher than that of the BA horizon, namely, 3.50me/100g. The Bw_1 and Bw_2 horizons had exchangeable calcium amounts of 4.30 and 4.13 to 4.75me/100g, respectively. The Ap horizon had the least amount of exchangeable magnesium, that is 1.50me/100g and also the least base saturation, that is, 56%. The BA horizon had exchangeable magnesium content of 2.25me/100g and a base saturation of 59%. The Bw_1 horizon had the highest amount of exchangeable magnesium, namely, 3.50me/100g and also the highest base saturation, namely, 90%. The last horizon, the Bw_2, had exchangeable magnesium ranging between 2.25 and 2.75me/100g and a base saturation ranging between 66 and 75%.

The tendency for exchangeable potassium and sodium to decrease with depth further supports the earlier suggestion that Ferralsols replenish their plant nutrients mainly from the mineralization of organic matter and/or addition of inorganic fertilizers to the surface horizons. Calcium, magnesium and base saturation tendency to generally increase with depth, though not consistently, was unusual because Calcium and Magnesium are less prone to leaching than Sodium and Potassium.

Tables 2d and 2e show the exchangeable cations of both topsoil and subsoil in the field experiment site. The mean exchangeable potassium content of 1.70me/100g of the topsoil was high but the subsoil had a mean content of 1.30me/100g which was moderate for plant growth. The mean of 0.20me/100g exchangeable sodium in the topsoil and that of 0.15me/100g in the subsoil were moderate. The mean exchangeable calcium amounts of 6.80me/100g in the topsoil and 4.90me/100g in the subsoil were moderate, while the mean exchangeable magmesium of 3.50 and 3.10me/100g in the topsoil and subsoil, respectively, were high by NARL standards. The fact that the field experiment site was under natural pastures for grazing for about five years before the experiment was planted seems to have facilitated the recycling of plant nutrients resulting in moderate to high contents of exchangeable bases and hence high base saturation.

4.2. RHIZOBIAL POPULATION IN BROTH AND INOCULANTS

Table 3 shows that the two Bradyrhizobium japonicum strains, namely, NUM 504 and NUM 508 multiplied rapidly in broth fermenters and carrier material. NUM 504 strain

Table 3. Counts of rhizobia in broth and inoculants.

Strain	*Counts/ml broth	**Counts/g inoculant
NUM 504	$1x10^{9}$	$8.18x10^{9}$
NUM 508	$7.5x10^{8}$	$2.25x10^{9}$

*Each figure is a mean of two counts.

**Each figure is a mean of four counts.

produced $1x10^9$ cells per ml broth which were slightly higher than $7.5x10^8$ cells per ml broth produced by NUM 508 strain. However, the total counts of rhizobia per gram of inoculant, that is $8.18x10^9$ for NUM 504 strain and $2.25x10^9$ for NUM 508 strain are acceptable by Nairobi MIRCEN standard which rejects inoculants whose total rhizobia counts fall below $1.0x10^8$ cells per gram inoculant (Wangaruro, 1987).

4.3. GREENHOUSE POT EXPERIMENT

4.3.1. Effects of nitrogen and Bradyrhizobium japonicum inoculation on growth of soybeans under greenhouse conditions.

Tables 4, 5 and 6 show the effect of nitrogen level and Bradyrhizobium japonicum inoculation on mean root, shoot and total dry weights of soybeans grown in a greenhouse. Data on the mean root dry weights (Table 4) show no consistent trend in root dry matter production of both non-inoculated and inoculated plants treated with increasing N levels. However, at each level of N root dry weigts of the inoculated plants were generally slightly higher than those of non-inoculated plants but the difference due to inoculation and nitrogen treatments were not statistically significant. However, Essa and Dulaimi (1985) reported increased root dry weight due to inoculation and nitrogen with cv. Williams and nitrogen levels

Table 4. Effect of nitrogen level, *Bradyrhizobium japonicum* inoculation and variety on the mean root dry weight (g/3plants) of soybeans grown in a greenhouse.

Nitrogen	Inoculation			Variety		
kgN/ha	None	NUM 504	NUM 508	Duiker	Sable	Mean
0	3.15	3.41	3.25	2.55	4.00	3.27A
20	3.25	3.20	3.34	2.72	3.81	3.27A
40	3.22	3.31	3.68	2.73	4.07	3.40A
80	3.25	3.74	3.30	3.01	3.86	3.43A
160	3.37	3.40	3.33	3.03	3.71	3.37A
Variety				Mean		
Duiker	2.688	2.99	2.75	2.81^z		
Sable	3.82	3.84	4.01	3.89^y		
Mean	3.25a	3.38a	3.41a			

R-square = 0.70 C.V. = 14.67% General mean = 3.35

SE(means) Nitrogen levels = ±0.12
SE(means) Inoculation = ±0.09
SE(means) Variety = ±0.07
SE(means) Nitrogen levels vs Inoculation = ±0.2
SE(means) Nitrogen levels vs variety = ±0.16
SE(means) Inoculation vs variety = ±0.13

Nitrogen and inoculation means within a column or a row respectively, with the same letter and varietal means followed by the same superscripts are not significantly different according to DMRT (P=0.05).

of upto 160kgN/ha in a field study. It is worthwhile noting that root growth in potted plants is restricted especially in crop varieties with a lot of vegetative growth, hence the limited response of the plants to nitrogen and inoculation in the present study with regard to root dry matter production.

The effect of inoculation on shoot dry weight was similar to that of total dry weight (Tables 5 and 6). The increase in shoot dry weights due to inoculation was highly significant (P<0.001) but variety x strain interaction was not significant. At each level of N, shoot dry weights of inoculated plants were higher than those of the non-inoculated plants. NUM 504 strain increased the shoot dry weight by 22.4%, from 8.65g to 10.09g. The linear relationship between nitrogen levels and shoot dry weights of both inoculated and non-inoculated plants was highly significant (P<0.001), Fig.1.

The increase in total plant dry weight which was a summation of root and shoot dry weights due to inoculation was also highly significant (P<0.001). At each level of N, total plant dry weights of inoculated plants were generally higher than the total plant dry weights of non-inoculated plants. A similar observation was made by Bishnoi and Dutt (1983) who reported higher dry matter yields in inoculated treatments compared with those for non-inoculated treatments receiving same nitrogen rates. NUM 504 strain increased total plant dry weight by 17.9%, from 11.87g to 13.99g, while NUM 508 strain

Table 5. Effect of nitrogen level, <u>Bradyrhizobium japonicum</u> inoculation and variety on the mean shoot dry weight (g/3plants) of soybeans grown in a greenhouse.

Nitrogen	Inoculation			Variety		
kgN/ha	None	NUM 504	NUM 508	Duiker	Sable	Mean
0	7.08	9.92	9.59	8.04	9.69	8.86B
20	8.12	10.45	10.04	9.14	9.93	9.54BA
40	8.58	10.24	10.07	8.46	10.80	9.63BA
80	9.22	11.22	10.65	9.56	11.16	10.36A
160	10.24	11.14	10.11	10.89	10.11	10.50A
Variety				Mean		
Duiker	8.17	10.40	9.08	9.22^{z}		
Sable	9.12	10.79	11.10	10.34^{Y}		
Mean	8.65b	10.59a	10.09a			

R-square = 0.62   C.V. = 14.22%   General mean = 9.78

SE(means) Nitrogen levels = ±0.33
SE(means) Inoculation = ±0.25
SE(means) Variety = ±0.21
SE(means) Nitrogen levels vs Inoculation = ±0.57
SE(means) Nitrogen levels vs Variety = ±0.46
SE(means) Inoculation vs Variety = ±0.36

Nitrogen and inoculation means within a column or a row respectively, with the same letter(s) and varietal means followed by the same superscripts are not significantly different according to DMRT (P=0.05).

Table 6. Effect of nitrogen level, Bradyrhizobium japonicum inoculation and variety on the mean total dry weight (g/3plants) of soybeans grown in a greenhouse.

N	Inoculation			Variety		
kgN/ha	None	NUM 504	NUM 508	Duiker	Sable	Mean
0	10.21	13.30	12.83	10.57	13.66	12.11B
20	11.33	13.64	13.37	11.83	13.73	12.78BA
40	11.78	13.52	13.60	11.16	14.77	12.96BA
80	12.46	14.93	13.92	12.54	15.00	13.77A
160	13.60	14.53	13.41	13.91	13.79	13.85A
Variety				Mean		
Duiker	10.85	13.36	11.80	12.00^{z}		
Sable	12.90	14.61	15.05	14.19^{y}		
Mean	11.87b	13.99a	13.42a			

R-square = 0.62 C.V. = 13.58% General mean = 13.09

SE(means) Nitrogen levels = ±0.42
SE(means) Inoculation = ±0.32
SE(means) Variety = ±0.27
SE(means) Nitrogen levels vs Inoculation = ±0.73
SE(means) Nitrogen levels vs Variety = ±0.59
SE(means) Inoculation vs Variety = ±0.46

Nitrogen and inoculation means within a column or a row respectively, with the same letter(s) and varietal means followed by the same superscripts are not significantly different according to DMRT (P=0.05).

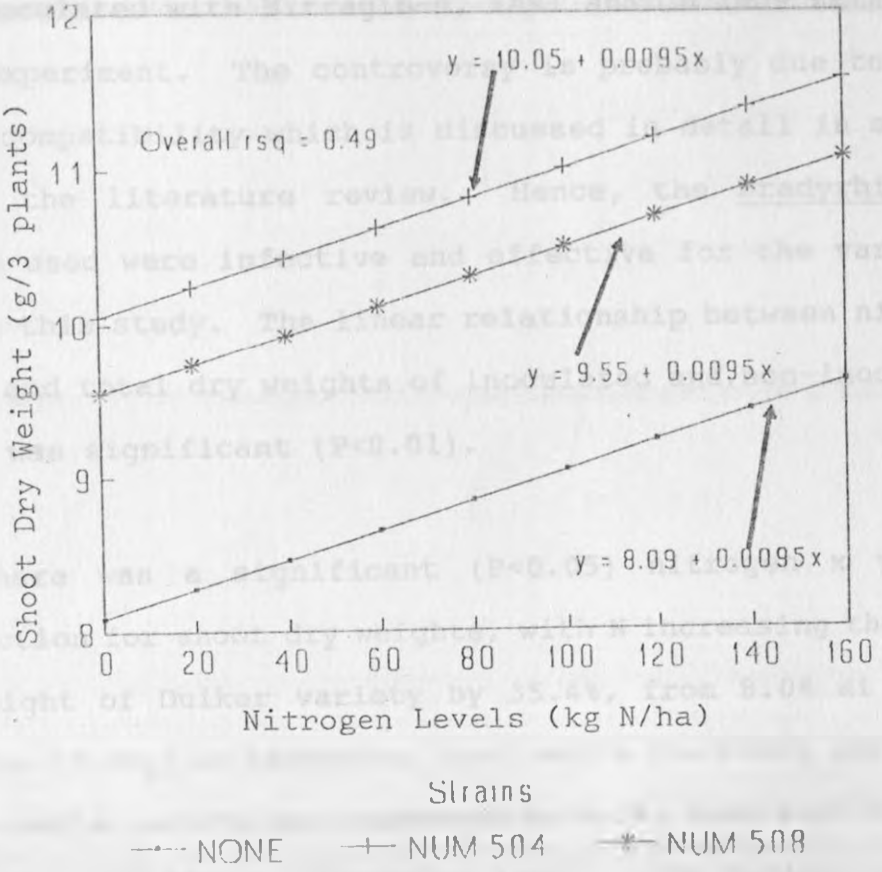

Fig.1. Relationship between N and shoot dry weight of soybeans grown with and without inoculation in a greenhouse

increased it by 13.42%, from 11.87g to 13.42g. This finding contradicts the results of Kang (1975) who reported non-significant differences in dry matter yield of soybean variety TK-5 inoculated with Nitragin-s, IARI and CB 1809 strains in a pot experiment. The controversy is probably due to host-strain compatibility which is discussed in detail in section 2.3 of the literature review. Hence, the Bradyrhizobium strains used were infective and effective for the varieties used in this study. The linear relationship between nitrogen levels and total dry weights of inoculated and non-inoculated plants was significant (P<0.01).

There was a significant (P<0.05) nitrogen x variety interaction for shoot dry weights, with N increasing the shoot dry weight of Duiker variety by 35.4%, from 8.04 at zero-N level to 10.89g, at 160kgN/ha level while the shoot dry weight of the Sable variety was increased by 4.3%, from 9.69 at zero-N level to 10.11g at 160kgN/ha level. The Duiker variety, which is shorter and has less vegetative growth, responded more to applied N than the Sable variety in the greenhouse experiment (Fig.2). Variety x nitrogen interaction for total dry weight was also significant (P<0.05). Total dry weight of Duiker variety was increased by 31.6% from 10.57g at zero-N level to 31.6g, at 160kgN/ha level and that of the Sable varity was increased by 1.0%, from 13.66g at zero-N level to 13.79g at 160kgN/ha level. The linear increase in total dry

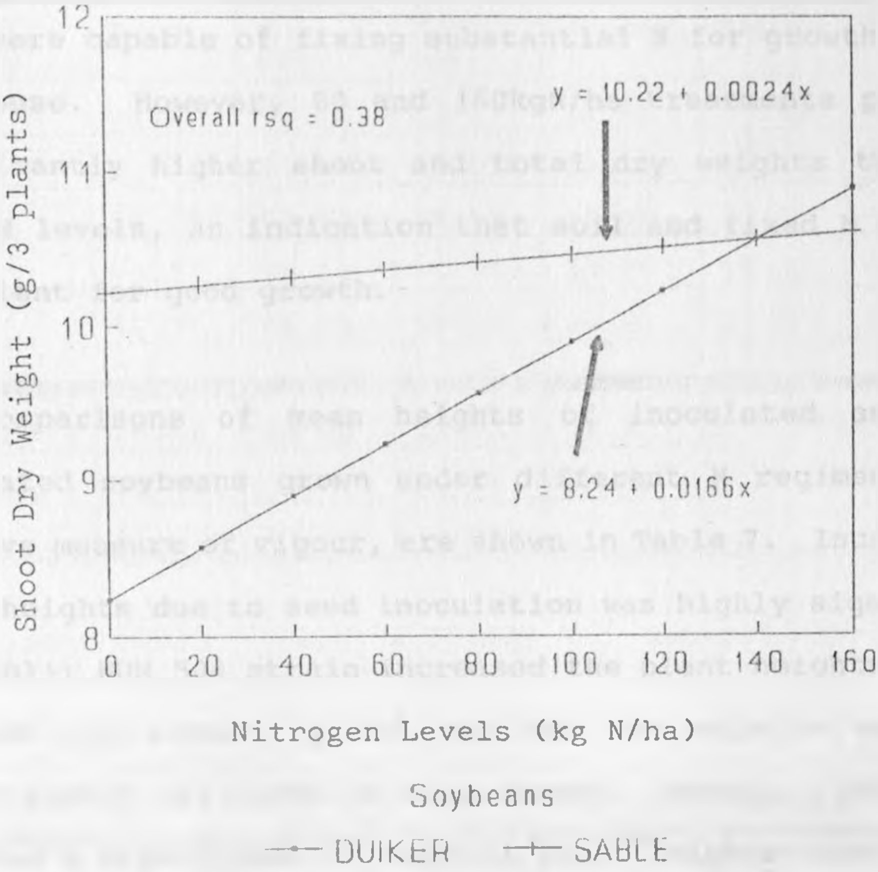

Fig.2. Relationship between N and shoot dry weight of Duiker and Sable varieties of soybeans grown in a greenhouse

weight with increase in N level was highly significant ($P<0.001$). It is interesting to note that both shoot and total dry weights of 0, 20 and 40kgN/ha levels are statistically the same, suggesting that soybeans in the zero-N level were capable of fixing substantial N for growth in the greenhouse. However, 80 and 160kgN/ha treatments produced significantly higher shoot and total dry weights than the lower N levels, an indication that soil and fixed N was not sufficient for good growth.

Comparisons of mean heights of inoculated and non-inoculated soybeans grown under different N regimes, as a relative measure of vigour, are shown in Table 7. Increase in plant heights due to seed inoculation was highly significant ($P<0.001$); NUM 504 strain increased the plant heights by 15% and NUM 508 strain by 22% but the two strains were not significantly different in this aspect. Nangju (1980) also reported a significant increase in plant heights due to seed inoculation under field conditions. Inoculation x nitrogen interaction was significant ($P<0.05$) as non-inoculated plants attained optimum height of 47.23cm at 80kg/ha level, while plants inoculated with NUM 504 attained their maximum height of 53.62cm at 40kgN/ha level. Plants inoculated with NUM 508 were also relatively tall (51.18cm) at 40kgN/ha level but at 80kg/ha level the plant heights declined to 41.18cm then increased up to 57.98cm at 160kgN/ha level.

Table 7. Effect of nitrogen level, <u>Bradyrhizobium japonicum</u> inoculation and variety on the mean plant height (cm) of soybeans grown in a greenhouse.

Nitrogen	Inoculation			Variety		
kgN/ha	None	NUM 504	NUM 508	Duiker	Sable	Mean
0	36.50	43.45	50.77	30.52	56.62	43.57A
20	42.42	44.88	50.43	32.14	59.68	45.91A
40	38.15	53.62	51.18	31.98	63.32	47.65A
80	47.23	49.18	41.18	33.24	58.49	45.87A
160	41.77	46.05	57.98	36.20	61.00	48.60A
Variety				Mean		
Duiker	29.52	35.00	33.93	32.82^{z}		
Sable	52.91	59.87	66.69	59.82^{y}		
Mean	41.21b	47.44a	50.31a			

R-square = 0.83 C.V. = 18.53% General mean = 46.32

SE(means) Nitrogen levels = ±2.02
SE(means) Inoculation = ±1.57
SE(means) Variety = ±1.28
SE(means) Nitrogen levels vs Inoculation = ±3.50
SE(means) Nitrogen levels vs Variety = ±2.86
SE(means) Inoculation vs Variety = ±2.22

Nitrogen and inoculation means within a column or a row respectively, with the same letter and varietal means followed by the same superscripts are not significantly different according to DMRT (P=0.05).

These results indicate that non-inoculated plants needed more nitrogen (80kgN/ha) to attain their optimum heights of 47.23cm while inoculated plants attained the heights of between 51.18 and 53.62cm at 40kgN/ha.

4.3.2. Effects of nitrogen and Bradyrhizobium japonicum Inoculation on the nodulation of soybeans grown in the greenhouse.

At 63 days after planting, the two inoculants had formed nodules with the plants (Table 8) which were mainly distributed on the crown of tap roots and a few scattered on the lateral roots. The nodules were large and medium sized with few small ones. A few nodules dissected from each pot showed that most of them were effective in biological nitrogen fixation as they had pink coloration, which is a usual sign, though some had turned green while others were black. The possibility of some nodules having being lost through decay prior to harvesting could not be ruled out, thus for proper nodulation assessment in the greenhouse plants should have been harvested earlier, probably 45 days after planting. The high ambient temperature (maximum 41°C) at the time of sowing and the subsequent days before the rhizosphere effect of young soybean seedlings induced multiplication of the rhizobia could also have interfered with nodulation. Brockwell et al (1988) found that inoculant mortality in the first 24 hours was 20 times greater when ambient temperature on the day of sowing was 38° than when it was 28°C.

The high coefficient of variation (51.42%) of the mean number of nodules when transformed which signal caution in interpreting the data could not easily be attributed to one particular factor. However, nodule decay in some treatments together with the sample size of only theee plants, which is small, presumably contributed to the high variability of the data. The effect of inoculation in increasing the nodule numbers in the soil, which had not been cropped with soybeans for a period of over ten years, was highly significant ($P<0.001$). This concurs well with the finding of Singleton and Tavares (1986) who reported a highly significant increase in nodule numbers due to inoculation of soybeans planted in four different soils in a greenhouse. Most of the non-inoculated plants did not form nodules at all except in a few isolated cases which were attributed to contamination either during planting or while uprooting the weeds. The soil had no native <u>Bradyrhizobium japonicum</u> bacteria as shown by the MPN counts. On average, NUM 504 strain formed 18.73 nodules per three plants while NUM 508 strain formed 16.67 nodules per the same number of plants which are significantly higher than the 2.21 nodules per three plants formed by non-inoculated plants.

Table 8. Effect of nitrogen level, <u>Bradyrhizobium japonicum</u> inoculation and variety on the mean[δ] number of nodules formed per three plants of soybeans grown in a greenhouse.

N	Inoculation			Variety		
kgN/ha	None	NUM 504	NUM 508	Duiker	Sable	Mean
0	2.27	21.00	32.25	15.62	12.43	13.97A
20	2.16	17.76	21.10	9.08	12.11	10.53BAC
40	3.02	18.56	23.62	10.07	15.32	12.50BA
80	2.03	20.06	7.66	8.35	6.08	7.15BC
160	2.03	16.59	6.97	4.37	8.76	6.33C
Variety				Mean		
Duiker	2.25	18.65	13.29	9.02^y		
Sable	2.17	18.89	20.49	10.64^y		
Mean	2.21b	18.73a	16.67a			

R-square = 0.75 C.V. = 51.42% General mean =9.78

SE(means) Nitrogen levels = ±0.34
SE(means) Inoculation = ±0.26
SE(means) Variety = ±0.21
SE(means) Nitrogen levels vs Inoculation = ±0.59
SE(means) Nitrogen levels vs Variety = ±0.48
SE(means) Inoculation vs Variety = ±0.37

Nitrogen and inoculation means within a column or a row respectively, with the same letter(s) and varietal means followed by the same superscripts are not significantly different according to DMRT (P=0.05).

[δ] These data were subjected to √x transformation before analysis of variance was done. The treatment means presented in the table have been retransformed.

Nodule numbers showed a significant ($P<0.05$) decrease with application of N. The mean number of nodules formed per three plants at 80kgN/ha level (7.15) are significantly lower than those formed by unfertilized plants (13.97). Plants supplied with the highest rate of nitrogen which was of 160kgN/ha, formed the least number of nodules, namely, 6.33 per three plants, which was significantly lower than the number formed by unfertilized plants as well as those fertilized with 40kgN/ha. The inverse relationship between nitrogen levels and nodule numbers was significant ($P<0.01$). Franklin and Allison (1934) reported similar results with alfalfa seedlings grown in a greenhouse for 12 days. They observed that increasing the concentration of nitrate from zero-N treatment to 80mgN/pot decreased nodule production proportionately from 4.52 in the zero-N treatment to 0.34 in the highest concentration. The decrease was associated with inadequate carbohydrate supply in the roots as the effect of N on the bacteria themselves plays a secondary role. Where nitrogen is abundant the synthesized carbohydrate is used for growth of the tops and little is available for growth of the roots or nodules.

4.3.3. Effects of nitrogen and Bradyrhizobium japonicum inoculation on percent nitrogen in shoots of soybeans grown in the greenhouse.

Inoculation and nitrogen had no significant effect on nitrogen concentration in shoots of plants grown in the greenhouse when expressed as percent on dry weight basis (Table 9). Similar observations were made by Alaides et al (1979) at 35 and 55-day harvests for percent nitrogen of shoots of soybeans grown in the greenhouse. The low R-square figure of 0.41 was an indication that the standard model used for the analysis of variance, Section 3.6.5. was not good enough for the statistical analysis of nitrogen concentration data when expressed as percent nitrogen. While it was not easy to speculate why the model did not fit these particular data well, it could be presumed that some of the assumptions made while formulating it were not true in this case. In additon, it was difficult to include equal proportions of leaf lamina, pod, flower, petiole and stem which constitute the shoot in every 0.5g sample used for nitrogen analysis. Singh and Saxena (1978) reported mean percent nitrogen contents of 4.31, 3.42, 2.85, 1.16 and 0.91 in leaf lamina, pod, flower, petiole and stem, respectively for soybean in 1969 season at 65 days after sowing but nitrogen content of the flower was determined at 50 days after sowing. Therefore apart from the effect of the treatment, nitrogen concentration in every sample most likely depended additionally on the proportion of each of the five portions of the shoot which confounded the interpretation of the results.

Table 9. Effect of nitrogen level, <u>Bradyrhizobium japonicum</u> inoculation and variety on the mean [*]percent nitrogen content in shoots of soybeans grown in a greenhouse.

Nitrogen	Inoculation			Variety		
kgN/ha	None	NUM 504	NUM 508	Duiker	Sable	Mean
0	2.26	2.35	2.39	2.28	2.39	2.33A
20	2.08	2.44	2.27	2.25	2.27	2.26A
40	2.26	2.30	2.20	2.30	2.20	2.24A
80	2.21	2.31	2.19	2.28	2.20	2.23A
160	2.41	2.24	2.44	2.31	2.42	2.36A
Variety				Mean		
Duiker	2.26	2.36	2.23	2.28^{Y}		
Sable	2.22	2.30	2.36	2.29^{Y}		
Mean	2.24a	2.33a	2.29a			

R-square = 0.41 C.V. = 10.02% General mean = 2.29

SE(means) Nitrogen levels = ±0.05
SE(means) Inoculation = ±0.04
SE(means) Variety = ±0.03
SE(means) Nitrogen levels vs Inoculation = ±0.09
SE(means) Nitrogen levels vs Variety = ±0.08
SE(means) Inoculation vs Variety = ±0.06

Nitrogen and Inoculation means within a column or a row respectively, with the same letter and varietal means followed by the same superscripts are not significantly different according to Scheffe's test (P=0.05).

[*] All values were expressed on dry matter bases.

Generally there was an improvement in the nitrogen concentration of inoculated plants over non-inoculated ones. Averaged over the nitrogen levels, non-inoculated plants had a mean percent nitrogen content of 2.24 while plants inoculated with NUM strains 504 and 508 had mean percent nitrogen contents of 2.33 and 2.29, respectively. Effect of inoculation was most conspicuous in the absence of nitrogen fertilizer (Plate 1). As the level of nitrogen fertilizer was increased, the difference due to inoculation became smaller. The antagonism between the high supply of nitrogen in solution culture in the greenhouse and symbiosis reported by Allos and Bartholomew (1955) should be responsible for this observation; soybean plants supplied with varying quantities of nitrogen, namely, 0, 108, 216 and 432mgNpot fixed 310, 289, 195 and 135mgN/pot, respectively. Therefore as nitrogen level increased, the percentage of nitrogen derived from symbiosis decreased.

Although there was no consistent trend in percent nitrogen concentration of the shoot with increasing levels of nitrogen treatment, plants supplied with the highest nitrogen rate of 160kgN/ha had the highest percent nitrogen concentration in most cases. The non-inoculated plants that were planted without N-fertilizer had percent nitrogen concentration of 2.26 while the non-inoculated plants supplied with 160kgN/ha had nitrogen content of 2.41%. Plants that were sown without N-fertilizer but inoculated with NUM 504 strain had percent nitrogen concentration of 2.35 which was just slightly lower than the 2.44% of the plants inoculated with the same strain but supplied with 20kgN/ha. Increasing the N levels above 20kgN/ha did not improve the percent nitrogen content of plants inoculated with NUM 504 further. This observation was amazing because plant dry matter yield increased linearly with increasing levels of N. Possibly the use of the whole shoot instead of its various components separately which have different nitrogen contents as already mentioned above contributed to this anomaly. Plants inoculated with NUM 508 had the highest nitrogen content (2.44%) at 160kgN/ha. Similarly, Duiker and Sable varieties had the highest nitrogen contents, namely, 2.31 and 2.42%, respectively, at 160kgN/ha. Similar increases were also observed by Singh and Saxena (1978) on percent nitrogen concentration of leaf lamina, pod, flower, petiole and stem of inoculated and non-inoculated soybean variety Bragg, 65 days after planting in the field.

All plants were uniform in size and healthy upto about 35 days after which non-inoculated plants in the zero nitrogen treatments became slightly chlorotic but N deficiency symptoms became more severe with time. Near harvest time marked differences were observed between non-inoculated plants receiving different rates of N with those at the lower levels

(0, 20 and 40kgN/ha) looking slightly chlorotic compared with those receiving 80 and 160kgN/ha which were green. At the end of the study, all inoculated plants were green with no discernible colour differences irrespective of the nitrogen rate applied.

Plate 1. Non-inoculated plants (left) grown without N fertilizer showed nitrogen deficiency symptoms and were less vigorous in growth than inoculated ones (right) which were not supplied with N fertilizer under greenhouse conditions.

4.4. FIELD EXPERIMENT

4.4.1. Effects of nitrogen and *Bradyrhizobium japonicum* inoculation on percent nitrogen concentration in shoots of soybeans grown in the field.

Data for nitrogen contents in shoots of the plants grown in the field are shown in Table 10. Like for the greenhouse results for percent nitrogen concentration in shoots, the R-square figure for the field experiment results was low (0.29), hence it was not surprising that nothing turned out significant in the analysis of variance. However, according to Duncans Multiple Range Test (DMRT) P=0.05, percent nitrogen content of 3.77 in plants inoculated with NUM 508 strain was significantly higher than the 3.59% in shoots of the non-inoculated plants. Kang (1975) also reported a significant increase in nitrogen uptake due to inoculation of soybean variety TK-5 with nitragin-S grown in the field. Nitrogen content of 3.66% in shoots of plants inoculated with NUM 504 was higher but not significantly different from that of the non-inoculated plants. Since the mean percent nitrogen contents of inoculated and non-inoculated plants have been averaged over the nitrogen levels and soil nitrogen (Tables 2d and 2e) occurred fairly uniformly in the field site, the extra nitrogen in inoculated plants was most likely derived from atmospheric nitrogen fixaion. The two strains of

fixation

Table 10. Effect of nitrogen level, <u>Bradyrhizobium japonicum</u> inoculation and variety on the mean percent nitrogen content in shoots of soybeans grown in the field.

Nitrogen	Inoculation			Variety		
kgN/ha	None	NUM 504	NUM 508	Duiker	Sable	Means
0	3.63	3.74	3.66	3.53	3.83	3.68A
20	3.51	3.70	3.76	3.69	3.62	3.66A
40	3.62	3.67	3.77	3.77	3.61	3.69A
80	3.67	3.49	3.88	3.75	3.61	3.68A
160	3.52	3.72	3.78	3.70	3.64	3.67A
Variety				Mean		
Duiker	3.59	3.72	3.75	3.69^y		
Sable	3.59	3.60	3.78	3.66^y		
Mean	3.59b	3.66ba	3.77a			

R-square = 0.29 C.V. = 8.80% General mean = 3.67

SE(means) Nitrogen levels = ±0.08
SE(means) Inoculation = ±0.06
SE(means) Variety = ±0.05
SE(means) Nitrogen levels vs Inoculation = ±0.13
SE(means) Nitrogen levels vs Variety = ±0.11
SE(means) Inoculation vs Variety = ±0.08

Nitrogen and inoculation means within a column or a row respectigely, with the same letter(s) and varietal means followed by the same superscripts are not significantly different according to DMRT (P=0.05).

All values were expressed on dry matter bases.

Bradyrhizobium japonicum used as sources of inoculants, namely NUM 504 and NUM 508, were not significantly different in biological nitrogen fixation.

Application of N fertilizer had no significant effect on percent nitrogen concentration of the shoots. However, various workers have shown that N fertilizer can increase the nitrogen uptake of non-nodulated soybean plants (Dean and Clark 1980; Hatum 1976; Pal and Norman 1987) as well as of nodulated cultivals (Matsunaga et al 1983). The reason for the failure by the plants in the present study to respond to N application as far as percent nitrogen was concerned, although they responded in terms of shoot dry weight, grain size, number of grains and pods per plant together with grain nitrogen, was not clear. But the processing of the whole shoot for nitrogen analyses as discussed in Section (4.3.3.) probably contributed to the behaviour of the results.

Plants had similar growth patterns to those for the greenhouse experiment. All plants were uniform in size and healthy upto 60 days after planting, when the leaves of non-inoculated plants at zero nitrogen treatments started turning pale yellow and N deficiency symptoms worsened with time. Towards physiological maturity, some differences were observed between uninoculated plants receiving lower levels of nitrogen (0, 20 and 40kgN/ha), which were slightly pale yellow, and those receiving high N levels (80 and 160kgN/ha), which were dark green. Most of the inoculated plants remained green irrespective of the N fertilizer applied until they attained physiological maturity. At physiological maturity, plants from the inoculated treatments showed less leaf senescence than those from uninoculated treatments even at the highest rate of N fertilizer.

4.4.2. Effects of nitrogen and Bradyrhizobium japonicum inoculation on the growth of soybeans in the field.

Table 11 shows the effects of N treatment and Bradyrhizobium japonicum inoculation on the mean shoot dry weights of soybeans grown in the field. Despite the fact that inoculation did not increase the dry weights significantly, its effect cannot be ignored as it improved the dry matter yield of the plants substantially. NUM 504 strain increased the shoot dry weights by 8.5%, from 152 to 164.87g, while NUM 508 strain increased them by 14%, from 152 to 173.23g. Higher percent increase (36-47) in fresh weights of soybeans due to inoculation have been reported (Nangju, 1980). Possibly the nitrogen content of the topsoil in the experimental site, which was 0.2% and regarded as just sufficient for plant growth according to NARL standards, contributed to the low percent increase in shoot dry weights due to inoculation.

Table 11. Effect of nitrogen level, *Bradyrhizobium japonicum* inoculation and variety on the mean shoot dry weight (g/3plants) of soybeans grown in the field.

Nitrogen	Inoculation			Variety		
kgN/ha	None	NUM 504	NUM 508	Duiker	Sable	Mean
0	137.33	148.83	141.67	117.33	167.89	142.61B
20	139.17	136.17	132.67	131.22	140.78	136.00B
40	126.33	166.67	157.83	132.22	168.33	150.28B
80	182.50	181.83	216.00	150.56	236.33	193.44A
160	174.67	190.83	218.00	142.88	246.11	194.50A
Variety				Mean		
Duiker	125.00	141.00	138.53	134.84^{z}		
Sable	179.00	188.73	207.93	191.89^{y}		
Mean	152.00a	164.87a	173.23a			

R-square = 0.55 C.V. = 32.25% General mean = 163.37

SE(means) Nitrogen levels = ±12.42
SE(means) Inoculation = ±9.62
SE(means) Variety = ±7.85
SE(means) Nitrogen levels vs Inoculation = ±21.51
SE(means) Nitrogen levels vs Variety = ±17.56
SE(means) Inoculation vs variety = ±13.60

Nitrogen and inoculation means within a column or a row respectively with the same letter and varietal means followed by the same superscripts are not significantly different according to DMRT (P=0.05).

Decrease in symbiotic nitrogen fixation when N is available has already been documented (Allos and Bartholomew, 1955; Sears and Lynch, 1951).

Nitrogen significantly ($P<0.01$) increased the shoot dry weights. The dry weights produced by the application of 20 and 40kgN/ha levels, were statistically similar and significantly lower than the dry weights produced by the application of 80 and 160kgN/ha levels. The results compare well with those of other researchers; Afza et al (1987) reported a significant increase in the dry matter yield of soybeans due to the application of 60kgN/ha while Pasaribu et al (1987) reported a significant increase due to the application of 80kgN/ha in field experiments. Unlike the results for the pot experiment for shoot dry matter yields, variety x nitrogen interaction was not significant. Duiker and Sable varieties responded similarly to N application dispelling the notion that N application has very little effect on the vegetative growth of the Sable variety.

There was a significant ($P<0.01$) linear relationship between the shoot dry weights of both the inoculated and non-inoculated plants and increasing levels of N (Fig. 3). Although the relationship was weak ($r^2=0.27$), it indicated that a further increase in shoot dry weights would have been expected with N levels that were even higher than 160kgN/ha.

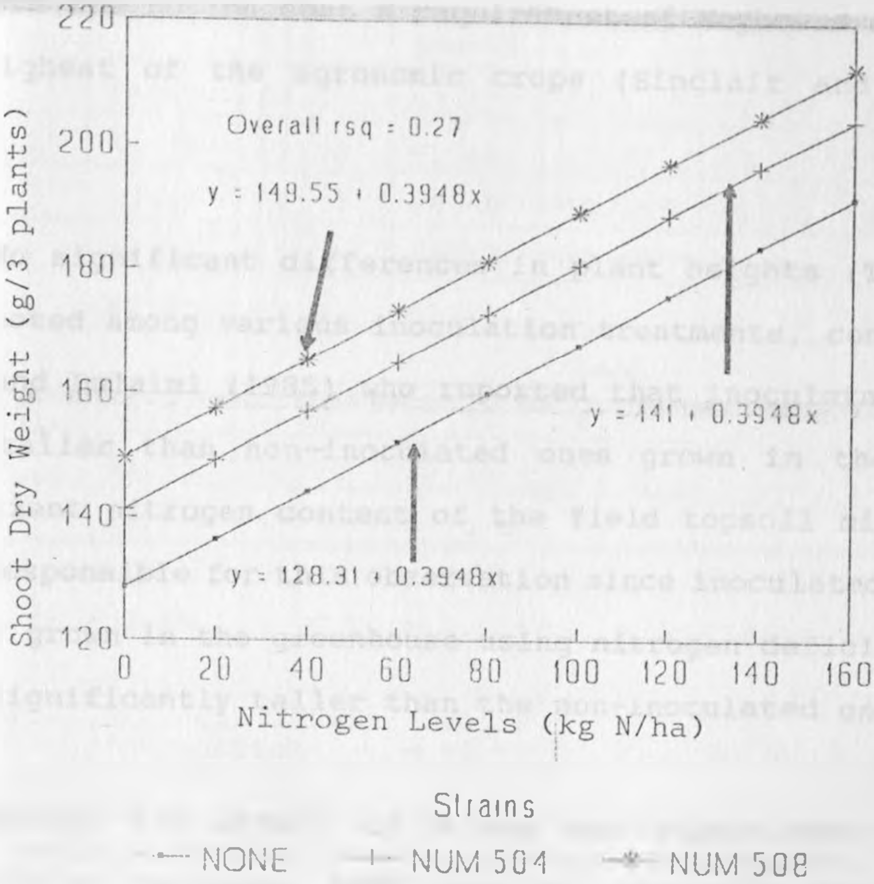

Fig.3. Relationship between N and shoot dry weight of soybeans grown with and without inoculation in the field

In view of the fact that the topsoil was not deficient in nitrogen, the linear response of the plant to a nitrogen level as high as 160kgN/ha with regard to dry matter production supports the notion that N requirement of soybeans is one of the highest of the agronomic crops (Sinclair and de Wit, 1975).

No significant differences in plant heights (Table 12) were noted among various inoculation treatments, contrary to Essa and Dulaimi (1985) who reported that inoculated plants were taller than non-inoculated ones grown in the field. Sufficient nitrogen content of the field topsoil might have been responsible for this observation since inoculated soybean plants grown in the greenhouse using nitrogen-deficient soil were significantly taller than the non-inoculated ones.

Though the effect of N was not significant in the analysis of variance, DMRT ($P=0.05$) indicated that plants treated with 40kgN/ha were statistically similar in height to those treated with 0, 20 and 80kgN/ha but significantly taller than those treated with 160kgN/ha. These results disagree with Olsen et al's (1975) suggestion that N fertilizer has little influence on soybean plant height provided the plants are well nodulated and other nutrients are not limiting. A closer look at the data reveals a favourable combination of Bradyrhizobium japonicum inoculation and 40 kgN/ha which has

Table 12. Effect of nitrogen level, <u>Bradyrhizobium japonicum</u> inoculation and variety on the mean plant height (cm) of soybeans grown in the field.

N	Inoculation			Variety		
kgN/ha	None	NUM 504	NUM 508	Duiker	Sable	Mean
0	63.05	64.33	63.48	56.83	70.41	63.62BA
20	65.32	61.98	61.60	57.42	68.51	62.97BA
40	63.45	64.17	66.88	58.27	71.40	64.83A
80	64.68	62.28	63.10	56.47	70.24	63.36BA
160	60.67	62.73	62.82	55.96	68.19	62.07B
Variety				Mean		
Duiker	56.94	56.20	57.82	56.99^z		
Sable	69.93	70.00	69.33	69.75^y		
Mean	63.43a	63.10a	63.57a			

R-square = 0.89 C.V. = 4.55% General mean = 63.37

SE(means) Nitrogen levels = ±0.68
SE(means) Inoculation = ±0.53
SE(means) Variety = ±0.43
SE(means) Nitrogen levels vs Inoculation = ±1.18
SE(means) Nitrogen levels vs Variety = ±0.96
SE(means) Inoculation vs Variety = ±0.74

Nitrogen and inoculation means within a column or a row respectively, with the same letter(s) and varietal means followed by the same superscripts are not significantly different according to DMRT (P=0.05).

contributed to this increased plant height.

4.4.3. Effects of nitrogen and *Bradyrhizobium japonicum* inoculation on nodulation of soybeans grown in the field.

Table 13 shows that many plants at zero-N and 20kgN/ha levels had excellent nodulation after inoculation with either NUM 504 or NUM 508 strains of *Bradyrhizobium japonicum* in the field compared with the greenhouse plants. The natural temperatures of the field experiment (Appendix 11) coupled with moderate organic matter content of the topsoil possibly provided a favourable environment for the rhizobia. In a study by Weaver and Frederick (1970) it was reported that percent organic matter and population of *Bradyrhizobium japonicum* were highly correlated with greater *Bradyrhizobium japonicum* populations present in soils with higher organic matter. The nodules from the field experiment which were pigmented with legheamoglobin were mainly distributed on the lateral roots but a few of them were found distributed on the crown and the rest of the taproot. In most cases, the plants had a mixture of large, medium and small nodules but where the nodules were few, they tended to be large or medium in size.

The effect of inoculation in increasing the nodule numbers was highly significant (P<0.001) in this field where soybeans had not been grown before. These results agree quite well with those of Muldoon et al (1980) and generally with those of other researchers including Papastylianou (1986); Rao and Patil, (1977). NUM 504 and NUM 508 strains produced an avarage of 97.20 and 96.25 nodules per three plants, respectively. The presence of a few nodulated plants in the non-inoculated treatments where the indigenous rhizobia had been found lacking by MPN counts might have been due to either rhizobia being carried by the seed or cross-contamination from adjacent plots.

Application of N fertilizer resulted in a highly significant (P<0.001) decrease in nodule numbers. The finding is consistent with most of the data in literature which show that a reduction in nodulation occurs whenever N fertilizer is applied (Abdalla et al, 1985; Bishnoi and Dutt, 1980; Sunarlim et al, 1980). The number of nodules formed by unfertilized plants and those receiving 20kg N/ha were statistically similar and significantly higher than the number of nodules formed by plants fertilized with 40, 80 and 160kgN/ha. The nodule numbers showed a highly significant (P<0.001) inverse relationship with increase in the rate of applied N for both varieties. The mean nodule numbers decreased from 73.08 and 73.73 at zero-N treatments to 29.72 and 24.75 at 160kgN/ha level for Duiker and Sable varieties, respectively.

Table 13. Effect of nitrogen level, <u>Bradyrhizobium japonicum</u> inoculation and variety on the mean [δ]number of nodules formed per three plants of soybeans grown in the field.

Nitrogen	Inoculation			Variety		
kgN/ha	None	NUM 504	NUM 508	Duiker	Sable	Mean
0	6.62	163.00	133.92	73.08	73.73	73.40A
20	6.03	120.06	177.61	55.28	85.92	69.68A
40	6.03	83.97	91.19	29.43	59.44	42.97B
80	6.11	65.29	71.12	35.81	33.77	34.84B
160	6.00	70.16	39.41	29.72	24.75	27.16B
Variety				Mean		
Duiker	6.11	81.52	90.64	42.97^{y}		
Sable	6.06	114.37	101.84	52.51^{y}		
Mean	6.08b	97.20a	96.25a			

R-square = 0.86 C.V. = 38.00% General mean = 47.60

SE(means) Nitrogen levels = ±0.58
SE(means) Inoculation = ±0.45
SE(means) Variety = ±0.37
SE(means) Nitrogen levels vs Inoculation = ±1.00
SE(means) Nitrogen levels vs Variety = ±0.82
SE(means) Inoculation vs Variety = ±0.63

Nitrogen and inoculation means within a column or a row respectively, with the same letter and varietal means followed by the same superscripts are not significantly different according to DMRT (P=0.05).

[δ] This data was subjected to √x transformation before analysis of variance was done. The treatment means presented in the table have been retransformed.

There was a significant ($P<0.05$) interaction between Bradyrhizobium japonicum strains and nitrogen for nodule numbers. Such an interaction was reported by Olufajo (1990). NUM 508 strain was affected more than NUM 504 strain by increasing levels of N (Fig.4) though the nodule numbers produced by both strains showed a highly significant ($P<0.001$) inverse relationship with increase in the rate of applied N. The mean nodule numbers produced by inoculation with NUM 508 strain were decreased from 177.61 at 20kgN/ha to 39.41 at 160kgN/ha while those produced by inoculation with NUM 504 strain were decreased from 163.00 at zero-N to 70.16 at 160kgN/ha. The observation implies that NUM 504 strain can tolerate high N levels much better than NUM 508 strain can.

The mean nodule dry weights (Table 14) generally showed a similar trend to the mean nodule numbers. The increase in nodule dry weights due to inoculation was highly significant ($P<0.001$). Such an increase in dry weights of nodules due to inoculation of soybean seeds with commercial inoculants of Bradyrhizobium japonicum have been reported by some workers e.g., Essa and Dulaimi, 1985; Rao and Patil, 1977. Averaged over the nitrogen levels, the dry weights of nodules produced by NUM 504 and NUM 508 were 0.68 and 0.67g, respectively, while the dry weights of nodules formed by non-inoculated plants were negligible. There was a significant ($P<0.05$) interaction between variety and Bradyrhizobium japonicum

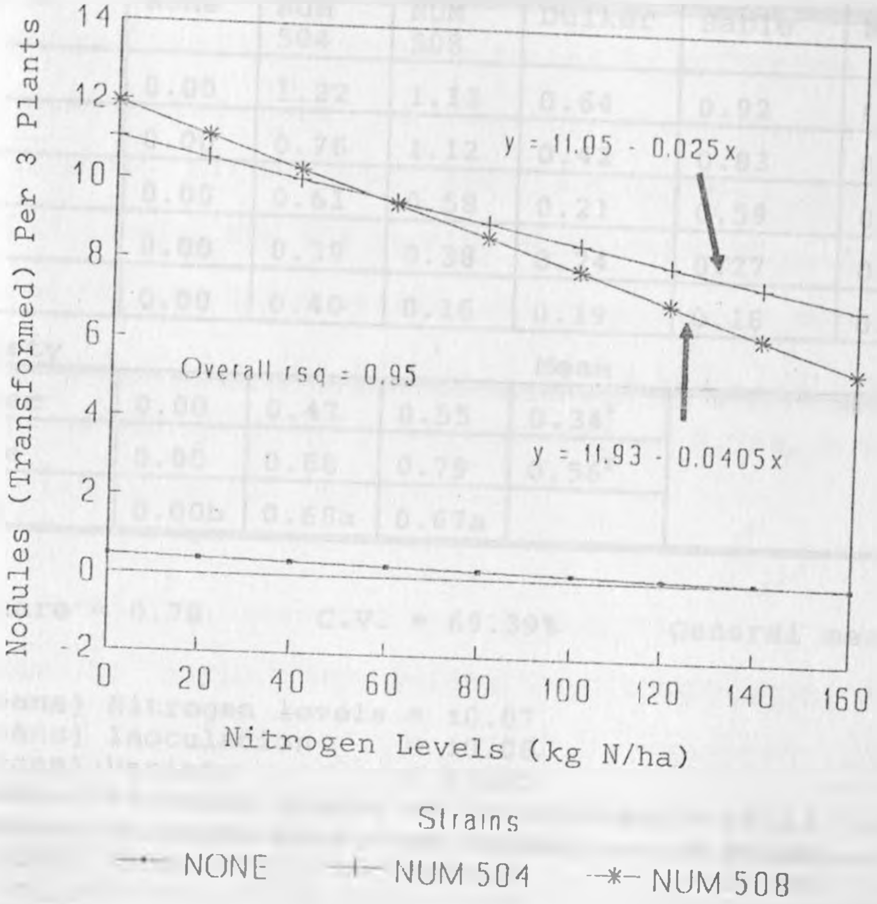

Fig.4. Relationship between N and nodule count of soybeans grown with and without inoculation in the field

Table 14. Effect of nitrogen level, *Bradyrhizobium japonicum* inoculation and variety on the mean nodule dry weight (g/3plants) of soybeans grown in the field.

Nitrogen	Inoculation			Variety		
kgN/ha	None	NUM 504	NUM 508	Duiker	Sable	Mean
0	0.00	1.22	1.13	0.64	0.92	0.78A
20	0.00	0.76	1.12	0.42	0.83	0.62A
40	0.00	0.61	0.58	0.21	0.59	0.40B
80	0.00	0.39	0.38	0.24	0.27	0.25B
160	0.00	0.40	0.16	0.19	0.18	0.19B
Variety				Mean		
Duiker	0.00	0.47	0.55	0.34^z		
Sable	0.00	0.88	0.79	0.56^y		
Mean	0.00b	0.68a	0.67a			

R-square = 0.78 C.V. = 69.39% General mean = 0.45

SE(means) Nitrogen levels = ±0.07
SE(means) Inoculation = ±0.06
SE(means) Variety = ±0.05
SE(means) Nitrogen levels vs Inoculation = ±0.13
SE(means) Nitrogen levels vs Variety = ±0.10
SE(means) Inoculation vs Variety = ±0.08

Nitrogen and inoculation means within a column or a row respectively, with the same letter and varietal means followed by the same superscripts are not significantly different according to DMRT (P=0.05).

strains for nodule dry weight. Similar variations in nodulation responses between soybean genotypes and Bradyrhizobium japonicum strains have been reported (Cregan and Keyser, 1986; Ganacharya and Nirmal 1978; Khurana, 1981). The mean dry weight of nodules formed by Duiker variety after inoculation with NUM 508 strain, which was 0.55g, was significantly higher than that for nodules formed after inoculation with NUM 504 strain, which was 0.47g. The reverse was true for the Sable variety which produced a significantly higher mean nodule dry weight of 0.88g when inoculated with NUM 504 strain compared with a weight of 0.79g formed after inoculation with NUM 508 strain.

N fertilizer resulted in a highly significant ($P<0.001$) decrease in nodule dry weight, an observation which is consistent with the findings of other researchers (Jamro et al, 1990; Matsunaga et al, 1983; Papastylianou, 1986). The mean nodule dry weights of 0.78 and 0.62g at zero and 20kgN/ha levels, respectively, were statistically similar and significantly higher than the nodule dry weights of 0.40, 0.25 and 0.19g at 40, 80 and 160kgN/ha, respectively. The nodule dry weights showed highly significant linear ($P<0.001$) and also significant quadratic ($P<0.05$) inverse relationship with increase in the level of applied N for both varieties. The dry weights were decreased linearly by upto 80kgN/ha but the decrease was less pronounced with further increase in N.

There was a significant (P<0.01) interaction between Bradyrhizobium japonicum strains and nitrogen for nodule dry weight. Such an interaction has been reported by Olufajo (1990). The mean dry weights of the nodules produced by inoculation with NUM 504 strain decreased consistently from 1.22g at zero-N to 0.39g at 80kgN/ha level but increased slightly to 0.40g at 160kgN/ha level. The mean dry weights of the nodules produced by inoculation with NUM 508 strain decreased consistently from 1.13g at zero-N treatment to 0.16g at 160kgN/ha treatment. Therefore the dry weights of the nodules produced by inoculation with both strains showed a highly significant (P<0.001) inverse and also significant (P<0.01) quadratic relationship with increase in the level of applied N. The decrease was linear as N levels increased from zero upto 80kgN/ha but became less pronounced and quadratic in nature thereafter, especially for nodules formed by NUM 508 strain, while those for NUM 504 strain even tended to increase as N levels approached 160kgN/ha (Fig.5). This suggested that NUM 504 strain could tolerate high N levels much better than NUM 508 strain.

4.4.4. Effect of nitrogen and Bradyrhizobium japonicum inoculation on grain yield and yield components of soybeans grown in the field.

Table 15 shows that the number of grains per pod was not

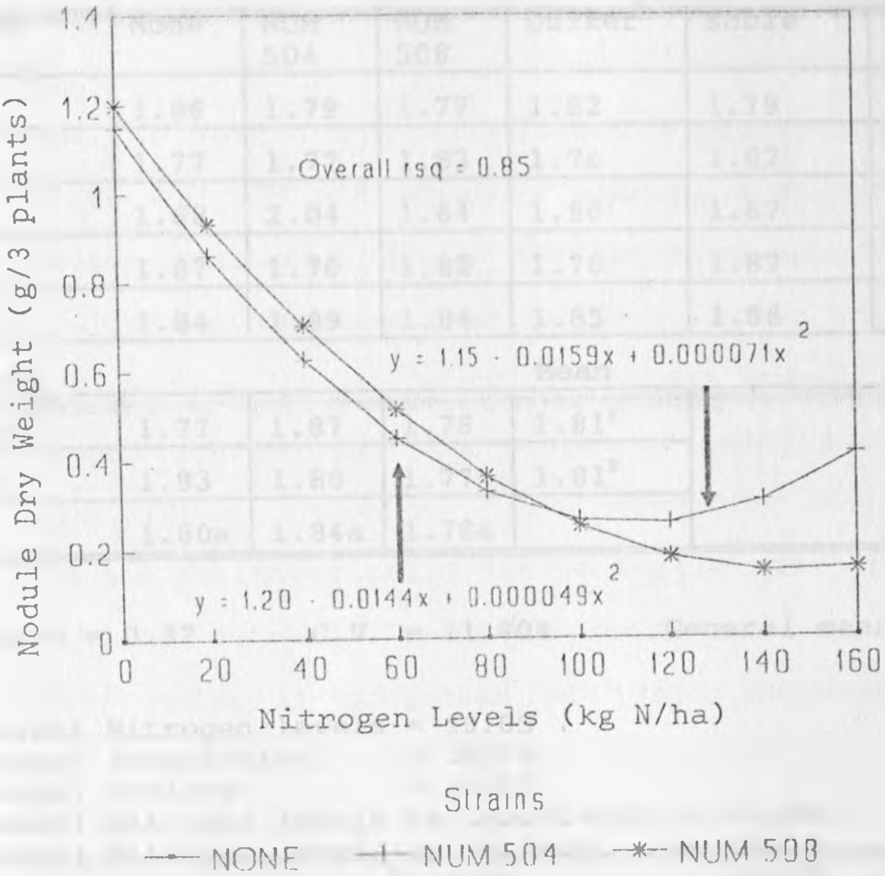

Fig.5. Relationship between N and nodule dry weight of soybeans grown with and without inoculation in the field

Table 15. Effect of nitrogen level, Bradyrhizobium japonicum inoculation and variety on the mean number of grains per pod of soybeans grown in the field.

Nitrogen	Inoculation			Variety		
kgN/ha	None	NUM 504	NUM 508	Duiker	sable	Mean
0	1.86	1.79	1.77	1.82	1.79	1.80A
20	1.77	1.77	1.83	1.76	1.82	1.79A
40	1.68	2.04	1.64	1.90	1.67	1.79A
80	1.87	1.70	1.82	1.70	1.87	1.80A
160	1.84	1.89	1.84	1.85	1.86	1.85A
Variety				Mean		
Duiker	1.77	1.87	1.78	1.81^{y}		
Sable	1.83	1.80	1.77	1.81^{y}		
Mean	1.80a	1.84a	1.78a			

R-square = 0.37 C.V. = 11.40% General mean = 1.81

SE(means) Nitrogen levels = ±0.05
SE(means) Inoculation = ±0.04
SE(means) Variety = ±0.03
SE(means) Nitrogen levels vs Inoculation = ±0.08
SE(means) Nitrogen levels vs Variety = ±0.07
SE(means) Inoculation vs Variety = ±0.05

Nitrogen and inoculation means within a column or a row respectively, with the same letter and varietal means followed by the same superscripts are not significantly different according to DMRT (P=0.05).

influenced by any of the treatments, contrary to the finding of Jamro et al (1990) who reported increased seeds per pod in cv. Bossier as N rate increased from zero to 90kg/ha. Averaged over nitrogen levels, the mean number of grains per pod, namely, 1.80 for the non-inoculated treatments, are not statistically different from those formed in plants inoculated with NUM 504 and NUM 508 strains, namely, 1.84 and 1.78, respectively. Similarly, grains per pod from each level of nitrogen, which was averaged over the inoculation treatments were not significantly different from each other. These observations suggested that the number of seeds per pod in Duiker and Sable varieties were probably genetically controlled and therefore could not be easily manipulated by changing the environment. Hence the number of seeds per pod has limited value as predictor of yield response to N fertilization and inoculation in this experiment.

There was no significant difference between mean pod numbers due to Bradyrhizobium japonicum inoculation (Table 16). A similar finding has been reported by other researchers (Kang, 1975; Awai, 1981). Averaged over N levels, non-inoculated plants formed 95.87 pods per plant while those inoculated with NUM 504 and NUM 508 strains formed 89.47 and 92.87 pods per plant, respectively. Though NUM 508 strain tended to form slightly more pods per plant than NUM 504 strain with the Duiker variety while the latter strain tended

Table 16. Effect of nitrogen level, *Bradyrhizobium japonicum* inoculation and variety on the mean number of pods per plant of soybean grown in the field.

N	Inoculation			Variety		
kgN/ha	None	NUM 504	NUM 508	Duiker	Sable	Mean
0	73.17	79.50	70.83	72.00	77.00	74.50B
20	112.00	95.50	92.67	91.56	108.56	100.06A
40	88.33	85.67	97.67	87.78	93.33	90.56A
80	111.00	75.00	88.00	89.56	93.11	91.33A
160	94.83	111.67	115.17	105.56	108.89	107.22A
Variet				Mean		
Duiker	88.33	83.73	95.80	89.29^{y}		
Sable	103.40	95.20	89.93	96.18^{y}		
Mean	95.87a	89.47a	92.87a			

R-square = 0.43 C.V. = 24.97% General mean = 92.73

SE(means) Nitrogen levels = ±5.46
SE(means) Inoculation = ±4.23
SE(means) Variety = ±3.45
SE(means) Nitrogen levels vs Inoculation = ±9.46
SE(means) Nitrogen levels vs Variety = ±7.72
SE(means) Inoculation vs Variety = ±5.98

Nitrogen and inoculation means within a column or a row respectively, with the same letter and varietal means followed by the same superscripts are not significantly different according to DMRT (P=0.05).

Variet = Variety

to form slightly more pods per plant than NUM 508 strain with the Sable variety, there was no variety x strain interaction.

Application of N significantly ($P<0.01$) increased the number of pods per plants. A similar finding of increased number of pods due to increase in N supply has been reported by other workers (EL-Kady et al, 1982; Rabie et al, 1979). Plants at zero-N treatments formed 74.50 pods per plant, which were significantly fewer than the 100.06 pods per plants formed by plants supplied with 20kgN/ha. However, higher N levels gave no further increase in pod number. A significant linear ($P<0.01$) relationship between applied N and pod number for both varieties was found. There was also a significant linear ($P<0.01$) relationship between applied nitrogen and pod number for both non-inoculated and inoculated plants.

Data on the number of grains per plant (Table 17) generally showed a trend similar to that for pod number per plant. The effect of inoculation on the number of grains per plant was not significant. Infact plants inoculated with NUM 504 and NUM 508 strains showed relatively fewer grains per plant, namely, 163.03 and 165.87, respectively, compared with the 173.40 grains per plant produced by non-inoculated treatments. NUM 508 strain tended to produce slightly more grains per plant than NUM 504 strain with the Duiker variety while NUM 504 strain tended to produce slightly more grains

Table 17. Effect of nitrogen level, Bradyrhizobium japonicum inoculation and variety on the mean number of grains per plant of soybean grown in the field.

Nitrogen	Inoculation			Variety		
kgN/ha	None	NUM 504	NUM 508	Duiker	Sable	Mean
0	134.67	141.00	125.17	130.00	137.22	133.61C
20	197.83	171.83	169.50	161.00	198.44	179.72BA
40	148.00	163.33	162.33	159.56	156.22	157.89BC
80	209.00	126.17	160.67	153.44	178.44	165.94B
160	177.50	210.83	211.67	197.11	202.89	200.00A
Variety				Mean		
Duiker	155.13	153.53	172.00	160.22^{Y}		
Sable	191.67	172.53	159.73	174.64^{Y}		
Mean	173.40a	163.03a	165.87a			

R-square = 0.47 C.V. = 27.15% General mean = 167.43

SE(means) Nitrogen levels = ±10.72
SE(means) Inoculation = ±8.30
SE(means) Variety = ±6.78
SE(means) Nitrogen levels vs Inoculation = ±18.56
SE(means) Nitrogen levels vs variety = ±15.15
SE(means) Inoculation vs variety = ±11.74

Nitrogen and inoculation means within a column or a row respectively, with the same letter(s) and varietal means followed by the same superscripts are not significantly different according to DMRT (P=0.05).

per plant than NUM 508 strain with the Sable variety but the interaction between variety and strains for grains per plant did not reach a significant level.

Nitrogen application caused a highly significant ($P<0.001$) increase in the number of grains per plant. The 179.72 grains per plant produced from the 20-kgN/ha level were significantly more than the 133.61 grains per plant produced from the zero-N level. Hovever, the 157.89 and 165.94 grains per plant produced from 40 and 80-kgN/ha levels, respectively, were not significantly different from the number produced from the 20-kgN/ha level. The 200 grains per plant at 160-kgN/ha level were significantly more than the number for zero, 40 and 80-kgN/ha levels. The linear relationship between nitrogen levels and number of grains per plant for both varieties was significant ($P<0.01$). The linear relationship between nitrogen levels and number of grains per plant for non-inoculated and inoculated treatments was also significant ($P<0.01$).

The results of the effects of nitrogen and Bradyrhozobium japonicum strains on 1000-bean weight are given in Table 18. The increase in bean weight due to inoculation was highly significant ($P<0.001$) and in agreement with the results of Ibrahim and Mahmoud (1989) and Okon et al. (1978). Both NUM 504 and NUM 508 strains performed equally well as the former

Table 18. Effect of nitrogen level, Bradyrhizobium japonicum inoculation and variety on the mean grain size (g per 1000) oven-dried seeds of soybeans grown in the field .

Nitrogen	Inoculation			Variety		
kgN/ha	None	NUM 504	NUM 508	Duiker	Sable	Mean
0	188.50	202.33	202.67	187.89	203.78	195.83C
20	197.00	205.33	198.50	192.33	208.22	200.28CB
40	197.00	200.17	212.00	195.44	210.67	203.06B
80	207.17	208.67	210.00	198.89	218.33	208.61A
160	207.33	213.50	218.17	206.22	219.78	213.00A
Variety				Mean		
Duiker	188.67	196.60	203.20	196.16^{z}		
Sable	207.73	215.40	213.33	212.16^{y}		
Mean	198.20b	206.00a	208.27a			

R-square = 0.80 C.V. = 3.75% General mean = 204.16

SE(means) Nitrogen levels = ±1.80
SE(means) Inoculation = ±1.40
SE(means) Variety = ±1.14
SE(means) Nitrogen levels vs Inoculation = ±3.13
SE(means) Nitrogen levels vs Variety = ±2.55
SE(means) Inoculation vs Variety = ±1.98

Nitrogen and inoculation means within a column or a row respectively, with the same letter(s) and varietal means followed by the same suporscripts are not significantly different according to DMRT (P=0.05).

increased the bean weight by 4% while the latter increased it by 5% over the non-inoculated control. The interaction between variety and Bradyrhizobium japonicum strains for bean weight was significant ($P<0.05$). A similar interaction has already been reported above, for nodule dry weight (Section 4.4.3). These two interactions strongly support the claim that Bradyrhizobium strains have specificities for different varieties (Khurana, 1986). The weight of 203.20g for 1000 beans of Duiker variety inoculated with NUM 508 strain was significantly higher than the weight of 196.60g for 1000 beans produced by inoculation with NUM 504 strain. The reverse was true for the Sable variety whose weight of 215.40g for 1000 beans produced by inoculation with NUM 504 was significantly higher than that of 213.33g for 1000 beans produced by inoculation with NUM 508 strain. The finding was not unusual as significant cultiver x strain interaction for grain yield has been reported (Chowdhury, 1977; Nangju, 1980).

Nitrogen caused a highly significant ($P=<0.001$) increase in 1000-bean weight which was in agreement with Kang (1975), Sorensen and Penas (1978) who observed significant increases in grain weight from increased nitrogen rates. The 1000-bean weights of 195.83 and 200.28g from zero and 20-kgN/ha treatments, respectively, are statistically similar. However, the 1000-bean weight produced by the 40-kgN/ha level, viz., 203.06g, was significantly higher than the bean weight from the zero-N treatment. Application of 80kgN/ha produced bean weight of 208.61g which is similar to the bean weight of 213.00g from the 160-kgN/ha treatment but significantly higher than all the bean weights from lower N levels. A highly significant ($P<0.001$) linear relationship between 1000-bean weight and increasing levels of N was observed. This was an indication that higher bean weights from the two varieties would be expected from higher levels of nitrogen. The interaction between nitrogen and inoculation for 1000-bean weight was significant ($P<0.05$). Non-inoculated and inoculated plants responded differently to N application; while the 1000-bean weight of non-inoculated plants tended to increase steadily only upto 80kgN/ha then became constant and finally drop as N levels approached 160kgN/ha, the bean weights of inoculated plants continued to increase even with the highest N level of 160kgN/ha (Fig.6). This was an interesting observation which suggested that there was a synergistic effect of nitrogen fixation on soybean seed development and also supported Webber's (1966) suggestion that the energy required for seed production, respiration of nodule bacteria and the reduction of N_2 to $-NH_2$ is less than that required for absorption of NO_3^- and its subsequent reduction to $-NH_2$ in the non-nodulating line. The positive linear relationship between 1000-bean weight and nitrogen levels was highly significant ($P<0.001$) while the quadratic component was significant (<0.05).

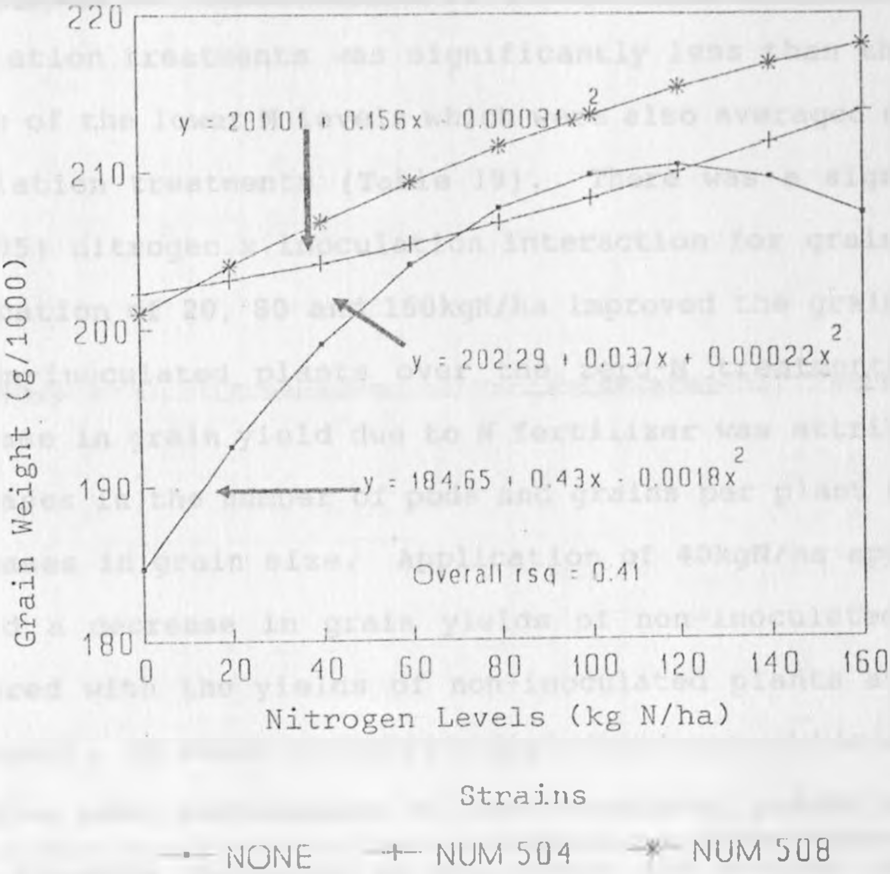

Fig.6. Relationship between N and 1000 oven-dried grains of soybeans grown with and without inoculation in the field

The main effects of nitrogen and Bradyrhizobium japonicum inoculation on grain yields were not significant, (Appendix V11). However, according to Scheffe's test (P= 0.05) the grain yield of 3523.09kg/ha at 160kgN/ha averaged over the inoculation treatments was significantly less than the grain yields of the lower N levels which were also averaged over the inoculation treatments (Table 19). There was a significant ($P<0.05$) nitrogen x inoculation interaction for grain yield. Application of 20, 80 and 160kgN/ha improved the grain yields of non-inoculated plants over the zero-N treatments. The increase in grain yield due to N fertilizer was attributed to increases in the number of pods and grains per plant and also increases in grain size. Application of 40kgN/ha apparently caused a decrease in grain yields of non-inoculated plants compared with the yields of non-inoculated plants at zero-N treatment. No sound scientific explaination could be advanced for the poor performance of non-inoculated plants supplied with 40kgN/ha reflected in both shoot dry weights and grain yield.

Inoculation improved the grain yield of zero and 40-kgN/ha levels which was attributed to an increase in the grain size, but decreased the grain yields of 20, 80 and 160-kgN/ha treatments (Fig.7). Inverse relationship of fertilizer N and inoculation has been documented by Vasilas and Ham (1985) who reported that application of 100kgN/ha decreased the yields of

Table 19. Effect of nitrogen level, <u>Bradyrhizobium japonicum</u> inoculation and variety on the mean grain yield (kg/ha) of soybeans grown in the field.

Nitrogen	Inoculation			Variety		
kgN/ha	None	NUM 504	NUM 508	Duiker	Sable	Mean
0	3846.20	4135.58	4413.67	4303.02	3960.61	4131.82A
20	4161.09	4044.05	3994.83	4262.66	3870.65	4066.65A
40	3484.33	4254.51	4241.62	4406.11	3580.86	3993.49A
80	4117.79	3896.26	3686.94	4418.24	3382.42	3900.33A
160	4066.33	3264.51	3238.42	4109.99	2936.18	3523.09B
Variety				Mean		
Duiker	4186.23	4335.92	4377.86	4300.00^{y}		
Sable	3684.07	3502.04	3452.33	3546.15^{z}		
Mean	3935.15a	3918.98a	3915.09a			

R-square = 0.82 C.V. = 13.75% General mean = 3923.06

SE(means) Nitrogen levels = ±127.14
SE(means) Inoculation = ±98.48
SE(means) Variety = ±80.41
SE(means) Nitrogen levels vs Inoculation = ±220.21
SE(means) Nitrogen levels vs Variety = ±179.80
SE(means) Inoculation vs Variety = ±139.27

Nitrogen and inoculation means within a column or a row respectively, with the same letter and varietal means followed by the same superscripts are not significantly different according to Scheffe's test (P=0.05).
All values were adjusted to 13% moisture content.

nodulating soybeans. At zero-N level, inoculated plants had many nodules and hence fixed substantial amounts of N that promoted higher grain yields in inoculated than non-inoculated plants. The rate of 20kgN/ha slightly decreased nodulation of inoculated plants compared with nodulation of plants at zero-N treatment. This slight decrease in nodulation probably caused a decrease in fixed N which 20kgN/ha could not compensate for as it was a bit low. Hence the lower yields obtained from inoculated plants supplied with 20kgN/ha compared with inoculated ones at zero N level.

Application of 40 kgN/ha significantly reduced nodulation of inoculated plants, therefore less N was fixed which in conjunction with the applied rate proved favourable for seed production. Further increases in N to 80 and 160 kgN/ha continued to decrease plant nodulation but did not eliminate it completely. Thus, in addition to the application of 80 and 160kgN/ha the nodulated plants fixed some N from the air which generally resulted in higher N content in nodulated plants compared with non-inoculated ones at the same N level. This high N content seems to have stimulated excessive vegetative growth in inoculated plants at the expense of the seeds. Karyagin (1980) found that N fertilizer at 120kgN/ha in conjunction with inoculation increased the yield of green matter and hay in soybean but decreased its yields. These results emphasize the importance of judiciously defining the optimum dose of N when used in conjunction with Bradyrhizobium japonicum inoculation for soybean seed production.

Regression analysis of the grain yields of non-inoculated and inoculated Duiker and Sable varieties over N levels showed inversely linear relationships with increasing levels of N for both varieties (Fig.8). The grain yield of the Sable variety was reduced more by increasing levels of N than that of the Duiker variety. This phenomenum could easily be explained by the fact that the Sable variety tends to produce a lot of vegetative growth, probably at the expense of the seeds, with increasing levels of N compared with the Duiker variety.

Regression analysis of the grain yields of non-inoculated and inoculated plants over the N levels suggested a positive linear relationship between grain yields of non-inoculated plants and increasing levels of N but the relationship between the grain yield of inoculated plants and increasing levels of N was inversely linear (Fig.9). The grain yields of plants inoculated with NUM 508 strain were more reduced, though slightly, by increasing levels of N than the yields of plants inoculated with NUM 504 strain. However, it should be remembered that plants inoculated with NUM 508 strain had slightly higher N contents than those inoculated with NUM 504 strain, thus strongly supporting the suggestion that the grain yield decrease in inoculated plants with increasing levels of

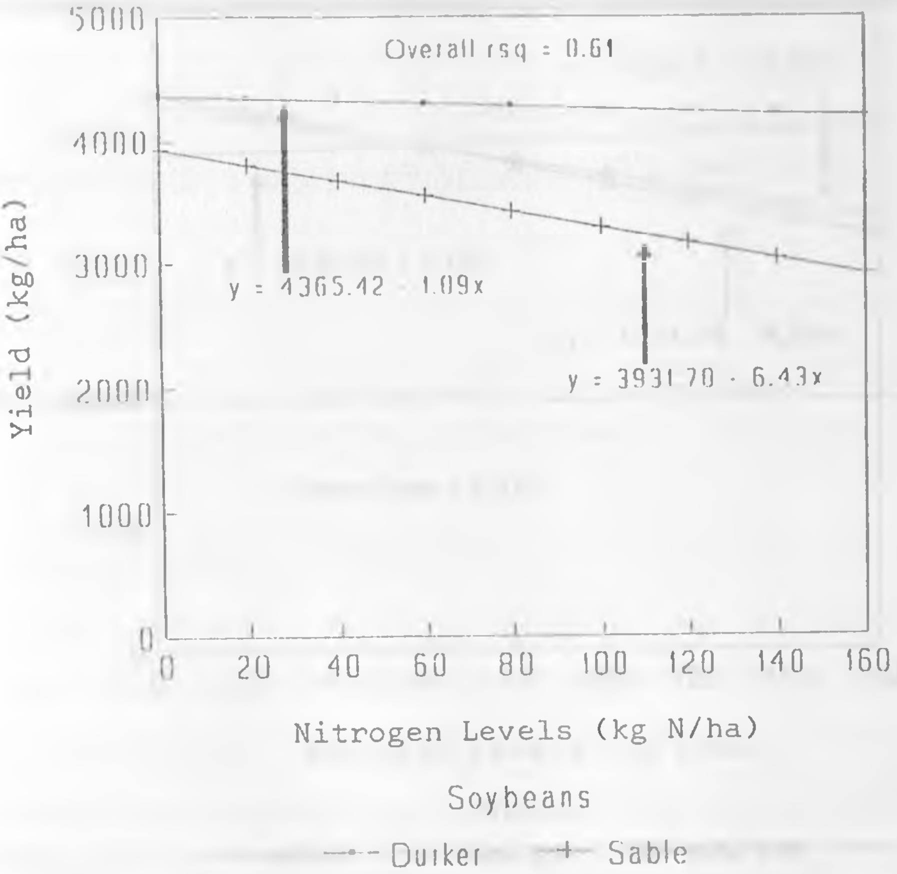

Fig.7. Relationship belwwen N and grain yield of Duiker and Sable varieties of soybeans grown in the field

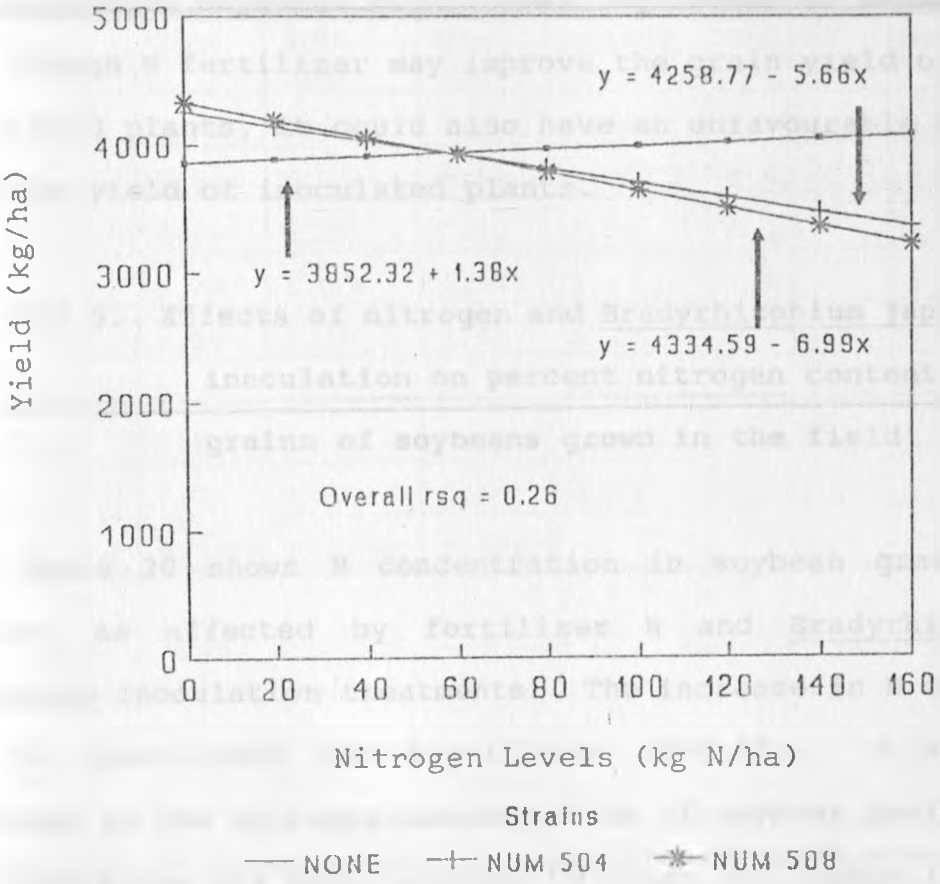

Fig.8. Relationship between N and grain yield of soybeans grown with and without inoculation in the field

N is due to their high N content which seems to have stimulated excessive vegetative growth at the expense of the seeds. The grain yield behavioural pattern of inoculated and non-inoculated soybeans with increasing levels of N suggests that though N fertilizer may improve the grain yield of non-inoculated plants, it could also have an unfavourable effect on grain yield of inoculated plants.

4.4.5. Effects of nitrogen and *Bradyrhizobium japonicum* inoculation on percent nitrogen content of grains of soybeans grown in the field.

Table 20 shows N concentration in soybean grains at harvest as affected by fertilizer N and *Bradyrhizobium japonicum* inoculation treatments. The increase in N content due to inoculation was significant ($P<0.05$). A similar increase in the nitrogen concentration of soybean grains due to inoculation has been reported by Singh and Saxena (1978). The grains from plants inoculated with NUM 508 strain had a mean percent nitrogen content of 6.47 which was significantly higher than the mean percent nitrogen content of 6.09 for the grains from the non-inoculated plants. However, grains from plants inoculated with NUM 504 strain had a mean nitrogen content of 6.26%, which was higher than that of grains from non-inoculated plants but not significantly different.

Table 20. Effect of nitrogen level, Bradyrhizobium japonicum inoculation and variety on the mean *percent nitrogen content in grains of soybeans grown in the field.

Nitrogen	Inoculation			Variety		
kgN/ha	None	NUM 504	NUM 508	Duiker	Sable	Mean
0	5.60	5.97	6.40	6.06	5.92	5.99C
20	5.92	6.10	6.50	5.92	6.42	6.17BC
40	6.03	6.33	5.92	6.05	6.13	6.09BC
80	6.38	6.33	6.57	5.97	6.59	6.43BA
160	6.52	6.57	6.98	6.17	7.21	6.69A
Variety				Mean		
Duiker	5.81	6.03	6.25	6.03^z		
Sable	6.37	6.49	6.69	6.52^y		
Mean	6.09b	6.26ba	6.47a			

R-square = 0.62 C.V. = 8.58% General mean = 6.27

SE(means) Nitrogen levels = ±0.13
SE(means) Inoculation = ±0.10
SE(means) Variety = ±0.08
SE(means) Nitrogen levels vs Inoculation = ±0.22
SE(means) Nitrogen levels vs Variety = ±0.18
SE(means) Inoculation vs Variety = ±0.14

Nitrogen and inoculation means within a column or a row respectively, with the same letter(s) and varietal means followed by the same superscripts are not significantly different according to DMRT (P=0.05).

*All values were expressed on dry matter bases.

Nitrogen treatments significantly (P<0.01) increased the mean percent N content of the grains, a similar finding has been documented by other researchers (EL-Kady et al, 1982; Kang et al., 1977; Watanabe, 1985) who reported increased seed protein content with increasing N supply. The mean N contents of grains from the plants supplied with zero, 20 and 40kgN/ha, viz., 5.99, 6.17 and 6.09, respectively, are statistically similar but the mean percent N content of 5.99 for grains of plants at zero-N treatments was significantly less than that for the grains of plants supplied with 80kgN/ha, viz., 6.43%. Further increase in N from 80 to 160kgN/ha did not increase the N content of grains significantly. The mean percent N content of 6.69 for the grains from plants supplied with 160kgN/ha was significantly higher than that of 5.99, 6.17 and 6.09 for the grains of plants supplied with zero, 20 and 40kgN/ha, respectively.

The variety x nitrogen interaction was significant (P<0.01) for percent N content in the grains. Nitrogen fertilizer increased the N content of the grains of Sable variety almost consistently, with increasing levels, from 5.92 at zero-N level to 7.21 at 160-kgN/ha level. The N content of the grains of Duiker variety remained almost constant with increasing levels of fertilizer N. At zero-N level, the mean percent N content of Duiker variety was 6.06 while that at 160-kgN/ha level was 6.17. This interaction implied that N fertilizer could only increase the grain protein content of Sable variety but not for Duiker variety. However, an alternative and cheaper way of increasing the protein content of the grains for both varieties would be by use of inoculants.

4.5. COMPARISON OF GREENHOUSE AND FIELD EXPERIMENTS

The lack of literature concerning nodulation characteristics of Duiker and Sable varieties was a major reason for conducting preliminary investigations in a greenhouse pot experiment to ascertain whether the two varieties contain the dominant allele Rj2 which restricts nodulation with Bradyrhizobium japonicum strains or the recessive allele rj2 which permits normal nodulation. The greenhouse experiment, which was relatively easier and cheaper to conduct than the field experiment which was expensive and more involving, provided the vital information about nodulation characteristics of the two varieties used before the field experiment was undertaken. In addition, the greenhouse experiment provided the necessary inoculation skills which helped in minimizing experimental errors in the field experiment.

Application of 80kgN/ha in both greenhouse and field experiments produced significant increases in shoot dry weights over the zero-N level. The shoot dry weights of inoculated and non-inoculated plants increased linearly with the increase in N levels in both experiments. The nitrogen x variety interaction for shoot dry weight was significant in the greenhouse experiment but such an interaction was not observed in the field experiment. Sable variety is taller with more vegetative growth compared with Duiker variety, hence the growth of the former must have been restricted in pots resulting in a significant nitrogen x variety interaction under greenhouse conditions. Generally, inoculated plants attained their maximum heights at 40kgN/ha in both experiments which seems to favour the view that inoculation in conjunction with 40kgN/ha can improve the growth of soybeans.

Application of 80kgN/ha caused a significant decrease in nodule numbers of the plants grown in the greenhouse while the nodule numbers of the plants grown in the field were significantly reduced by the application of 40kgN/ha. The soil used in the greenhouse experiment was deficient in nitrogen and therefore a much higher rate of N (80kgN/ha) suppressed nodulation unlike the field topsoil which had sufficient nitrogen and hence just a little extra N (40kgN/ha) was enough to suppress nodulation. In both experiments nitrogen fertilizer had no significant effect on mean nitrogen content of the shoots averaged over the inoculation treatments. Plants treated with lower N levels formed more nodules and hence probably fixed more nitrogen than those treated with higer N levels which inturn probably derived more nitrogen from the high N levels supplied in form of fertilizer.

Inoculation caused a significant increase in shoot dry weights of plants grown in the greenhouse but the increase in the shoot dry weights of plants grown in the field did not reach a significant level. Similarly, inoculation caused a significant increase in plant heights only in the greenhouse but did not affect those grown in the field. Thus the plants grown in the greenhouse responded more to inoculation in terms of growth than those grown in the field. The better response of greenhouse plants to inoculation was probably due to the fact that the soil used in the greenhouse was low in N.

The results suggest that the environmental conditions in the greenhouse were not as favourable as those prevailing in the field with regard to plant nodulation as the inoculated plants in the greenhouse formed fewer nodules compared with inoculated plants grown in the field despite the fact that the same inoculation rate of 0.25kg inoculant per hactare was used in both experiments. Generally, this rate of inoculant application increased the N content of the shoots compared with the non-inoculated control, although the increase was not significant except where the plants were inoculated with NUM 508 in the field experiment.

CHAPTER FIVE

CONCLUSIONS

Both greenhouse and field experiments were conducted on soils that were completely devoid of native Bradyrhizobium bacteria. Nitrogen fertilizer significantly increased the shoot dry weight of the soybean plants. There was a positive linear relationship between shoot dry weights and increasing levels of N. Application of 40kgN/ha in conjunction with inoculation produced the tallest plants. Increasing levels of N decreased nodulation of the plants but did not eliminate it completely. The nitrogen contents of the shoots averaged over inoculation treatments were not affected by different N levels as the plants treated with lower N levels formed more nodules than the ones treated with higher N levels and hence fixed more nitrogen to offset the differences due to the different N rates. However, application of 80 and 160kgN/ha significantly increased the nitrogen contents of the grains over the zero-N treatments, though the nitrogen contents corresponding to the two rates were not significantly different. Application of 20, 80 and 160kgN/ha increased the grain yields of non-inoculated plants compared to the zero-N level through increases in the number of pods and the number of grains per plants as well as increase in the grain size.

The two *Bradyrhizobium japonicum* strains used, namely, NUM 504 and NUM 508 performed equally well in all the parameters studied both in the greenhouse and in the field. There was a significant variety x strain interaction for nodule dry weights and grain size in the field experiment. The NUM 508 strain formed heavier nodules with Duiker variety than the nodules formed by NUM 504 strain with the same variety. The reverse was true for NUM 504 strain which formed heavier nodules with sable variety than the nodules formed by NUM 508 strain with the Sable variety. A similar trend was repeated for grain size whereby NUM 508 strain produced heavier grains with Duiker variety than the grains produced by NUM 504 strain with the same variety while NUM 504 produced heavier grains with Sable variety than the grains produced by NUM 508 strain with the Sable variety.

Bradyrhizobium japonicum inoculation significantly increased the shoot dry weights and heights of the plants grown in the greenhouse but the increase in shoot dry weights and plant heights was not significant in the field experiment. Inoculation significantly increased nodulation of the plants in both experiments. The results obtained under greenhouse and field conditions indicate that *Bradyrhizobuium japonicum* inoculation was absolutely necessary for soybean nodulation. Generally, inoculation increased the nitrogen contents of the shoots but only NUM 508 strain increased it significantly over the non-inoculated control under field conditions. Similarly, inoculation increased the nitrogen contents of the grains but only NUM 508 strain caused a significant increase over the non-inculated control in the field. Bradyrhizobium japocnicum inoculation alone without any addition of N fertilizer produced the highest grain yields followed by inoculation in conjunction with 40kgN/ha. The increase due to inoculation was attributed to increase in grain size. However, inoculation in conjunction with 20, 80 and 160kgN/ha decreased the grain yields through improper balancing of inoculation and nitrogen rates.

SUGGESTIONS FOR FUTURE RESEARCH

Studies on inoculation under field conditoons are difficult, yet this is the most appropriate place to conduct them. Data collected from the field study suggested the areas listed below as important for future research.

(1) A comprehensive study on the effects of nitrogen and Bradyrhizobium japonicum inoculation on the grain yields of soybeans should be conducted on different soils especially those that are deficient in nitrogen in order to avoid waste of N fertilizers together with the possible unfavourable consequences on the environment and the economy. Such a study should be geared towards substantial reduction in soybean production costs by promoting the use of inoculants which are cheap and have no adverse effects on the soil.

(2) Investigation of the physiological response of non-inoculated soybean plants to application of 40kgN/ha which caused reduction in shoot dry weights and grain yields in comparison with unfertilized plants and those supplied with 20kgN/ha.

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Appendix Table 1. Some physical properties of the soil used in the greenhouse experiment.

Profile pit		Moist colour		Particle size-%			Textural class	Classification FAO/UNESCO (1974)
Horizon	Depth (cm)	Munsell notation	Colour name	Sand	Silt	Clay		
Ap	0-9	7.5YR2/3	Very dark brown	66	2	32	Sandy clay loam	Humic Ferralsol
AB	9-30	5YR2/3	Very dark reddish brown	64	2	34	Sandy clay loam	
Bw_1	30-61	5YR3/4	Dark reddish brown	56	4	40	Sandy clay	
Bw_2	61-132+	5YR3/6	Dark reddish brown	62	4	34	Sandy clay loam	

Appendix Table 11. Some physical properties of the soil of the field experiment.

Profile pit		Moist colour		Particle size %			Textural class	Classification FAO/UNESCO (1974)
Horizon	Depth (cm)	Munsell notation	Colour name	Sand	Silt	Clay		
Ap	0-15	5YR2/3	Very dark reddish brown	66	4	30	Sandy clay loam	Rhodic Ferralsol
BA	15-36	2.5YR3/6	Dark reddish brown	64	2	34	Sandy clay loam	
Bw$_1$	36-80	2.5YR3/6	Dark reddish brown	60	2	38	Sandy clay	
Bw$_2$	80-115	2.5YR3/6	Dark reddish brown	58	4	38	Sandy clay	
Bw$_2$	115-150+	2.5YR3/6	Dark reddish brown	58	4	38	Sandy clay	

Appendix Table 111. Low, Moderate and High Soil Nutrients.

Nutrient	Low	Moderate	High
Na(me/100g)	____________	0 _____ 2.0	2.0+
K(me/100g)	Less than 0.2	0.2 _____1.5	1.5+
Ca(me/100g)	Less than 2.0	2.0______ 10.0	10.0+
Mg(me/100g)	Less than 1.0	1.0 ______3.0	3.0+
P(ppm)	Less than 20	20 __ 80 Mehlich	80+
C(%)	Less than 1.0	1.0 __ 2.0 Moderate 2.0 __ 4.0 Adequate	4.0+
N(%)	Less than 0.2	____________________	_____

This generalised guide is adopted from a technical circular entitled, "some important values for soil fertility evaluation" released by National Agricultural Research Laboratories (NARL), Nairobi.

Appendix Table 1V. Weather conditions for National Agricultural Research Centre, NARC-Kitale.

Month	Total Rainfall(mm)	Rainy Days	Mean max. Temp.(°C)	Mean min. Temp.(°C)	Mean Relative Humidity %	
					9.00 a.m	3.00 p.m
Jan.	68.6	7	27.0	10.8	73	39
Feb.	13.5	4	29.0	11.5	71	32
Mar.	130.0	8	28.5	12.6	66	40
April	128.5	16	25.6	13.1	78	54
May	152.0	21	25.1	13.9	86	59
June	129.1	16	24.3	13.2	87	62
July	187.1	20	23.0	12.2	89	63
Aug.	195.1	20	24.0	12.1	88	64
Sept.	46.6	9	25.8	10.8	78	49
oct.	156.6	18	25.1	11.7	77	56
Nov.	49.6	11	24.8	11.3	72	54
Dec.	7.9	2	26.0	10.4	71	40
Mean	105.4*	152*	25.7	12.0	78	51

* Total

Source: Kenya Meteorological Department.

Appendix Table V. The analysis of variance tables for most variables except chemical and grain yield analysis are as shown below for 1000-grain weight.

Dependent variable: 1000-Grain weight.

Source	DF	Sum of squares	Mean square	F-value	P>F
Model	31	13763.8	443.9	7.6	0.0001
Error	58	3400.5	58.6		
C.T.	89	17163.8			

R-square = 0.8 C.V. = 3.8% Mean = 204.2

Source	DF	Anova SS	Mean Square	F-Value	P>F
Replicate	2	242.8	121.4	2.1	0.1353NS
V	1	5760.0	5760.0	98.2	0.0001^{***}
I	2	1673.2	836.6	14.3	0.0001^{***}
VxI	2	387.5	193.7	3.3	0.0437^{*}
N	4	3304.5	826.1	14.1	0.0001^{***}
VxN	4	83.1	20.8	0.4	0.8399NS
VxN Linear SS	2	3069.0	1534.5	26.2	0.0001^{***}
VxN Quad. SS	2	279.1	139.6	2.4	0.1015NS
VxN Cubic.SS	2	30.9	15.4	0.3	0.7694NS
VxN Quar. SS	2	8.6	4.3	0.1	0.9297NS
IxN	8	1292.5	161.6	2.8	0.0119^{*}
IxN Linear SS	3	3228.9	1076.3	18.4	0.0001^{***}
IxN Quad. SS	3	671.3	223.8	3.8	0.0145^{*}
IxN Cubic.SS	3	59.2	19.7	0.3	0.7988NS
IxN Quar.SS	3	637.6	212.5	3.6	0.0181^{*}
VxIxN	8	1019.8	127.5	2.2	0.0428^{*}

C.T. = Corrected Total
V = Variety
I = Inoculation
N = Nitrogen

*** Highly significant at P≤ 0.001
* Significant at P≤ 0.05
NS Not significant P>0.05

Appendix Table VI. The general analysis of variance tables for chemical analyses are as shown below for nitrogen content of the grains.

Dependent variable: Grain nitrogen

Source	DF	Sum of squares	Mean square	F-Value	P>F
Model	31	27.3	0.9	3.0	0.0001
Error	58	16.8	0.3		
C.T.	89	44.1			

R-square = 0.6 C.V. = 8.6% Mean = 6.3

Source	DF	Anova SS	Mean square	F-Value	P>F
Replicate	2	0.4	0.2	0.7	0.5185NS
V	1	5.2	5.2	18.1	0.0001***
I	2	2.2	1.1	3.8	0.0276*
VxI	2	0.1	0.03	0.10	0.9056NS
N	4	5.8	1.4	5.0	0.0016**
VxN	4	4.7	1.2	4.1	0.0055**
IxN	8	2.3	0.29	1.0	0.4509NS
VxIxN	8	6.6	0.82	2.8	0.0098**

C.T. = Corrected Total
V = Variety
I = Inoculation
N = Nitrogen

***	Highly significant at $P \leq 0.001$
**	Significant at $P \leq 0.01$
*	Significant at $P \leq 0.05$
NS	Not significant $P > 0.05$

Appendix Table VII. Grain yield analysis adjusted for stand count.

Dependent Variable: Grain Yield

Source	DF	Sum of squares	Mean square	F-value	P>F
Model	33	1458441869	44195208	151.9	0.0001
Error	57	16584367	290954		
UT	90	1475026237			

R-square = 0.8 C.V. = 13.7% Mean = 3923.1

Source	DF	TypeIII SS	Mean square	F-Value	P>F
Replicate	2	853252.3	426626.1	1.47	0.2394NS
HSTD	1	3054531.3	3054531.3	10.50	0.0020^{**}
V	1	4222253.6	4222253.6	14.51	0.0003^{***}
I	2	5949.8	2975.0	0.01	0.9898NS
VxI	2	613628.9	306814.8	1.05	0.355NS
N	4	2274361.0	568590.3	1.95	0.1139NS
VxN	4	2523935.7	630984.0	2.17	0.084NS
IxN	8	6468648.7	808581.1	2.78	0.0115^{*}
VxIxN	8	3981372.0	497671.5	1.71	0.1157NS

UT = Uncorrected Total
HSTD = Harvested stand
V = Variety
I = Inoculation
N = Nitrogen

*** Highly significant at $P \leq 0.001$
** Significant at $P \leq 0.01$
* Significant at $P \leq 0.05$
NS Not significant $P > 0.05$