EFFECT OF *Bradyrhizobium* INOCULATION AND METHODS OF MOLYBDENUM APPLICATION ON GROWTH, NITROGEN FIXATION AND YIELD OF TWO SOYABEAN *(Glycine max* (L.) Merrill) CULTIVARS

BY

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DEPARTMENT OF AGRONOMY

FACULTY OF AGRICULTURE

AHMADU BELLO UNIVERSITY

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MAY, 1999

DECLARATION

I hereby declare that this thesis has been written by me and that it is a record of my own research work. It has not been presented before in any previous application for a higher degree.

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CERTIFICATION

This thesis entitled "EFFECT OF *Bradyrhizobium* INOCULATION AND METHODS OF MOLYBDENUM APPLICATION ON GROWTH, NITROGEN FIXATION AND YIELD OF TWO SOYABEAN (*Glycine max* (L.) Merrill) cultivars" by Cecilia, Isibhakhomen Iyamabo meets the requlations governing the degree of Master of Science (M.Sc) of Ahmadu Bello University, Zaria and is approved for its contribution to science knowledge and literary presentation.

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DEDICATION

This work is affectionately dedicated to

my beloved parents

Mr. and Mrs. S.U. Iyamabo

who have always been there for me.

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"To God be the glory, great things he has done". My profound gratitude goes to God the Heavenly Father, for the graces showered on me throughout the course of this work.

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ABSTRACT

Field experiments were conducted at the Institute for Agricultural Research Farm, Samaru, Zaria during the wet seasons of 1995 and 1996 to study the effect of *Bradyrhizobium* inoculation and methods of molybdenum application on two soyabean {*Glycine max* (L.) Merrill) cultivars. The treatments consisted of two soyabean cultivars (SAMSOY-2 and TGx 1019-2EN), two methods of molybdenum application (seed treatment with 217-gMo/ha, foliar spray with 217g Mo/ha) and a no molybdenum control) and three levels of *Bradyrhizobium* inoculation (uninoculated, inoculation with *Bradyrhizobium)* strain IRJ 2184 and R.25B). Maize given no molybdenum, molybdenum as seed treatment and foliar spray was included as a reference crop. The twenty-one treatments of which three were reference treatments were arranged in all possible factorial combination in a randomized complete block design and replicated four times.

Bradyrhizobium inoculation with strain R.25B isolated from the nodules of soyabean at Samaru increased nitrogen fixation. Leaf area and leaf area index were significantly increased by strain R.25B. Strain R.25B increased grain yield by 15%. Yield increases were as a result of higher pod weight per plant, number and weight of seeds per plant and one hundred seed weight. Strain IRJ 2184A isolated from nodules of soyabean plant at Ibadan proved to be ineffective.

Molybdenum applied either as seed treatment or foliar spray significantly increased nitrogen fixation by 15% and 19% respectively. Also significantly increased were actual and relative uriede nitrogen, leaf dry weight, leaf area and leaf area index. Yield increases of 15% and 19% were obtained with the application of molybdenum as seed and foliar treatment respectively. This yield increase was as a result of higher number and weight of pods per plant, number and weight of seeds per plant and one hundred seed weight. When applied at the pre-bloom stage, foliar applied molybdenum was as effective as seed treatment.

The shorter determinate SAMSOY-2 cultivar exhibited more vigorous vegetative growth in terms of leaf number and weight, leaf area and leaf area index than the taller indeterminate TGx 1019-2EN cultivar. SAMSOY-2 out yielded TGx 1019-2EN by 25%.

Soyabean fixed between 37-115kgN/ha which was 24-92% of its total nitrogen.

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Chapter One

1.0 INTRODUCTION

1.1 Origin and Distribution

Soyabean (*Glycine max* (L.) Merrill) also known as Manchuria or Chinese beans is an ancient foodcrop belonging to the family leguminosea and subfamily papilionoidea. Although the exact centre of origin is uncertain, evidence based on distribution of *Glycine ussuriensis* which is believed to be the progenitor of *Glycine max*, suggests that it originated from China proper, probably north and central region. (Piper and Moorse, 1923; Nagata, 1959; 1960), where it has been grown for over 5,000 years (Hymowitz, 1970). Historical evidence also shows that soyabean has been recorded in China as far back as 2,838 BC (Leakey and Wills, 1977), where Chinese writings dated 2,800 BC named it as one of the five sacred crops.

The distribution of soyabean is however more certain. It spread from north China to east and southern countries of Manchuria, Korea and Japan between 2,000 BC and third century (Hymowitz and Kaizuma, 1979). Cultivation remained confined to this region until 1904, when there was exportation of the crop to Europe (Arnon, 1972; Probst and Judy, 1973). However, there are evidences that it had been known in Europe, since 17th Century (Bening, 1951) and in the United States of America since 1804 (Mease,1804), as an exotic crop from the orients. In the United State of America, it was initially used

exclusively as a forage crop until 1915, when the first oil was extracted from home-grown soyabean. The war in 1945 stimulated its increased production, because of war time demand for edible oils and fats (Purseglove, 1977).

Soyabean was introduced into Brazil by missionaries in 1882 (Dafferet, 1892) but did not become an important crop until about one hundred years latter. It was brought to England in 1709 and cultivated in Royal Botanical Garden, Kew (Aiton, 1814).

Soyabean was introduced into Africa by colonial officers and missionaries in the twentieth century. It reached Tanzania in 1907 and Uganda in 1913, but was not established as a crop until 1940 (Ezedinma, 1964; Auckland, 1970). It was introduced into Nigeria in 1905 (Ezedinma, 1964), and Malayan variety was the first to be recommended in the soyabean growing area of Nigeria (Leleji and Adedzuwa, 1983). Research work started on the crop in Samaru, Nigeria in 1930's and this led to the development of new cultivars SAMSOY- 2, SAMSOY- 1 (Leleji and Adedzuwa, 1983).

1.2 World Production of Soyabean

World production of soyabean increased by 47% from 86,078,000 metric tonnes in 1979-81 to 126,957,000 metric tonnes in 1995 (FAO, 1995). Total harvested area also increased by 24% from 50,542,000 hectares in 1979-81 to 62,462,000 hectare in 1995. Similarly, yield per hectare

increased by 19% from 1703 kg/ha in 1979-81 to 2033 kg/ha in 1995. Of the total production in 1995, the major producing countries of United State of America, Brazil, China and Argentina accounted for 87% of the total world production from 51,338,000 hectares, while the United States of America alone accounted for 54% from 24,980,000 hectare. Similarly, Brazil, China and Argentina produced 23%, 12% and 11% respectively from 26,358,000 hectares. Production in Africa was lowest at 554,000 metric tonnes and yield of 1134 kg/ha. Nevertheless, this is an improvement on total production and mean yields of 1979-81 which was 326,000 metric tonnes and 926 kg/ha respectively. (FAO,1995).

1.3 Soyabean Production in Nigeria

Nigeria is the leading producer of soyabean in Africa, with about 35% of Africa's production in 1995 (FAO, 1995). Production increased from 4,000 metric tonnes in 1948-1952 to 32,000 metric tonnes from 28,000 hectares in 1971 (Leakey and Wills, 1977). Further increase in production was reported from 60,000 metric tonnes in 1979-81 to 196,000 metric tonnes from 130,000 hectares in 1995. This was accompanied by increased yield per hectare from 300 kg/ha in 1979-81 to 1,508 kg/ha in 1995 (FAO, 1994). However, there was sharp decline in production between 1967 and 1969 due to the Nigerian civil war and from 1970 to 1973, which was attributed to lack of interest by farmers, drought and change in crop price (Ashaye

et al., 1974).

Benue State is the leading producer of soyabean in Nigeria with production centred around Gboko, Kwande, Katsina-Ala, Gwer and Makurdi Local Government areas of the State (Nyieakura, 1982). However, production which was hitherto confined to the traditional production areas of Benue and southern parts of Kaduna has spread to virtually every state of the country.

1.4 Economic Importance of Soyabean

1.4.1 Food

Soyabean is considered to be the most important grain legume because of its high protein and unsaturated oil content. It contain 40% protein (Ashaye *et al.*, 1974) and has a better balance of amino acid than other vegetable sources (Parman, 1975; Purseglove, 1977). Its amino acid content in gm/gm total nitrogen for isoleucine is 4.5 and 4.8 in seed and cake respectively, 7.8 of leucine in both seed and cake and 6.1 of lysine in seed and cake. Similar patterns were recorded for phenylanaline, alanine, aspartic acid, glutamic acid, proline and serine in both seed and cake of soyabean (Taylor, 1980).

It also contains 20% oil (Ashaye *et al.*, 1974) of which 85% is unsaturated and cholesteral free (Nelson *et al.*, 1978). Such unsaturated fatty acid are oleic, linolenic, linoleic, arachidonic, and tetradecanoic acids and low content of

palmitic, arachidic and stearic acid. It has a total digestable nutrient percentage of 92%, implying that it is an excellent energy source (Mijindadi, 1987). The soyabean cake gotten after oil extraction is a source of minerals, particularly calcium, phosphorus and iron. It has a good to excellent content of vitamin E and K (Taylor,1980).

The dried beans can be eaten, roasted, made into cheese substitute, vegetable milk, yoghurt and soyabean meat. It can also be made into local foods such as pap, porridge, "akara", "moi-moi" and condiment such as "daddawa". The ground paste is a good substitute for melon seed in soup preparation.

The soyabean cake when made into flour is used as wheat substitute in making confectioneries, bread, pastries, breakfast food, macaroni and spaghetti, while the soyabean oil is used as edible products such as margarine, salad oil, cooking oil, butter and lard.

1.4.2 Industrial uses

Soyabean is of various industrial uses. The oil is used in the manufacture of glycerine, varnishes, enamels, waterproof goods, linoleum, soap stock, printing ink, rubber substitute, lubricants, celluloids, glue, core wire, disinfectants and insecticides (Delorit *et al.*, 1974). The cake is also used as fertilizer constituent (Norman, 1978).

1.4.3 Soil improvement

Soyabean improves soil fertility by fixing nitrogen into the soil. This is possible because of the presence of nitrogen fixing bacteria in the root nodules of the plant. It fixes about 49-480 kg nitrogen per hectare for the subsequent crop planted (Rajaralnam, 1973; Hardy and Havelka, 1975; Peoples and Crasswell, 1992; Wanni and Lee, 1992).

Soyabean could be used as a cover crop for protecting the soil against erosion and adverse condition such as temperature. This is because of its fast growth, and ability to form canopy over the soil surface. The roots make the soil compact, therefore, improving soil structure. It is also very useful in crop rotation, and as green manure for soil organic matter enrichment .

1.4.4 Livestock feed

Soyabean could be used as a forage crop and fed to animals in form of hay and sillage. The cake is a major protein concentrate for livestock and poultry feed, as it contains up to 45% protein (Delorit *et al.*, 1974).

1.5 Constraints to Soyabean Production

Until recently, soyabean production in Nigeria was low. This was due to factors such as, availability of more palatable edible grain legumes, eg. cowpea (*Vigna unguiculata* (L.) Walp), difficulty associated with its cooking, flatulence

and lack of recognition of the potential of the crop (Bichi, 1982). Other reasons are poor seed viability (Joshua and Ogundipe, 1988), poor relative pricing offered by defunct Soyabean marketing board (Emechebe, 1983;Yuwa,1983) and poor agronomic characteristics of the available varieties. The variety such as "Malayan" which was widely grown in Nigeria from 1930, had a high shattering percentage (50%). However, of two low shattering varieties "SAMSOY-1" (M-79) with less than 5% shattering and "SAMSOY-2" (M-16) with less than 2% shattering and relatively high yielding have been released.

There is now an increased awareness of the use of the crop. The introduction in Nigeria of on farm trials in Nationally Co-ordinated Research Programmes from 1984 has resulted in adoption of soyabean as a commercial crop. Thus soyabean has spread to areas, previously considered unsuitable for its production.

1.6 Justification and Objective

The Northern Guinea savanna agroecological zone of Nigeria is considered to be the most suitable area for soyabean production because of its unimodal pattern of rainfall. However, savanna soils are generally low in native fertility particularly nitrogen and phosphorus (Heathcote and Stockinger, 1970; Mokwunye, 1979). It is therefore necessary to apply nutrients to crops in form of chemical fertilizer. Fertilizer use per hectare in Nigeria is very low due to

problems associated with fertilizer scarcity caused by poor distribution system and high cost of fertilizer. Thus, there is need to search for alternatives to chemical fertilizer.

In contrast to chemical inorganic fertilizer, the use of legumes in farming systems if the legumes are nodulated by efficient nitrogen fixing rhizobia is an attractive alternative. Their ability to fix atmospheric nitrogen allows them grow in nitrogen impoverished soils with very little nitrogen. The nodulated roots and above ground residue left after the seed and other components of the crop has been harvested represents a valuable source of replenishment of soil organic nitrogen. In the tropics over a period of 66-110 days, soyabean has been reported to fix 26-188 kg N/ha which is 70-87% of its total nitrogen using ^{15}N-natural abundance, ^{15}N-isotope dilution and difference methods (Eaglesham *et al.*, 1982, Boddey *et al.*, 1990; People *et al.*, 1991).

Soyabean is reported to be nodulated by *Bradyrhizobium japonicum* (Halwakar *et al.*, 1981; Moawad *et al.*, 1988; Olufajo *et al.*, 1989; Yoneyema, 1992), and the fast growing *Rhizobium fredii* (Giller and Wilson, 1991; Somasegaran and Hoben, 1994)and yield increases of 0.8t/8ha - 1.75 t/ha has been reported by these investigators. Though *Bradyrhizobium japonicum* and *Rhizobium fredii* are specific to soyabean, some soyabean varieties grown in Africa has been shown to have a wide range of compatibility with native Rhizobia strains indigenous to African soils. They nodulate and fix nitrogen

with these indigenous strains of *Rhizobium* isolated from the nodules of other legume hosts such as cowpea, when grown on the same field. Though nitrogen fixation occurs, there is limited information on the amount of nitrogen fixed especially in Nigerian soils.

There are various methods of measuring nitrogen fixation, some of which are isotopic method; ^{15}N isotope dilution method, ^{15}N-natural abundance, uriede assay, acetylene reduction assay and difference method. Despite active research on this subject, the measurement of the amount of nitrogen fixed, remains a problematic. This is largely because such methods used in determining nitrogen must be capable of differentiating nitrogen gained from the atmosphere from that absorbed from the soil. Sometimes, this is difficult to do as each method of measuring nitrogen fixation has its limitation.

The isotopic method has been named as the best method for determining nitrogen fixed, but it has been shown to be very expensive. The uriede technique on the other hand has been shown to be a cheap and easy method of measuring nitrogen fixed, and it gives estimates of nitrogen fixation that correlates very well with estimates obtained using other methods (Herridge *et al.*, 1990).

It is also known that the application of some mineral elements, enhance nitrogen fixation. Molybdenum in particular is a constituent of the nitrogen fixing enzyme, nitrogenase

(Chatt *et al.*, 1969). It is also an essential element of the enzyme nitrate reductase, which reduces nitrate to nitrite in protein synthesis (Nicholas and Nason, 1955; Evans, 1956). As a result, its presence at the appropriate level enhances nitrogen fixation and efficient functioning of the nitrogen fixing bacteria. Results of previous experiments conducted in the Nigerian Savanna to examine the effect of molybdenum on soyabean have been inconsistent (Pal *et al.*, 1989), probably because of the method of Molybdenum application. There is therefore need to determine the most effective method of Molybdenum application to enhance nitrogen fixation and increase soyabean yield.

This study was therefore undertaken to:

1. Quantify the amount of nitrogen fixed by soyabean inoculated with *Bradyrhizobium* strains and its effect on growth, yield and nitrogen fixation.
2. Determine the effect of different methods of Molybdenum application on growth, nitrogen fixation and yield of soyabean.

Chapter Two

2.0 LITERATURE REVIEW

The essential micronutrient (Zinc, Manganese, Iron, Boron, Molybdenum and Chlorine) are needed in small quantities but they are essential for plant growth and development. Molybdenum is indispensable for plant and animal growth and its content in soil ranges from trace to 20 ppm. Soils with high humus content and soil of the semi-arid zones, generally contain the highest concentration of molybdenum (Aubert and Pinta, 1977). Although molybdenum concentration ranges from 0-20 ppm, while available concentration in the plant ranges from 0.01-12 ppm (Aubert and Pinta, 1977).

There has been variation in the response of soyabean to rhizobial inoculation. This has been attributed to genetic factors, compatibility of rhizobia strain, co-evolution of adaptable host plant genotype (Lie *et al.*, 1987) and cropping history of the land on which soyabean was grown (Semu and Hume, 1979; Chowdury *et al.*, 1983).

2.1 Effect of Molybdenum on Soyabean

Over the past sixty years, molybdenum has been transformed from an element of unknown biological significance to a fertilizer of widespread importance. Bortel (1930) first established its function in biological process, when he showed that it was need by free-living Azotobacter to fix symbiotic nitrogen.

Molybdenum is essential for symbiotic nitrogen fixation by legumes (Evans, 1956). It is a constituent of the nitrogen fixing enzyme, nitrogenase (Chatt *et al.*, 1969; Burns *et al.*, 1970; Vandecasteel and Burns, 1970). This enzyme catalysis the fixation of atmospheric dinitrogen gas to ammonia, which is needed by the host plant. Molybdenum is also needed for nitrate reduction being an essential constituent of the enzyme nitrate reductase, which reduces nitrate to nitrite in protein synthesis (Nicholas and Nasson, 1955; Evans, 1956). However, less molybdenum is needed for nitrate reduction than to support nitrogen fixation (Parker and Harris, 1977). Consequently, legumes are particularly susceptible to molybdenum deficiency when relying on nitrogen fixation.

Deficiency of molybdenum is more widely spread on soyabean than any other field crop (Walter and Samuel, 1970). It differs from other micronutrient in that its availability increases with soil pH. Its deficiency is therefore most common on acid soil with low pH, as low pH makes molybdenum unavailable (Plant, 1951). Molybdenum is unavailable at pH below 6 (Anthony, 1967) and most available at pH of 6-6.5. Symptoms resembling nitrogen deficiency are shown by plants deficient in Molybdenum (Parker and Harris, 1962; Hagstrum and Berger, 1963). This is because symptoms occur due to lack of nitrogen in the plant tissue, rather than lack of molybdenum in the plant tissue. Deficiency, therefore, does not occur on soils with enough nitrogen to make up for the lack of nodule

nitrogen fixation. Such deficiency symptoms are yellow spots of chlorosis on the older leaves starting from the leaf tip, necrosis, impaired development of the vascular system (Bussler, 1970) and low yield (Atwal and Sidhu, 1964). Molybdenum deficiency also results in decreased chlorophyll concentration in the leaf (Das Gupta and Basuchaudhuri, 1977; Agarwala *et al.*, 1978), which leads to decreased photosynthetic efficiency. The lack of chlorophyll production is most likely a secondary effect on the disrupted nitrogen metabolic system, as molybdenum has not been shown to play a direct role in chlorophyll synthesis.

Molybdenum toxicity often occurs in heavy textured soils, formed from calcareous materials, marine sediments formed from calcareous materials, marine sediment of clay, schist found in peaty and certain gley soils (Ryan *et al.*, 1967).

2.1.1 Effect of molybdenum on growth

The application of molybdenum has been shown to significantly increase growth of soyabean (Boswell and Anderson, 1969). Nayak *et al.* (1989) observed that the application of 73g molybdenum per hectare on the field significantly enhanced soyabean growth. Parker and Harris (1962) also reported that 224g/ha molybdenum applied as seed treatment and foliar spray increased vegetative growth of soyabean. Similarly, in pot trials, Rao *et al.* (1978) observed that 50ppm molybdenum applied as foliar spray

increased plant dry weight, while Alphonso and Jimenex (1985) observed plant height and flower number where greatest in plants grown in complete nutrient solution in the presence of 0.5ppm molybdenum. Sokolova (1966) reported decrease in number of days to maturity. This beneficial effects of molybdenum were attributed to nitrogen fixation (Weerartha, 1980).

2.1.2 Effect of molybdenum on nodulation

Molybdenum has been reported to enhance nodulation in soyabean (Hagstrum and Berger, 1963; Sokolova, 1966). Parker *et al.* (1987) found that application of 15-30 grams molybdenum per hectare increased soyabean nodulation in acid soils. In pot trials, Huceng (1979) reported that the application of molybdenum increased the size and number of root nodules per plant. Similarly, Rao *et al.* (1978) observed increase in nodulation and nodule dry weight with application of 50ppm molybdenum as foliar spray.

2.1.3 Effect of molybdenum on yield and yield components

Molybdenum has been found to significantly increase soyabean grain yield (Sokolova, 1966; Boswell and Anderson, 1969; Huceng, 1979). Dermory (1970) reported that soyabean yield increased by 269kg/ha to 336kg/ha with application of 93g/ha molybdenum to soil with pH range of 5.0-6.7. Similar increases were reported by Harris *et al.* (1965) who found that

27g Molybdenum/67kg of seed gave yield response of 377-387kg/ha (20-43% increase) on soils with pH of 5.4. Parker and Harris (1962) also recorded up to 1,123kg/ha (50%) yield increase with application of 224g molybdenum and seed treatment compared with the zero molybdenum seed treatment on soil with pH of 5.1.

Molybdenum has been reported to increase seed size and weight (Parker and Harris, 1962; Sokolova, 1966), number of seeds per plant (Huceng, 1979) and number of pods per plant (Parker and Harris, 1962; Huceng, 1979). However, there are reports that molybdenum reduced oil content of soyabean, (Parker and Harris, 1962; Chu *et al.*, 1963; Boswell and Anderson, 1969). Although molybdenum decreased oil seed percentage per seed, total oil per hectare was increased (Sokolova, 1966). There was also improvement in water soluble protein and protein content of seed (Parker and Harris, 1962; Sokolova, 1966).

In contrast, molybdenum application did not result in significant yield increase in Sierra Leone (Rhode and Nangju, 1979) and Korea (Park *et al.*, 1987). In Nigeria, the application of 146g/ha molybdenum as 270g/ha ammonium molybdate increased yield of soyabean in Yandev (southern Guinea Savanna) only in the first year, but there was no response in the subsequent two years (Pal *et al.*, 1989). This lack of response was attributed to availability rather than deficiency of molybdenum and further investigation was

suggested. However, Bhattarri (1977) reported decrease in grain yield from 3.9-9.4 tonnes/ha to 3.7 tonnes/ha on application of 20g molybdenum/ha when soyabean was grown in Khuwar soil with pH of 6.5 in Nepal.

2.1.4 Effect of molybdenum on nutrient concentration

Molybdenum has been shown to increase nutrient concentration in soyabean plant. Hagstrum and Berger (1963) reported that nitrogen content of leaf was positively correlated to molybdenum supply. Similarly, Hagstrum and Berger (1963), Boswell and Anderson (1969) observed increases in nitrogen concentration of plant with increase after the end of flowering increasing from 2.4%-4.0%, while Chu *et al.* (1963) and Lee *et al.* (1967) observed decrease in nitrate content of the plant. This was attributed to enzyme reductase activity in converting nitrate to nitrite.

Molybdenum has also been reported to increase chlorophyll content of soyabean leaves (Smith, 1970). Pozsar (1965) reported improved total phosphorous uptake due to molybdenum application. The nucleic acid phosphorous and acid labile phosphorous in leaves also increased by 50% and 100% when molybdenu0m was applied to soil and sprayed respectively. Similarly, molybdenum application increased molybdenum content of leaves (Boswell and Anderson, 1969).

In contrast, Alphonso and Jimenex (1985) reported that increasing molybdenum concentration reduced nitrogen uptake

and in the presence of 0.5ppm molybdenum, the leaves became chlorotic and growth and development inhibited.

Lee *et al.* (1967) found that nitrogen, potassium and calcium content of soyabean leaf was not affected by molybdenum application. They suggested that the nutrient content of leaves is related to developmental stages, as differences at the young stage may disappear at the end of flowering.

2.1.5 Effect of method of molybdenum application

Some of the methods of molybdenum application are seed treatment with molybdenum salt, soil application and foliar spray.

Seed treatment with molybdenum salt is the most common method of molybdenum application (Caldwell, 1973). Parker and Harris (1962) in United States of America and (Petkova, 1968) in USSR found seed treatment with molybdenum salt more effective than foliar spray at the latter stages of growth. However, Boswell and Anderson (1969) reported that spraying 217g molybdenum at plant height of 20cm and at pre-bloom stage was as effective as seed treatment, and that response decreased significantly only when spraying was postponed until bloom and early pod formation stages.

Philips and Nugent (1970) observed that seed treatment at a rate of 0.5kg/ha molybdenum was less effective than molybdenum foliar spray of 210g molybdenum on unlimed soil.

Furthermore, seed treatment at the rate of 35g/ha molybdenum over a period of five years, left no residual effect, but the residual effect of foliar and soil applied molybdenum was as effective as liming.

2.2 Effect of *Bradyrhizobium* inoculation on Soyabean

In the centres of diversity of soyabean and countries of South Eastern Asia, where soyabean has been grown for several centuries, soyabean nodulates without rhizobial inoculation (Giller and Wilson, 1991). However, when grown in fields for the first time, soyabean did not nodulate in many parts of Africa, America, Europe and other parts of the world. In North America, soyabean yield was low, until soils containing compatible rhizobia where introduced from Japan (Allen and Allen, 1981). There is therefore need to inoculate here and positive response to inoculation has been reported. The failure to nodulate outside their area of domestication has been attributed to co-evolution of adaptable host plant, genotype and compatible rhizobia strain (Lie *et al.*, 1987). In tropical soils symbiotic nitrogen is important and inoculation with *Bradyrhizobium japonicum* has been shown to be essential for soyabean production.

Inoculation is therefore necessary only when soyabean is introduced to new areas (Semu and Hume, 1979). However, this need for inoculation is determined by the need of introduced soyabean crop for its specific strain of rhizobia. When the

introduced soyabean crop nodulates effectively with the rhizobia present in the soil in sufficient number, inoculation is not necessary or effective. However, where compatible rhizobia are absent, where the population of compatible rhizobia is too small to give sufficiently rapid nodulation or where the indigenous rhizobia are ineffective or less effective in nitrogen fixation with the host plant legume of interest than selected inoculant strains, inoculation is essential and effective.

Though inoculation increased yield when soyabean was introduced to new areas, it may be beneficial only in the first few years (Kalia *et al.*, 1984). Using two soyabean cultivars and four different rhizobia strains, Chowdhury *et al.* (1983), found that inoculation increased nodulation at the flowering and pod filling stages, and grain yield in the first year. Over the next few years, nodulation decreased ten folds and inoculation had no significant effect on grain yield.

2.2.1 Effect of *Bradyrhizobium* inoculation on growth

In pot trials inoculation of soyabean cultivar V9, Duocrop and Jupitar, with *Bradyrhizobium* strain 3412 in sand increased plant dry weight by 20-40% (Romero *et al.*, 1988). Similar observations were made by Muktar and Naib, 1987; Moawad *et al.*, 1988; Dou *et al.*, 1991, when soyabean was inoculated with and *Bradyrhizobium japonicum*.

Also soyabean varieties chip and clay when inoculated with different *Bradyrhizobium japonicum* strains produced more dry matter than control and non-nodulating isoline. Uninoculated control plants produced an average dry matter yield similar to those from non-nodulating plants (El-Mallah, 1978).

Inoculation with *Bradyrhizobium spp* also increased plant height (Yazdi - Samadi and Zali, 1978; Halwakar *et al.*, 1981) and dry weight of root by 20 - 80% (Romero *et al.*, 1988), but the number of days to flowering was reduced (Yazdi-Samadi and Zali, 1978).

2.2.2 Effect of *Bradyrhizobium* inoculation on nodulation

In field trials in Samaru, *Bradyrhizobium* spp has been shown to consistently enhance nodulation (Olufajo *et al.*, 1989). Similar observations were made by (Halwaker *et al.*, 1981), who reported that inoculation of soyabean with five *Rhizobium* strains significantly increased nodulation.

Similarly, inoculation with *Bradyrhizobium japonicum* increased nodulation by increasing number of nodules per plant (Yazdi-Samadi and Zali, 1978; Bishnor and Dutt, 1980; Dou *et al.*, 1991), weight of nodules per plant (Halwaker, 1981; Bishnor and Dutt, 1980, Muktar and Naib, 1987; Dou *et al.*, 1991) and nitrogenase activity in the nodule (Dou *et al.*, 1991).

2.2.3 Effect of *Bradyrhizobium* inoculation on yield and yield component

Inoculation with the appropriate strains of rhizobia, has been shown to substantially increase the yield of soyabean cultivar Bossier at different locations in Africa (I.I.T.A., 1980). Yield increase was comparable to or larger than those with the addition of 90kgN/ha of nitrogen fertilizer (IITA, 1980; Chowdhury *et al.*, 1983). In field experiments in the State of Rio Grande in Brazil, Freiere (1970) reported yield of eight soyabean varieties inoculated with multistrain inoculant were increased by 6%-12% over an uninoculated control. Kang *et al.* (1977) also reported works of Bonnier and Seeger (1958) in which yield increases of between 80-300% were obtained with inoculation when compared with uninoculated control in the Equatorial Region of Zaire.

Similarly, Sexen and Tilak (1975) showed that inoculated soyabean yields were increased from 73-94%. With no application of nitrogen fertilizer, yield of wheat following an inoculated crop of soyabean was more than 65% higher than wheat following a soyabean crop that had not been inoculated. The response was equivalent to about 30 Kg N/ha as fertilizer.

Similarly, inoculation with *Bradyrhizobium* strain has been reported to increase grain yield by a margin of 0.8t/ha - 1.75t/ha (Yazdi-Samadi and Zali, 1978; Muldoon *et al.*, 1980; Halwaker *et al.*, 1981; Wey, 1983). Other parameters significantly increased by rhizobial inoculation were: number

of seeds per plant (Halwakar *et al.*, 1981), weight of seed per plant (Yazdi-Samadi and Zali, 1978) and number of pods per plant (Khodabander and Yazdi-Samadi, 1978). Seed oil yield was increased from 0.85t/ha to 4.7t/ha (Chamber, 1979).

Inoculation with rhizobia in the root nodules have also been shown to provide sufficient nitrogen from soyabean yield of 4,000 Kg/ha with fixed nitrogen ammounting to 18 Kg/ha (Hardy and Havelka, 1975). A figure of 1,112 Kg./ha was quoted from Malaysia (Rajaratnam, 1973).

In contrast, response of soyabean to *Bradyrhizobium japonicum* has been observed in some years in Yandev (southern Guinea Savanna), but not Samaru (northern Guinea savanna) in Nigeria, despite the fact that the indigenous soil population of bacteria is low (Pal *et al.*, 1989). This lack of response in Samaru was attributed to incompatible and ineffective stains of *Bradyrhizobium* strains, more competitive strains or low survival rate of introduced strain due to failure to adapt to soil environmental condition.

2.2.4 Effect of *Bradyrhizobium* inoculation on nitrogen fixation

The quantity of nitrogen fixed by soyabean rhizobia is dependent on many environmental factors which affect the host plant (macrosymbiont), the rhizobia (microsymbiont) and the development as well as effective functioning of the nodules. Such factors are soil pH, temperature, moisture, organic

matter, nutrient content, inoculum rate and pesticide (Giller and Wilson, 1991).

Soyabean varieties chip and clay when inoculated with different *Bradyrhizobium japonicum* strain produced higher nitrogen content than uninoculated and non-nodulating isoline. Uninoculated control plant produced an average nitrogen content similar to those of non-nodulating plants (El Mallahi, 1978).

Schroder and Hinson (1974) grew nodulating and non-nodulating soyabean in rotation with winter rye in mixture with rye to study the contribution of legumes to soil nitrogen. Their results supported the idea that roots of nodulating soyabean leaves significant amount of nitrogen in the soil.

Soyabean has been reported to fix 0-450 kgN/ha of which is 0-95% of the total nitrogen (Peoples and Crasswell, 1992; People *et al.*, 1994a). Similarly, Peoples *et al.* (1994b) reported that soyabean had 330-348 KgN/ha crop nitrogen of which 44-250KgN/ha was by nitrogen fixation. This represented 13-72% of the plant total nitrogen.

Peoples *et al.* (1991) in Thailand observed that soyabean fixed 26-57KgN/ha, which was 78-87% of the total nitrogen over a period of 64-73 days using ^{15}N natural abundance. Also in Brazil, Boddey *et al.*(1990) reported that soyabean fixed 85-154KgN/ha which was 70-80%N of the total nitrogen over a period of 110 days using ^{15}N Isotope dilution method.

In Nigeria, Eaglesham *et al*, (1982), observed that soyabean fixed 114-188 KgN/ha which was 84-87% of its total nitrogen content over a period of 66 days using ^{15}N Isotope dilution and nitrogen difference method. In Egypt, Ritz (1966) showed a gain of soil nitrogen of 59 Kg nitrogen after soyabean crop was grown. The amount of nitrogen fixed in Kent variety of soyabean was 74-82KgN/ha from estimates based on acetylence reduction technique (Hardey *et al.*, 1968), while Webber *et al.* (1971) calculated that the amount of nitrogen fixed by soyabean was about 100Kg/ha. A conservative average of 70KgN fixed by soyabean *Bradyrhizobium japonicum* symbiosis in Brazil was reported by (Friere, 1976).

2.3 Interaction between *Bradyrhizobium* Inoculation and Molybdenum

Lyasnko (1980) reported that seed inoculation with *Bradyrhizobium* and molybdenum applied as soil treatment, increased nitrogen content of plant and protein content of seed. Also, applied molybdenum increased molybdenum content in leaves and seed, but uptake of molybdenum was highest when seed inoculation was combined with molybdenum application. Tripathski and Edward (1978) obtained higher seed yield when molybdenum application was combined with rhizobial inoculation, when compared with rhizobial inoculation alone.

Alphonso and Jimenex (1985) found that soyabean cultivar INIFAT 382 had the highest rate of nitrogen fixation when inoculated with *Bradyrhizobium japonicum* in the presence of molybdenum.

Sabel'nikova *et al.* (1977) also observed that nitragin (*Rhizobium* specie) and molybdenum increased fodder and seed yield of soyabean when grown as two crops per year under rainfed and irrigated conditions. Nitrogen crude protein and free amino acid content of the plant was also increased.

2.4 Methods of Determining Nitrogen Fixation

There are various methods of determining the amount of nitrogen fixed. Some of these methods are: ^{15}N Isotope dilution method (Thomas *et al.*, 1977), uriede assay (Herridge and Peoples, 1990) acetylence reduction assay (Dilworth, 1966), difference methods (Williams *et al.*, 1977) and nitrogen accumulation (Bremmer, 1965).

The ^{15}N isotope dilution technique provides the most precise quantitative determination of nitrogen fixation. However, the cost of spectrometer for isotope ratio analysis or of paying for isotope ratio analysis makes it prohibitive to some investigators. The need for less expensive methods is therefore desirable.

The uriede technique has been shown to be good (McClure *et al.*, 1980; Pate *et al.*, 1980) and uriede method has been shown to give estimate of nitrogen fixation that correlated

very well with estimates obtained using other methods such as ^{15}N natural abundance method (Herridge and Peoples 1990).

2.4.1 Uriede assay

The uriede technique was based on the fact that newly fixed nitrogen is assimilated in legume nodule and transported to other plant parts as uriede (allantoin and allantoic acid) or as amide. Nitrogen is absorbed from the soil predominantly as nitrate and to a lesser extent as ammonium. Nitrate is either reduced to ammonium in the root by subsequent action of enzyme nitrate reductase and nitrite reductase and assimilated into amino acid before being transported, or transported directly as nitrate to the stem before reduction and assimilation. Ammonium ion is however always rapidly assimilated before being transported, as they are toxic. Uriede producing legumes such as soyabean fixes most of its nitrogen in form of uriede, whilst nitrogen absorbed from the soil is in form of nitrate and to a lesser extent in form of amide. The proportion of nitrogen in the xylem sap present as uriede has been developed as an index of nitrogen fixation (McClure *et al.*, 1980; Pate *et al.*, 1980). Soyabean has been shown to fix between 6-56% nitrogen using uriede method (Herridge *et al.*, 1990).

Detailed tracer experiments using ^{15}N and ^{14}C (Herridge *et al.*, 1978; Matsumoto *et al.*, 1977) revealed that uriede (allantoin and allantoic acid) were products of nitrogen

fixation and responsible for transporting approximately 80% of fixed nitrogen from the root nodule of fully symbiotic plants. Also, they were present only in minor amounts in plants dependent on mineral nitrogen.

Samples for uriede analysis were collected as vacuum extracted sap, root bled sap and stem extract. Stem extract has been shown to be more suitable for determining nitrogen fixation when compared with root bled sap, as shoot axis provides the most suitable target organ when using this method because of the relative ease of sampling (Herridge, 1982).

Relative uriede of root bled sap was shown to be insensitive to diurnal fluctuation (Herridge, 1982a), thus simplifying the sampling procedure. Plant material could be stored at ambient temperature of 20-30°C for up to 24 hours without affecting the relative concentration of uriede and nitrate (Herridge, 1982a). Also, uriede technique has been shown to be possible with the standard curve presented, irrespective of genotype of strain or rhizobia occupying the nodules (Herridge and Peoples, 1990).

Like other methods of measuring nitrogen fixation this method has its limitation. Such limitations are: Variation in composition of sap with age of the plant and difficulty in extraction of sap (Peoples *et al.*, 1989) and the fact that bleeding sap can be extracted from cut stump of plant only if there is sufficient soil moisture. Other limitations are: Sap can be extracted from segment of the shoot tissue by the

use of mild vacuum or by stem extract, through the composition of such extract may differ from that of bleeding sap and may require separate calibration (Herridge and Peoples 1990). Nevertheless, the method has been shown to give estimates of nitrogen fixation that correlated well with estimates obtained using other methods like ^{15}N isotope dilution (Herridge and Peoples, 1990) and ^{15}N natural abundance method (Herridge *et al.*, 1990).

2.4.2 Nitrogen difference method

Legumes take up nitrogen as soil mineral nitrogen (from the soil and fertilizer) and by nitrogen fixation. However, non-nitrogen fixing plants take up nitrogen only as mineral nitrogen (from the soil and fertilizer). Therefore measurement of the contribution of nitrogen to the plant by nitrogen fixation is estimated by growing a nitrogen fixing crop with a non-nitrogen fixing crop with similar nitrogen uptake capability. The total nitrogen of the non-nitrogen fixing crop (derived solely from soil and fertilizer is subtracted from the total nitrogen content of nitrogen fixing legume. The difference between the value is assumed to be the quantity of nitrogen derived from nitrogen fixation. This method is called the difference method (Williams *et al.*, 1977). The method consists of comparing the total nitrogen content of a legume and non-legume crop, nodulating and non-nodulating legume or a rhizobia inoculated and un-inoculated

legume grown separately in replicated plots.

However, there are limitations in the use of this method. In the use of a nitrogen fixing and non-nitrogen fixing crops, limitation occurs as there is variation in the uptake of mineral nitrogen from the soil and fertilizer because of differential rooting system and uptake of nutrients. Also, the use of nodulating and non-nodulating legume is limited to areas where non-nodulating legumes are available. In the use of inoculated and uninoculated legume, availability, proper handling, use and adequate storage facility limits the use of inoculant.

2.4.2.1 **Comparism of legume with non-legume**

Soil nitrogen contribution by fixing legume is estimated by growing a non-legume concurrently with the legumes. Annual small grains such as wheat, barley (Rizk, 1966) have been used. Rizk (1966) used the difference method to calculate nitrogen fixation by soyabean in a calcarious sand at Kutch, Egypt. *Sesamum indicum* was the non-fixing control and soil nitrogen was measured before sowing and after harvesting. Rizk reported a fixation of 16.7KgN/ha and seed yield of 13.7KgN/ha. These low figures supports his statement that he had not found the most suitable tillage practice for the crop.

However, using a non-legume as a control in the difference methods requires the major assumptions. First, the nitrogen contained in the non-legume is assumed to arise

solely from soil nitrogen pool. Secondly, the legume and non-legume take up soil nitrogen in proportion to the amount available, and the differences due to growth patterns and root morphology would not be significant. The validity of this second assumption is open to question.

2.4. 2.2 Comparisim of nodulating and non-nodulating legume

The use of a non-nodulating line as control specie is based on the fact that the growth pattern, root morphology and nitrogen uptake patterns are assumed to be nearly identical. However, the nodulated isoline may absorb more soil nitrogen than the non-nodulated isoline (Ruschel *et al.*, 1979). This suggest a synergistic effect of fixed nitrogen on nitrogen derived from other sources. Also the use of this method requires host specificity i.e non-nodulating isoline.

However, Webber (1966) compared the above-ground nitrogen content of a nodulating and a near-isogenic non-nodulating soyabean cultiver Lee at Ames, lowa in the United States of America in soil low in nitrogen for 6 years. In 2 years when moisture was limiting the difference was 14.71 Kg N/ha representing 13% of the plant nitrogen. In 4 years with good growth conditions, the mean difference was 72.3KgN/ha in seed and 84kgN/ha in total dry matter. In both cases, this represented 40% of the total nitrogen in the nodulated plant.

Similarly, Bhangoo and Albritton (1976) compared nodulating and non-nodulating lines of cultivar Lee for 3

years at Pine Bluff, Arkansas. The nitrogen difference in seed and dry matter were 130, 161 and 56KgN/ha representing 51%, 64% and 22% respectively, of total plant nitrogen. In the first 2 years, the trials were on the same plot, whereas in the third year soyabean were planted on an area that had previously grown corn and had been heavily fertilized with NPK. This latter figure of 22% is probably more representative of the way soyabean is generally grown.

2.4.2.3 **Comparism of inoculated and uninoculated legume**

In comparism of cultivar grain of inoculated or un-inoculated soil. Separate plots inoculated with effective or ineffective strains of rhizobia (Nutman, 1973) was used. Here identical variety of soyabean was used for control and treatment with the variable only being the presence or absence of nodules (effective and ineffective nodules), due to the presence and absence of rhizobial inoculation. This method requires soil free of native rhizobia species capable of establishing an effective symbiosis with the legume.

Bezdicek *et al.* (1970) compared inoculated and uninoculated soyabean under field conditions. Uninoculated soyabean accumulated 76 and 105kgN/ha in the 2 years, while nodulated soyabean accumulated 387 and 368kgN/ha. By difference, the net fixation was calculated at 311 and 263KgN/ha.

In areas where legumes are routinely grown, soil lacking appropriate rhizobia specie may not be available. Care should also be taken to avoid contamination of uninoculated soils with rhizobia. These requirements makes the use of this procedure limited.

Like other methods, this method has its assumption. Here it is assumed that the root structure and function are not altered by the presence of nodules. This assumption has not yet been proven experimentally.

Chapter Three

3.0 MATERIALS AND METHODS

3.1 Site Description

A field experiment was conducted in the wet seasons of 1995 and 1996 at the Institute for Agricultural Research Farm, Samaru, Zaria (11°11^{1}N, 07° 38^{1}E), 686 meters above sea level to test the response of two soyabean cultivars to *Bradyrhizobium* inoculation and methods of molybdenum application. Samaru lies in the northern Guinea savanna ecological zone with a mean annual rainfall of 1100mm distributed between May and October and a peak in July/August. The hottest months are those preceding the rain (March/April), while the coldest months are in December/January.

The soil of the experimental Site in 1995 was silty loam, while that of 1996 was loam. Physiochemical properties of the soils of the experimental sites are presented in Appendix 1. The history of the land showed that the land used in 1995 and 1996 were cropped with maize the previous year.

3.2 Treatments and Experimental Design

The treatments consisted of two soyabean cultivars (SAMSOY-2 and TGx 1019-2EN), two methods of molybdenum application (seed treatment and foliar spray) and a no molybdenum control and three levels of *Bradyrhizobium* inoculation (uninoculated, inoculated with IRJ 2184A or inoculation with R.25B). Maize given molybdenum using the two

methods of molybdenum application as in the soyabean cultivar and no molybdenum control was included as a reference crop. These treatments were arranged in all possible factorial combination to give twenty-one treatments of which three were reference treatments in a randomized complete block design and replicated four times.

Each plot was 4m long and 4.5m wide, consisting of six rows 75cm apart. The gross plot was $18m^2$, while 3m length of the two centre rows ($4.5m^2$) was the net plot. The two outer rows of the plot were used as discard, whilst 3m length of the second and fifth rows of each plot were used for sampling. Plots and replications were separated by 1m unplanted area.

3.3 Treatment Details

3.3.1 Cultivars

Two soyabean cultivars were used SAMSOY-2 and TGx 1019-2EN.

The cultivar SAMSOY-2 (Line M216) was developed at the Institute for Agricultural Research Samaru by hybridization between soyabean cultivars "Malayan" and "Clemson" non-shattering. SAMSOY-2 is stout and short. It is determinate in growth habit and it bears its pods in clusters above the ground. It is resistant to shattering (less than 2%) and tolerant to pest and diseases. It has a large yellow seeds and is of medium maturity (112-118 days). (Leleji and Adedzuwa, 1983).

TGx 1019-2EN is determinate, medium maturing, moderately shattering resistant with medium height and low branching habit. The seeds are medium sized and light yellow in colour.

3.3.2 Molybdenum Application

Two hundred and seventeen grams Molybdenum as 399.34g ammonium molybdate was applied as seed treatment prior to planting. 20% sugar solution was used as sticker. For foliar spray, the same amount of ammonium molybdate was dissolved in water and sprayed on the soyabean leaves at the pre-bloom stage, about 20cm height (four weeks after planting) using a CP_3 knapsac sprayer.

3.3.3 Inoculant

Bradyrhizobium inoculation was done with strains R.25B and IRJ 2184A. Both inoculants were obtained from the International Institute for Tropical Agriculture, Ibadan.

Both inoculants belong to the genus *Bradyrhizobium*. They are gram negative "slow" growers with a generation time of 7-13 hours (Giller and Wilson, 1991). They require 3-5 days to produce moderate turbidity in liquid media and form visible colony of 1mm in 6-10 days. The cells are motile by a single polar or sub polar flagellum, and they utilize pentose sugars as their carbon source. They do not posses 6-phosphogluconate dehydrogenase, the key enzyme of the hexose monophosphate pathway. They also carry out nitrogen fixation in

microaerobic conditions, but the products cannot be assimilated (Giller and Wilson, 1991).

The inoculant R.25B was isolated at Zaria in 1994 from the nodules of soyabean plant. It is a promiscuous stain of rhizobia which nodulates all soyabean cultivars. It has also been shown to be effective on *Vigna unguiculata*

IRJ 2184A was isolated at the International Institute for Tropical Research Ibadan in 1979 from the nodules of soyabean. It has been shown to be effective on only *Glycine max*.

Seed inoculation with *Bradyrhizobium* was done by putting weighed amount of soyabean seeds in an air inflated polythene bags. 15 mls sticker of 20% sugar solution was gradually added per kilogram of seed in an air inflated polythene. The air inflated polythene was shaken until the seeds were evenly wet. 18 grams and 27 grams peat based *Bradyrhizobium* inoculant was added per kilogram of seed in 1995 and 1996 respectively. The sticky soyabean seed was then uniformly coated with *Bradyrhizobium* inoculum by shaking the soyabean seeds in the air inflated polythene to give uniform spread of the inoculant on the seed. This gave a rhizobia population of about $2.0x5^5$ rhizobia when based on inoculation of $1x10^9$ cells/grams. The inoculated seeds were immediately spread on a clean surface and allowed to dry in the shade before planting the seeds.

3.4 Determination Of Soil Nutrient Status

Soil samples were collected from the experimental site before ridging at a depth of 0-15cm and 15-30cm. The soils were analysed in the laboratory for physiochemical properties using standard procedures.

3.4.1 Particle size analysis

Particle size distribution was determined by the hydrometer method using calgon as dispersing agent (Day, 1965). The textural class was determined by the use of standard textural triangle.

3.4.2 Total soil nitrogen

Total soil nitrogen was determined by Macrokjeldah process (Bremmer, 1965; IITA, 1975), 5 grams of air dry soil was put in a macrokjeldah digestion flask with 30 mls of concentrated H_2SO_4 using mercury tablet and 10 gram potassium as catalyst. The flask was heated gently for 3-5 minutes. The digest was distilled into 50 ml boric acid using 150mls of 10N NaOH. The distillate was then titrated against 0.01N hydrochloric acid, until the colour changed from green to pink.

3.4.3 Soil pH

Soil pH was determined in 0.01m $CaCl_2$ and in water, using a soil solution ratio of 1:2 by means of a Philip analogue.

3.4.4 Available phosphorous

Five grams of less than 2mm diameter was put into an extracting bottle, 100mls of extraction solution (0.5m) $NaHCO_3$ at pH 8.5 and 1 teaspoon of carbon black (Darco E60 quality) was added to the extraction bottle. This was shaken for 30 minutes and the suspension filtered by use of a centrifuge at the speed of 600xg. The supernatant solution was decanted into a clean vial and the extracted phosphorous determined colorimetrically using molybdenum blue method (Bray and Kuntz, 1945) modified by (Murphy and Riley, 1962).

3.4.5 Exchangeable bases

The exchangeable bases were extracted with neutral IN acetic acid (NH_4OAC). Potassium and sodium were determined by atomic absorption spectrophotometry (Pratt, 1965a; Pratt, 1965b) using Perkin Elmer model 403.

3.4.6 Cation exchange capacity

Five grams of 2mm sieved air dry soil was weighed into a centrifuge tube. 30mls 1N ammonium acetate (NH_4OAC) solution was added using a measuring cylinder of 50 mls volume. The tubes were placed on a mechanical shaker and shaken for 1-2 hours at about 2500 rpm. The samples were then centrifuged for 10 minutes in a centrifuge at about 5,000 rpm. The extract was carefully decanted from the soil. With a pipette the clean clear solution was put into a clean glass vial. The

samples were read along side working standards on AAS (for Ca, Mg, Mo) and flame photometer (for Na and K). The carbon exchange capacity was then calculated.

3.4.7 Organic carbon

This was determined by chromic acid method (Allison, 1965). 1 gram of soil was weighed into 250 mls erlenmeyer flask and 10mls 1N potassium dichromate ($K_2Cr_2O_7$) was added and the flask swirled gently to dispense the soil. 10 mls concentrated H_2So_4 was rapidly added and swirled for 1 minute again. The flask was then allowed to stand on asbestors sheet for 30 minutes and 100 ml distilled water added to the flask and allowed to cool. The suspension was filtered with a filter paper. Five drops of indicator was added and it was titrated with ferrous ammonium sulphate solution with a white background and titration was done until colour change from blue to green to dark green and red.

3.5 Cultural Practices

3.5.1 Land preparation

The experimental site was ploughed to a depth of about 25cm and harrowed twice with a notch disc harrow, to break soil cloids, give fine tilth and bury planting materials. A rider attached to the draw-bar of a tractor was used to make ridges 75cm apart (interrow spacing).

3.5.2 Planting and planting material

Planting was done on 19 July, 1995 and 17 June, 1996 as soon as the rains were established. Seeds were sown at a depth of 2.5cm by hand drilling and thinned to 5cm intrarow spacing with a 5cm marked rope. This gave a plant population of about 266,666 plants per hectare.

3.5.3 Fertilizer application

All plots received a basal application of 10kg N/ha, 26kg P/ha and 33.3kg K/ha as urea (46% N), single superphosphate (18% P_2O_5 or 7.8% P) and muriate of potash (60% K_20 or 33.44%k) respectively.

3.5.4 Weed control

Weed control was done a day after planting with application of the preemergence herbicide (1:1 mixture of metolachlor and metobromuron) at a rate of five kilogram per hectare. Hoe weeding was done at 3 and 6 weeks after sowing.

3.5.5 Harvesting

Harvesting was done when the plants reached full maturity i.e. when 95% of the pods turned brown (Fehr *et al*, 1971).

3.6 Observation and Data Collection

Five plants samples were taken from the sampling rows of each plot beginning from 4 weeks after sowing and at 2 weeks

interval until maturity. At harvest, ten plants were taken as sample. The plants were uprooted with a garden fork to minimize the damage on nodules. The following parameters were measured.

3.6.1 Plant height (cm)

The height of the five sampled plants were taken from ground level to the top of the main stem using a metre rule. The mean height was recorded.

3.6.2 Number of leaves per plant

The leaves from the sampled plants were detached and counted. The mean number of leaves was recorded.

3.6.3 Weight of leaves per plant (g)

Leaves from the sampled plants were detached dried to constant weight in a gallenkemp oven model OV-420 at a temperature of 70°C for 48 hours and weighed with a mettler balance model P.1200. The mean of this weight was recorded.

3.6.4 Leaf area per plant (Cm^2)

Leaves from the sampled plants were detached. The leaf area was determined using cork borer by disc area and dry matter relationship as described by Watson (1958).

3.6.5 Leaf area index

This was determined by dividing the total leaf area by the ground covered by leaves (Watson, 1947; 1952).

3.6.6 Number of branches per plant

At each sampling period, the number of branches of sampled plants were counted and the mean number of branches recorded.

3.6.7 Dry matter accumulation per plant (g)

The sampled plants were oven dried in a gallenkemp oven model OV-420 at a temperature of 70°C for 48 hours and weighed with a mettler balance model P.1200. The mean weight was recorded.

3.6.8 Nodule number per plant

Nodules were detached from the roots of the sampled plants, washed and cleaned of soil particles. The nodules were counted and the mean number recorded.

3.6.9 Nodule dry weight per plant (g)

After counting the detached nodules, the nodules were dried to constant weight in a gallenkemp oven model OV-420 at a temperature of about 70°C for 48 hours and weighed with a mettler balance model P.1200

3.6.10 Number of days to 50% flowering

Days from sowing to the time when 50% of the plants produced flower was recorded.

3.6.11 Number of days to physiological maturity

Days from sowing to when pods became yellow and 50% of the leaves turn yellow (Fehr *et al.*, 1971).

3.6.12 Number of days to harvest maturity

Days from sowing to when 95% of the pods turned brown (Fehr *et al.*, 1971).

3.6.13 Number of pods per plant

After detaching the pods from the ten sampled plants the pods were counted and the mean number recorded.

3.6.14 Weight of pods per plant (g)

After detaching and counting the pods from the ten sampled plants, the pods were weighed with a mettler balance model P.1200. The mean weight was recorded.

3.6.15 Seed number per plant

At harvest, the seeds obtained from the ten sampled plants were counted and the mean number of seeds recorded.

3.6.16 Seed weight per plant (g)

At harvest pods from the ten sampled plants were detached and threshed. The seeds obtained were weighed using a mettler balance model P.1200 and the mean seed weight recorded.

3.6.17 One hundred seed weight (g)

After harvesting and threshing, one hundred seeds were randomly selected from each plot and weighed with a mettler balance model P.1200.

3.6.18 Seed number per pod

At harvest, the seeds obtained from the pods of the ten sampled plants were counted and divided by the number of pods.

3.6.19 Grain yield per hectare (Kg/ha)

The seeds from the net plot were weighed using a mettler balance model P.1200 and the weight obtained multiplied by 2.22 to convert from grams per plot to kilogram per hectare.

3.6.20 Shelling percentage (%)

The harvested pods were shelled and the following equation used:

$$\frac{\text{Weight of seed of x Pods}}{\text{Weight of x unshelled pods}} \times 100$$

3.6.21 Harvest index

This is derived from the formulae:

$$\frac{\text{Weight of seeds}}{\text{Weight of total dry matter}} \times 100$$

3.6.22 Phosphorous content

Plant phosphorous content was determined by the molybdenum blue colour method developed by (Bray and Kuntz, 1945) and modified by (Murphy and Riley, 1962).

3.6.23 Uriede assay

The uriede assay method was used to asses the proportion of nitrogen in soyabean derived from nitrogen fixation (Herridge and Peoples, 1990) This method was based on analysis of nitrogen collected as root-bleeding sap (RBS) and stem extract (SE) (Herridge, 1982a; Herridge, 1984; McClure *et al.*, 1980).

Root bleeding sap was collected at the early podding stage (R_3) ten weeks after sowing (Fehr *et al.*, 1971) from the same plant used to evaluate nodulation. The stem of the plant was

bled by decapitating the stem midway between ground level and the cotyledons, using a surgical blade. Silicon rubber tubes of about 2-3cm in length and appropriate diameter were inserted firmly but carefully to the cut root stump. Care was taken to prevent damage to the epidermis of the stem to avoid xylem sap leakage. The sap was then extracted using a syringe and put in a vacutainer, which was tightly corked. Xylem sap was collected for a period of 30 minutes to minimize starvation reaction (Pate, 1980). Xylem sap was then chilled on ice immediately after collection on the field and transferred to a freezer of about -15°C for storage until analysed for uriede (allantoin and allantoic acid) colorimetrically as hydrazone of glyphosate (Young and Conway, 1942) for nitrate by salicylic acid technique (Cataldo *et al.*, 1975) and alpha amino with ninhydrin (Herridge, 1984; Yemm and Cocking, 1955).

Stem xylem sap was collected between 9.00 am to 1.00 p.m, the period when relative nitrogen solute composition was most stable (Herridge *et al.*, 1990) and to decrease diurnal variation (Pate, 1982). Xylem sap exudation rate ranged from 0.01-0.5ml/plant/minute with the volume of exudate from root bleeding sap being between 0.3 - 15ml/plant in 30 minutes.

Stem extract was collected from the shoot of the plant from which xylem sap was collected. The leaflets were removed, and stem and petiole dried at a temperature of about 80°C for 48 hours in gallenkemp oven model OV-420. The stem

and petiole were then finely ground and samples (1g) of stem and petiole extracted with 25 mls boiling water for 2 minutes as described by (Avery *et al*, 1973; Herridge, 1982, Herridge and peoples, 1990). The extract was filtered, made up to volume (50 ml) and stored at -15°C to be analysed latter for uriede and nitrate.

The nodules from the sampled plans were dug up using a garden fork, washed, dried to constant weight in gallenkemp oven model OV-420 at a temprature of 70°C for 48 hours and weighed with a mettler balance model P.1200.

3.6.23.1 Uriede nitrogen

The concentration of uriede nitrogen (allantoin and allantoic acid) in stem extract and root-bled sap was estimated colorimetrically as hydrazone of glyphosate (Young and Conway, 1942).

0.05 ml of sap sample was dispensed into two testube. To check for consistency two testubes were used for each sample. 2.45 mls of distilled water was also dispensed into these sample tubes to make it up to 2.50 mls. 2.50 mls of water, was then dispensed into two other testubes as blank. Similarly, 2.50mls internal standard was dispensed into two testubes. 0.5ml (0.5N) sodium hydroxide (NaOH) was added to each testube and boiled in hot water bath with temperature of about 98°C for 10 minutes. The testubes were removed from the hot water bath and 1ml phenyl/HCl mixture added to all the

testubes. The testubes were put back in the hot water bath of temperature of 98°C for 2.5 minutes. After 2.5 minutes, the testubes were then removed and immediately placed on ice chips for 15 minutes to allow for rapid cooling. After 15 minutes, the testubes were removed from the ice chips and 2.5mls HCl/KFECN mixture added. It was left for 10 minutes on the bench to allow for optimum colour development. The spectrophotometer was set to a 525mm wavelength (absorbance). The blank sample was used to zero the spectophotometer. The optical density for absorbance of samples in the testubes was then read quickly at 120nm wavelenght on the digital spectrophotometer model CECIL CE 2000 series before colour fading of the samples occured, as colour starts fading after 15 minutes. The results obtained from the testube of the sample were averaged to obtain uriede content.

3.6.23.2 Nitrate nitrogen (nm)

Nitrate nitrogen in stem extract and root bled sap was measured by salicylic acid technique (Cataldo *et al.*, 1975). To check for consistency, two testubes were used for each sample 0.05ml of sap sample was dispensed into the two testubes. 0.05ml of distilled water was also dispensed into two other testubes as blank. Similarly, 0.05 ml internal standard was dispensed into two other testubes. 2mls H_2SO_4 and salicylic acid mixture was dispensed into the testubes gently and continuously because of its viscosity. Each testube was

well shaken to ensure proper mixing. The testubes were left on the bench for 20 minutes and 4.75mls of NaOH (2N) solution was added and left for 10 minutes. The spectophotometer was set to wavelength of 410nm (absorbance). The blank sample was used to zero the spectrophotometer. The optical density for absorbance of the samples were then read at 410 nm wavelength. The results obtained from the two testubes of the same sample were averaged to obtain the nitrate content.

3.6.23.3 Alpha amino nitrogen (nm)

Alpha amino nitrogen in root bled sap was determined colorimetrically with ninhydrin (Herridge, 1984; Yemm and Cocking, 1985) using a 1:1 asparagine: glutamine standard. 0.05ml of sap sample was dispensed into two testubes and 0.95ml of distilled water added to the two testubes to make it up to 1ml. 1ml distilled water was dispensed into two testubes as blank, and 1ml standard into two other testubes. 0.05ml citrate buffer was added into the two testubes. 1.25 mls of ninhydrin reagent was then also added into all the testube and boiled gently for 20 minutes. The testubes were then removed from the hot water bath and 3mls 60% ethanol added. The testubes were left on the bench and the samples allowed to cool to room temperature. The blank was used to zero the spectophotometer and the optical density of the samples read at 510nm wavelength (absorbance). Results obtained from the same sample were averaged to obtain the

alpha amino content.

3.6.23.4 Uriede nitrogen concentration (mM)

The concentration of uriede (allantoin and allantoic acid) in the root bleeding sap and stem extract was determined by the formulae:

$$\frac{\text{Standard Conc. x Optical density of sample (uriede)}}{\text{Optical density of standard}} \text{ x dilution}$$

3.6.23.5 Actual nitrogen uriede (mM)

Actual uriede was determined by the formulae:

4 x molar concentration of uriede

3.6.23.6 Nitrate nitrogen concentration (mM)

The nitrate concentration in the root bleeding sap and stem extract was determined by the formulae:

$$\frac{\text{Standard Conc. x Optical density of sample (nitrate)}}{\text{Optical density of standard}} \text{ x dilution}$$

3.6.23.7 Alpha amino nitrogen concentration (mM)

Alpha amino concentration of the root bled sap was determined by the formulae:

$$\frac{\text{Standard Conc. x Optical density of sample (amino-N)}}{\text{Optical density of standard}} \text{ x dilution}$$

3.6.23.8 Total sap nitrogen (mM)

Total sap nitrogen was determined by the formulae:

Root bleeding sap = actual uriede+nitrate-N + amino-N

Stem extract = actual uriede + nitrate-N

3.6.23.9 Relative uriede nitrogen(%)

Relative abundance of uriede nitrogen in the root bleed sap (RBS) was determined as described by (Herridge and People, 1990).

$$\text{Relative uriede-N(\%)} = \frac{4a\ (\text{actual uriede-N})}{(\ 4a+b+c)} \times 100$$

Where a,b,c are the molar concentration of uriede-N, nitrate-N and alpha amino-N respectively (uriede-N contains 4 nitrogen atom per molecule) (Herridge, 1984).
Relative abundance of uriede- N in stem extract (SE) was calculated as:

$$\text{Relative uriede N(\%)} = \frac{4\ (\text{actual uriede})}{(4a+b)}$$

Where a and b are respectively molar concentration of uriede-N and nitrate-N (Herridge and Pleoples, 1990).

3.6.23.10 Total Plant Nitrogen (kg/ha]

Total plant nitrogen analysis was determined at ten weeks after sowing. The oven dried sample (shoot) from each plot was properly ground in a mill to powder form for analysis. Total plant nitrogen was determined by
macro-kjeldahl (IITA, 1975).

3.6.23.11 Nitrogen fixed (%)

Percentage nitrogen fixed for root bled sap and stem extract was determined from the relative uriede nitrogen value, read from an already calibrated table based on regression established from greenhouse calibration (Herridge and People, 1990).

3.6.23.12 Nitrogen fixed (kg/ha)

Nitrogen fixed for uriede assay was calibrated as:

% Nitrogen fixed x total plant nitrogen (kg/ha)

For difference method:

Total plant nitrogen of soyabean - Total plant nitrogen of maize.

3.7 Statistical analysis of data

Data collected were subjected to analysis of variance (F-test) (Snedocor and Cochran, 1967). Significant differences among treatment means were evaluated using and Duncan Multiple Range Test (DMRT) (Duncan, 1955). Simple correlation coefficient was calculated from the means of each parameter according to Snedecor and Cochran (1967).

3.8 Meteorological information

Rainfall, temperature and relative humidity during the experimental period of 1995 and 1996 were obtained from the Institute for Agricultural Research, meteorological Station, Samaru, Zaria.

Chapter Four

4.0 RESULTS

4.1 Effect of *Bradyrhizobium* Inoculation, Methods of Molybdenum Application and Cultivar on Growth, Nitrogen Fixation and Yield Of Soyabean

Crop growth during the 1995 cropping season was good, though planting was done rather late (17 July), due to the short drought spell between 18 June and 15 July. Nevertheless, germination was good and crop growth was vigorous throughout the season.

In 1996, planting was done timely (17 June) but bacterial pustule *(Xanthomonas compestris)* (score 40%) occurred from the fifth week after planting with a peak at the ninth week. SAMSOY-2 was more susceptible than TGx 1019-2EN.

4.1.1. Number of leaves per plant

In both years, *Bradyrhizobium* inoculation had no significant effect on the number of leaves per plant, except at 12 WAS in 1995 when strain R.25B increased leaf number significantly compared with strain IRJ 2184A and the uninoculated control (Table 1).

Leaf number per plant was not influenced significantly by molybdenum throughout the sampling period (Table 1). However, in 1996, the number of leaves per plant at 8 WAS was

Table 1: Leaf number per plant of soyabean as influenced by *Bradyrhizobium* inoculation, method of molybdenum application and cultivar at 8, 10 and 12 WAS at Samaru in 1995 and 1996.

Treatment	Weeks after sowing					
	8		10		12	
	1995	1996	1995	1996	1995	1996
Inoculant (I)						
Uninoculated	18.50	16.67	20.08	20.67	17.21b	19.13
IRJ 2184A	20.42	15.97	19.96	21.97	17.71b	19.33
R.25B	19.92	16.33	22.33	19.00	19.71a	20.17
SE±	0.89	0.80	0.76	1.41	0.67*	1.25
Molybdenum (M)						
No molybdenum	18.29	13.71b	21.40	18.46	17.67	18.67
Seed treatment	19.58	19.17a	19.43	20.08	17.78	19.42
Foliar Spray	20.96	17.92a	21.54	22.92	19.25	20.88
SE±	0.89	0.80**	0.76	1.41	0.67	1.25
Cultivar (C)						
SAMSOY-2	23.22a	19.28a	23.78a	22.28a	20.47a	21.89
TGx 1019-2EN	16.10b	13.25b	17.81b	18.69b	15.94b	19.19
SE±	0.73**	0.66**	0.62**	1.15*	0.55**	1.02
Interactions						
I x M	NS	NS	NS	NS	NS	NS
I x C	NS	NS	NS	NS	NS	NS
M x X	NS	NS	NS	NS	NS	NS
I x M x C	NS	NS	NS	NS	NS	NS

Means with the same letter within the same treatment group, year and stage of growth are not significantly different at 5% level of probability using Duncan multiple range test.

Abbreviations
* Significant at 5% level of probability
** Significant at 1% level of probability
NS Not Significant
WAS Weeks after sowing
SE Standard error.

significantly higher when molybdenum was applied either as foliar spray or seed treatment when compared with no molybdenum control.

Cultivar effect was significant at all the sampling dates except at 12 WAS in 1996 (Table 1), when SAMSOY-2 producing more leaves than TGx 1019-2EN.

None of the interactions was significant (Table 1).

4.1.2 Leaf dry weight per plant (g)

Leaf dry weight was not significantly affected by *Bradyrhizobium* inoculation (Table 2).

Molybdenum application also had no significant influence on leaf dry weight in 1995 (Table 2). However, in 1996, seed AND Foliar application of molybdenum significantly increased leaf dry weight at all the sampling dates when compared with no molybdenum control.

Cultivar effect was significant at 8 and 12 WAS in 1995, and 8 and 10 WAS in 1996 (Table 2). In all cases, SAMSOY-2 produced heavier leaves than TGx 1019-2EN.

None of the interactions was significant (Table 2).

4.1.3. Leaf area per plant (cm^2)

Bradyrhizobium inoculation had significant effect on leaf area only at 10 WAS only in both years (Table 3). Strain R.25B increased leaf area significantly when compared to IRJ 2184A.

Table 2: Leaf dry weight per plant (g) of soyabean as influenced by *Bradyrhizobium* inoculation, method of molybdenum application and cultivar at 8,10,12 WAS at Samaru in 1995 and 1996.

Treatments	Weeks after sowing					
	8		10		12	
	1995	1996	1995	1996	1995	1996
Inoculant (I)						
Uninoculated	6.24	4.65	9.05	6.55	5.16	4.42
IRJ 2184A	6.21	4.56	10.66	6.75	4.46	5.67
R.25B	6.53	5.22	10.15	6.74	4.95	5.48
SE±	0.28	0.37	0.57	0.61	0.34	6.42
Molybdenum (M)						
No molybdenum	5.86	4.28b	9.44	5.12b	4.84	4.11b
Seed treatment	6.59	5.85a	10.00	7.38a	4.89	6.06a
Foliar Spray	6.53	5.30a	9.49	7.04a	4.87	5.40a
SE±	0.28	0.37**	0.57	0.61*	0.34	0.42**
Cultivar (C)						
SAMSOY-2	6.68a	5.58a	9.99	7.74a	5.36a	5.65
TGx 1019-2EN	5.97b	4.04b	9.83	5.62b	4.36b	4.73
SE±	0.23*	0.30**	0.47	0.50**	0.28**	0.35
Interactions						
I x M	NS	NS	NS	NS	NS	NS
I x C	NS	NS	NS	NS	NS	NS
M x X	NS	NS	NS	NS	NS	NS
I x M xC	NS	NS	NS	NS	NS	NS

Means with the same letter within the same treatment group, year and stage of growth are not significaly different at 5% level of probability using Duncan multiple range test.

Abbreviations
* Significant at 5% level of probability
** Significant at 1% level of probability
NS Not Significant
WAS Weeks after sowing
SE Standard error.

Molybdenum increased leaf area per plant significantly at 10 and 12 WAS in both years (Table 3). The application of molybdenum as either seed treatment of foliar spray resulted in significant increase in leaf area per plant when compared with the no molybdenum control.

Cultivar effect showed that SAMSOY-2 consistently produced significantly higher leaf area than TGx 1019-2EN (Table 3).

None of the interactions was significant (Table 3).

4.1.4. Leaf area index

Bradyrhizobium inoculation influenced leaf area index significantly at 10 was in 1995 and 1996 (Table 4). In both years inoculation with strain R.25B significantly increased leaf area index when compared with IRJ 2184A or the uninoculated control.

The effect of molybdenum was significant at 10 and 12 WAS in both years. Seed and foliar application of molybdenum resulted in significant increase in leaf area index when compared with no molybdenum control (Table 4).

produced significantly higher leaf area index than TGx 1019-2EN (Table 4).

None of the interactions was significant (Table 4).

4.1.5. Dry matter accumulation (g)

Bradyrhizobium inoculation did not significantly influence dry matter accumulation at all the sampling dates (Table 5).

Table 3: Leaf area per plan (cm^2) of soyabean as influenced by *Bradyrhizobium* inoculation, method of molybdenum application and Cultivar at 8, 10, 12 WAS at Samaru in 1995 and 1996.

Treatments	Weeks after sowing					
	8		10		12	
	1995	1996	1995	1996	1995	1996
Inoculant (I)						
Uninoculated	940	1239	1642ab	1348ab	1600	1900
IRJ 2184A	790	1085	1430b	1129b	1583	1832
R.25B	871	1171	1816a	1517a	1432	1733
SE±	62.12	59.15	87.80*	86.90*	68.45	66.49
Molybdenum (M)						
No molybdenum	850	1149	1410b	1100b	1427b	1732b
Seed treatment	879	1181	1779a	1485a	1640a	1948a
Foliar Spray	869	1165	1709a	1410a	1685a	1985a
SE±	62.12	59.15	87.80*	86.90**	68.45**	66.49*
Cultivar (C)						
SAMSOY-2	930	1230a	1863a	1762a	176a	2068a
TGx 1019-2EN	800	1100b	1421b	1269b	124b	1575b
SE±	50.97	47.77*	68.22**	55.86*	55.69b	52.78**
Interactions						
I x M	NS	NS	NS	NS	NS	NS
I x C	NS	NS	NS	NS	NS	NS
M x X	NS	NS	NS	NS	NS	NS
I x M x C	NS	NS	NS	NS	NS	NS

Means with the same letter within the same treatment group, year and stage of growth are not significantly different at 5% level of probability using Duncan multiple range test.

Abbreviations
* Significant at 5% level of probability
** Significant at 1% level of probability
NS Not Significant
WAS weeks after sowing
SE Standard error.

Table 4: Leaf area index (LAI) of soyabean as influenced by *Bradyrhizobium* inoculation, method of molybdenum application and cultivar at 8,10,12 WAS at Samaru in 1995 and 1996.

Treatments	Weeks after sowing					
	8		10		12	
	1995	1996	1995	1996	1995	1996
Inoculant (I)						
Uninoculated	2.51	3.02	3.80b	4.73b	3.79	4.70
IRJ 2184A	2.10	2.60	4.23b	4.60b	4.03	4.52
R.25B	2.37	2.88	4.89a	5.39a	3.81	4.30
SE±	0.16	0.19	0.19**	0.23**	0.19	0.18
Molybdenum (M)						
No molybdenum	2.31	2.82	3.73b	4.24b	3.18b	4.20b
Seed treatment	2.35	2.86	4.61a	5.12a	4.08a	4.89a
Foliar Spray	2.31	2.82	4.57a	5.08a	4.45a	4.95a
SE±	0.16	0.19	0.19**	0.23**	0.19**	0.18**
Cultivar (C)						
SAMSOY-2	2.51a	3.01a	4.82a	5.32a	4.64a	5.13a
TGx 1019-2EN	2.14b	2.64b	3.79b	4.30b	3.39b	3.89b
SE±	0.12*	0.16*	0.15**	0.19**	0.17*	0.15**
Interactions						
I x M	NS	NS	NS	NS	NS	NS
I x C	NS	NS	NS	NS	NS	NS
M x C	NS	NS	NS	NS	NS	NS
I x M x C	NS	NS	NS	NS	NS	NS

Means with the same letter within the same treatment group, year and stage growth are not significantly different at 5% level of probability using Duncan multiple range test.

Abbreviations
* Significant at 5% level of probability
** Significant at 1% level of probability
NS Not Significant
WAS weeks after sowing
SE Standard error.

Seed and foliar aplied molybdenum had no significant effect on dry matter production in 1995 (Table 5). In 1996, the application of molybdenum as seed and foliar treatment significantly increased dry matter accumulation when compared with no molybdenum control at 8, 12 WAS and at harvest.

Cultivar effect was significant at 8, 12 WAS in 1995 and 1996 and at harvest in 1995 with TGx 1019-2EN accumulating more dry matter than SAMSOY-2 (Table 5) Cultivar effect showed that SAMSOY-2 consistently.

The interaction between molybdenum and cultivar was significant at 8, 10 and 12 WAS in 1995 and at harvest in 1996 (Table 6). In all cases, molybdenum application had no effect on dry matter accumulation by TGx 1019-2EN. With SAMSOY-2 on the other hand, dry matter accumulation was significantly higher when molybdenum was applied either as seed treatment or foliar spray when compared with no molybdenum control. The only exception was at 10 WAS in 1995, when seed treatment was at par with no molybdenum control.

The interaction between inoculant and cultivar was significant at 10 WAS in 1996 (Table 7). *Bradyrhizobium* inoculation had no significant effect on dry matter accumulation by SAMSOY 2, whereas strain IRJ 2184A significantly increased dry matter accumulation of TGx 1019-2EN when compared with uninoculated control.

Table 5: Dry matter accumulation per plant of soyabean as influenced by *Bradyrhizobium* inoculation, method of molybdenum application and cultivar at 8,10,12 WAS and harvest at Samaru in 1995 and 1996.

Treatments	Weeks after sowing							
	(g/Plant)		(g/Plant)		(g/Plant)		(Kg/ha)	
	8		10		12		harvest	
	1995	1996	1995	1996	1995	1996	1995	1996
Inoculant (I)								
Uninoculated	13.58	10.44	18.06	17.49	21.14	19.34	5245	4741
IRJ 2184A	13.39	10.57	17.72	19.11	18.46	17.57	5361	5003
R.25B	12.35	11.03	18.89	19.26	21.26	18.12	5431	4704
SE±	0.44	0.85	0.73	1.39	0.87	1.02	204.64	183.10
Molybdenum (M)								
No molybdenum	12.34	9.10b	17.60	16.37	19.46	15.44b	5000	4610b
Seed treatment	13.63	12.79a	18.59	20.50	21.06	20.30a	5944	5165a
Foliar Spray	13.35	12.06a	18.47	18.98	21.33	19.39a	5440	5181a
SE±	0.44	0.85*	0.73	1.39	0.87	1.02**	204.64	183.10 *
Cultivar (C)								
SAMSOY-2	13.07b	9.88b	18.30	18.86	19.47b	16.46b	5042b	
TGx 1019-2EN	13.14a	11.48a	18.14	18.37	21.74a	20.23a	5649a	4799
SE±	0.36*	0.70**	0.60	1.14	0.71**	0.83**	167.09*	5099 147.49
Interactions								
I x M	NS	NS	NS	NS	NS	NS	NS	
I x C	NS	NS	NS	NS	NS	NS	NS	NS
M x C	**	NS	**	NS	**	NS	NS	NS
I x M x C	NS	NS	NS	NS	NS	NS	NS	** NS

Means with the same letter within the same treatment group, year and stage of growth are not significantly different at 5% level of probability using Duncan multiple range test.

Abbreviations
* Significant at 5% level of probability
** Significant at 1% level of probability
NS Not Significant
WAS weeks after sowing
SE Standard Error.

Table 6: Interaction between method of molybdenum application and cultivar on dry matter accumulation per plant (g) at 8, 10 and 12 WAS in 1995 and dry matter accumulation (kg/ha) of soyabean at harvest in 1996.

Molybdenum	Cultivar SAMSOY-2	TGx 1019-2EN
	8 WAS (1995)	
No molybdenum	10.91c	13.76ab
Seed treatment	13.52ab	13.75ab
Foliar spray	14.78a	11.92bc
SE±	0.62*	
	10 WAS (1995)	
No molybdenum	16.64b	18.57ab
Seed treatment	17.67ab	19.51ab
Foliar spray	20.59a	16.34b
SE±	1.03**	
	12 WAS (1995)	
No molybdenum	15.15c	23.78a
Seed treatment	22.26ab	20.87ab
Foliar spray	21.08ab	21.53ab
SE±	1.23**	
	Harvest (1996)	
No molybdenum	3889b	5315a
Seed treatment	5296a	4833a
Foliar spray	5213a	5148a
SE±	358.92**	

Means with the same letter within the same treatment group, year and stage of growth are not significantly different at 5% level of probability using Duncan multiple range test.

Abbreviations
* Significant at 5% level of probability
** Significant at 1% level of probability
NS Not Significant
WAS Weeks after sowing
SE Standard error.

Table 7: Interaction between *Bradyrhizobium* inoculation and cultivar on dry matter accumulation per plant (g) of soyabean at 10 WAS at Samaru in 1996.

	Cultivar	
Inoculant	SAMSOY-2	TGx 1019-2EN
Uninoculated	20.26ab	14.71b
IRJ 2184A	16.20ab	22.01a
R.25B	20.14ab	18.39ab
SE±	1.97**	

Means with the same letter within the same treatment group, year and stage of growth are not significantly different at 5% level of probability using Duncan multiple range test.

Abbreviations
* Significant at 5% level of probability
** Significant at 1% level of probability
NS Not Significant
WAS Weeks after sowing
SE Standard error.

4.1.6. Number of branches per plant

Branch number per plant was not significantly influenced by *Bradyrhizobium* inoculation in both years throughout the sampling period (Table 8).

Molybdenum also had no significant influence on branch number in both years (Table 8).

In both years SAMSOY-2 had more branches than TGx 1019-2EN throughout the sampling period (Table 8). Differences were however significant only at 12 WAS in both years and at 8 WAS in 1996.

None of the interactions was significant (Table 8).

4.1.7. Nodule number per plant

Nodule number was not significantly influenced by *Bradyrhizobium* inoculation at the various sampling stages (Table 9).

Similarly, molybdenum had no significant effect on nodule number per plant (Table 9) except at 10 WAS in 1996 when foliar applied molybdenum resulted in significantly higher number of nodules compared with either seed treatment or no molybdenum control.

Cultivar effect was significant at 8 and 10 WAS in 1995 and at 8 WAS in 1996 (Table 9), with SAMSOY-2 producing significantly higher nodule number per plant than TGx 1019-2EN.

Inoculant X Cultivar interaction was significant at 8 and 10 WAS in 1995 and at 10 WAS in 1996 (Table 10). At 8 WAS in 1995,

Table 8: Branch number per plant of soyabean as influenced by *Bradyrhizobium* inoculation, method of molybdenum application and cultivar at 8,10,12 WAS at Samaru in 1995 and 1996.

Treatment	Weeks after sowing					
	8		10		12	
	1995	1996	1995	1996	1995	1996
Inoculant (I)						
Uninoculated	4.96	2.75	6.25	8.63	4.04	4.38
IRJ 2184A	6.46	2.67	6.67	9.92	3.88	5.33
R.25B	6.00	2.29	6.96	11.13	4.33	4.67
SE±	0.48	0.23	0.33	0.71	0.16	0.46
Molybdenum (M)						
No molybdenum	5.17	2.13	6.92	8.88	3.92	4.58
Seed treatment	6.00	3.25	6.38	9.83	4.21	5.00
Foliar Spray	6.25	2.33	6.58	10.96	4.13	4.79
SE±	0.48	0.23	0.33	0.71	0.16	0.46
Cultivar (C)						
SAMSOY-2	6.11	3.36a	6.94	9.99	4.86a	5.83a
TGx 1019-2EN	5.50	1.78b	6.31	9.97	3.13b	3.75b
SE±	0.39	0.19**	0.27	0.57	0.13**	0.38**
Interactions						
I x M	NS	NS	NS	NS	NS	NS
I x C	NS	NS	NS	NS	NS	NS
M x C	NS	NS	NS	NS	NS	NS
I x M x C	NS	NS	NS	NS	NS	NS

Means with the same letter within the same treatment group, year and stage of growth are not significantly different at 5% level of probability using Duncan multiple range test.

Abbreviations
* Significant at 5% level of probability
** Significant at 1% level of probability
NS Not Significant
WAS weeks after sowing
SE Standard error.

Table 9: Nodule number per plant of soyabean as influenced by *Bradyrhizobium* inoculation, method of molybdenum application and cultivar at 8,10, and 12 WAS at Samaru in 1995 and 1996.

Treatments	Weeks after sowing					
	8		10		12	
	1995	1996	1995	1996	1995	1996
Inoculant (I)						
Uninoculated	30.30	13.23	24.42	18.00	16.67	19.54
IRJ 2184A	23.70	13.42	23.00	19.54	15.96	20.96
R.25B	27.50	15.42	23.58	18.54	14.92	20.67
SE±	2.07	1.41	1.72	0.84	1.39	1.40
Molybdenum (M)						
No molybdenum	30.10	13.08	27.67	17.92a	15.96	22.58
Seed treatment	23.80	14.54	23.25	17.71a	14.42	17.75
Foliar Spray	27.60	14.46	24.58	18.46b	17.17	20.83
SE±	2.07	1.41	1.72	0.84*	1.39	1.40
Cultivar (C)						
SAMSOY-2	30.60a	16.78a	29.39a	17.92	16.47	20.97
TGx 1019-2EN	23.80b	11.28b	21.39b	19.47	15.22	19.81
SE±	1.69**	1.15**	1.42**	0.68	1.14	1.41
Interactions						
I x M	NS	NS	NS	NS	NS	NS
I x C	**	NS	**	*	NS	NS
M x C	NS	NS	NS	NS	NS	NS
I x M x C	NS	NS	NS	NS	NS	NS

Means with the same letter(s) within the same treatment group, year and stage of growth are not significantly different at 5% level of probality using Duncan multiple range test.

Abbreviations
* Significant at 5% level of probability
** Significant at 5% and 1% level of probability
NS Not Significant
WAS weeks after sowing
SE Standard error.

strain R.25B significantly decreased nodule number of SAMSOY-2 when compared with strain IRJ 2184A and the uninoculated control. For TGx 1019-2EN, inoculation with strain R.25B resulted in significantly higher number of nodules when compared with the uninoculated control and IRJ 2184A.

At 10 WAS in 1995, inoculation with IRJ 2184A resulted in significant increase in the nodule number of SAMSOY-2 when compared to the uninoculated control. *Bradyrhizobium* inoculation had no effect on TGx 1019-2EN.

At 10 WAS in 1996, inoculation with strain IRJ 2184A increased nodule number of TGx 1019-2EN significantly when compared with the uninoculated control. For SAMSOY-2, nodule number was not affected by *Bradyrhizobium* inoculation.

4.1.8. Nodule dry weight per plant (g)

In each of the years, nodule weight per plant was not significantly influenced by *Bradyrhizobium* inoculation and molybdenum application throughout the sampling period (Table 11).

Cultivar effect was significant only at 10 WAS in both years (Table 11) with TGx 1019-EN producing 26% and 73% more nodules than SAMSOY-2 in 1995 and 1996 respectively.

Table 10: Interaction between *Bradyrhizobium* inoculation and cultivar on nodule number of soyabean at 8 and 10 WAS in 1995 and 10 WAS in 1996.

	Cultivar	
Inoculant	SAMSOY 2	TGx 1019-2EN
	8 WAS (1995)	
Uninoculated	37.30a	23.40b
IRJ 2184A	31.60a	15.70b
R.25B	22.80b	32.20a
SE±	2.93**	
	10 WAS (1995)	
Uninoculated	27.42b	21.42bc
IRJ 2184A	36.83a	19.17c
R.25B	23.92bc	23.25bc
SE±	2.44**	
	10 WAS (1996)	
Uninoculated	8.50ab	7.50b
IRJ 2184A	7.08b	12.00a
R.25B	8.17b	8.92ab
SE±	1.18**	

Means with the same letter within the same treatment group, year and stage of growth are not significantly different at 5% level of probability using Duncan multiple range test.

Abbreviations
* Significant at 5% level of probability
** Significant at 1% level of probability
NS Not Significant
WAS Weeks after sowing
SE Standard Error.

Table 11: Nodule dry weight per plant (g) of soyabean as influenced by *Bradyrhizobium* inoculation, method of molybdenum application and cultivar at 8,10, and 12 WAS Samaru in 1995 and 1996.

Treatments	Weeks after sowing					
	8		10		12	
	1995	1996	1995	1996	1995	1996
Inoculant (I)						
Uninoculated	0.32	0.15	0.21	0.15	0.16	0.05
IRJ 2184A	0.37	0.16	0.19	0.14	0.18	0.07
R.25B	0.31	0.17	0.20	0.15	0.21	0.13
SE±	0.03	0.01	0.02	0.02	0.03	0.03
Molybdenum (M)						
No molybdenum	0.32	0.15	0.21	0.15	0.16	0.05
Seed treatment	0.35	0.17	0.18	0.13	0.21	0.12
Foliar Spray	0.32	0.16	0.22	0.14	0.16	0.05
SE±	0.03	0.01	0.02	0.02	0.03	0.03
Cultivar (C)						
SAMSOY-2	0.35	0.15	0.19b	0.11b	0.18	0.06
TGx 1019-2EN	0.31	0.17	0.24a	0.19a	0.20	0.10
SE±	0.03	0.01	0.01**	0.01**	0.02	0.02
Interactions						
I x M	NS	NS	NS	NS	NS	NS
I x C	*	NS	*	*	NS	NS
M x C	NS	NS	NS	NS	NS	NS
I x M x C	NS	NS	NS	NS	NS	NS

Means with the same letter within the same treatment group, year and stage of growth are not significantly different at 5% level of probability using Duncan multiple range test.

Abbreviations

* Significant at 5% level of probability
** Significant at 1% level of probability
NS Not Significant
WAS weeks after sowing
SE Standard error.

Inoculant x cultivar interaction was significant at 8 and 10 WAS in 1995 and at 10 WAS in 1996 (Table 12). In all cases, *Bradyrhizobium* inoculation had no significant effect on the nodule weight of TGx 1019-2EN. For SAMSOY-2, at 8 WAS in 1995 inoculation with stain R.25B resulted in a significant decrease in nodule weight when compared with IRJ 2184A. At 10 WAS in 1995, nodule weight of SAMSOY-2 inoculated with IRJ 2184A was significantly lower than that of the uninoculated control.

At 10 WAS in 1996, inoculation with strain IRJ 2184A decreased nodule weight of SAMSOY-2 significantly when compared with uninoculated control and strain R.25B.

4.1.9. Plant height (cm)

Bradyrhizobium inoculation had no significant effect on plant height except at 8 WAS in 1995 and at harvest in both years (Table 13), when *Bradyrhizobium* inoculation significantly decreased plant height when compared with uninoculated control. Moreover, at harvest, in 1995, strain R.25B significantly decreased plant height when compared with IRJ 2184A.

The effect of molybdenum on plant height was significant only at harvest in both years (Table 13) with foliar spray resulting in a significant decrease in plant height compared to either no molybdenum or seed treatment.

Table 12: Interaction between *Bradyrhizobium* inoculation and cultivar on nodule dry weight per plant (g) of soyabean at 8 and 10 WAS in 1995 and 10 WAS in 1996.

Inoculant	Cultivar	
	SAMSOY 2	TGx 1019-2EN
	8 WAS (1995)	
Uninoculated	0.33ab	0.30b
IRJ 2184A	0.46a	0.27b
R.25B	0.27b	0.35ab
SE±	0.05*	
	10 WAS (1995)	
Uninoculated	0.21ab	0.22ab
IRJ 2184A	0.11c	0.27a
R.25B	0.16bc	0.23ab
SE±	0.03*	
	10 WAS (1996)	
Uninoculated	0.16ab	0.17ab
IRJ 2184A	0.06c	0.22a
R.25B	0.11ab	0.18ab
SE±	0.03*	

Means with the same letter within the same treatment group, year and stage of growthare not significantly different at 5% level of probability using Duncan multiple range test.

Abbreviations
* Significant at 5% level of probability
** Significant at 1% level of probability
NS Not Significant
WAS Weeks after sowing
SE Standard error.

Table 13: Plant height (cm) of soyabean as influenced by *Bradyrhizobium* inoculation, method of molybdenum application and cultivar at 8,10, 12 WAS and harvest at Samaru in 1995 and 1996.

Treatment	Weeks after sowing							
	8		10		12		harvest	
	1995	1996	1995	1996	1995	1996	1995	1996
Inoculants (I)								
Uninoculated	56.21a	40.57	60.51	52.77	62.53	50.60	58.68a	51.64a
IRJ 2184A	52.88b	40.57	58.87	52.50	60.43	51.20	55.25b	47.75b
R.25B	52.30b	39.30	57.67	52.40	60.03	51.10	51.93c	44.91b
SE±	0.91**	0.99	1.14	1.29	1.19	1.76	1.07**	1.06**
Molybdenum (M)								
No molybdenum	53.80	39.32	58.50	51.53	61.90	50.60	56.69a	49.70a
Seed treatment	54.15	40.61	58.70	52.83	60.63	51.70	57.50a	50.49a
Foliar Spray	53.45	40.52	59.85	53.31	60.45	50.70	51.60b	44.60b
SE±	0.91	0.99	1.14	1.29	1.19	1.76	1.07**	1.06**
Cultivar (C)								
SAMSOY-2	48.66b	35.84b	48.61b	40.19b	50.62b	37.80b	44.60b	37.57b
TGx 1019-2EN	58.94a	44.46a	69.42a	64.92a	71.38a	64.20a	65.90a	58.96a
SE±	0.75**	0.81**	0.93*	1.05**	0.97**	1.44**	0.87**	0.88**
Interactions								
I x M	NS	NS	NS	NS	NS	NS	**	**
I x C	NS	NS	NS	NS	NS	NS	**	*
I x C	NS	NS	NS	NS	NS	NS	NS	NS
I x M x C	NS	NS	NS	NS	NS	NS	NS	NS

Means with the same letter within the same treatment group, year and stage of growth are not significantly different at 5% level of probability using Duncan multiple range test.

Abbreviations
* Significant at 5% level of probability
** Significant at 1% level of probability
NS Not Significant
WAS weeks after sowing
SE Standard Error.

Cultivar effect showed that TGx 1019-2EN was consistently taller than SAMSOY-2 (Table 13).

Inoculant x Cultivar interaction was significant on plant height at harvest in 1995 and 1996 (Table 14). In both years, strain R.25B decreased plant height of TGx 1019-2EN significantly when compared with either IRJ 2184A or the uninoculated control. In contrast, *Bradyrhizobium* inoculation had no significant effect on SAMSOY-2.

Molybdenum x Inoculant interaction was significant at harvest in 1995 and 1996 (Table 15). The height of uninoculated plot was not affected by molybdenum in both years. Whereas for plots inoculated with strain IRJ 2184A, the tallest plants were produced when molybdenum was applied as seed treatment and plants were significantly higher than control. Foliar application of molybdenum depressed the height of plants inoculated with strain R.25B in both years.

4.1.10. Height to the lowest pod (cm)

In both years, *Bradyrhizobium* inoculation had no significant effect on height to the lowest pod (Table 16).

Molybdenum application either as seed or foliar treatment significantly decreased height to the lowest pod in both years (Table 16).

Table 14: Interaction between *Bradyrhizobium* inoculation and cultivar on plant height (cm) of soyabean at harvest in Samaru in 1995 and 1996.

	Cultivar	
Inoculant	SAMSOY 2	TGx 1019-2EN
	1995	
Uninoculated	46.84c	70.44a
IRJ 2184A	42.64c	67.95a
R.25B	44.32c	59.50b
SE±	1.49**	
	1996	
Uninoculated	37.72c	63.37a
IRJ 2184A	33.57c	62.86a
R.25B	37.20c	54.49b
SE±	1.51*	

Means with the same letter within the same treatment group, year and stage of growth are not significantly different at 5% level of Probability using Duncan multiple range test.

Abbreviations
* Significant at 5% level of probability
** Significant at 1% level of probability
NS Not Significant
WAS Weeks after sowing
SE Standard error.

Table 15: Interaction between *Bradyrhizobium* inoculation and method of molybdenum application on plant height of soyabean at harvest in Samaru in 1995 and 1996.

	Inoculant		
Molybdenum	uninoculated	IRJ 2184A	R.25B
		1995	
No molybdenum	61.24a	52.64c	56.22abc
Seed treatment	57.90abc	60.07ab	54.50bc
Foliar spray	56.79abc	53.03c	45.00d
SE±		1.85**	
		1996	
No molybdenum	54.31a	43.71c	49.29abc
Seed treatment	49.97abc	53.14ab	45.55bc
Foliar spray	50.86abc	46.10abc	36.00
SE±		2.00**	

Means with the same letter within the same treatment group, year and stage of growth are not significantly different at 5% level of probability using Duncan multiple range test.

Abbreviations
* Significant at 5% level of probability
** Significant at 1% level of probability
NS Not Significant
WAS Weeks after sowing
SE Standard Error.

Table 16: Height to the lowest pod (cm) as influenced by *Bradyrhizobium* inoculation, method of molybdenum application and cultivar at harvest Samaru in 1995 and 1996.

Treatment	1995	1996
Inoculant (I)		
Uninoculated	14.47	11.45
IRJ 2184A	13.93	10.83
R.25B	14.08	11.11
SE±	0.45	0.40
Molybdenum (M)		
No molybdenum	15.23a	12.20a
Seed treatment	13.57b	10.60b
Foliar Spray	13.67b	10.73b
SE±	0.45*	0.40*
Cultivar (C)		
SAMSOY-2	12.58b	9.85b
TGx 1019-2EN	15.46a	12.46a
SE±	0.37*	0.37**
Interaction		
I x M	NS	NS
I x C	NS	NS
M x C	NS	NS
I x M x C	NS	NS

Means with the same letter within the same treatment group, year and stage of growth are not significantly different at 5% level of probability using Duncan multiple range test.

Abbreviations
* Significant at 5% level of probability
** Significant at 1% level of probability
NS Not Significant
WAS Weeks after sowing
SE Standard error.

Cultivar effect showed that TGx 1019-2EN had significantly greater height to the lowest pod than SAMSOY-2 (Table 16).

None of the interactions was significant (Table 16).

4.1.11. Uriede nitrogen (nm)

Bradyrhizobium inoculation had no significant influence on uriede nitrogen of root bled sap (Table 17) and stem extract (Table 18) in both years.

Similarly, molybdenum application had no significant effect on uriede nitrogen of root bled sap (Table 17). However, in 1995 and 1996 seed and foliar applied molybdenum increased uriede nitrogen of stem extract when compared with no molybdenum control (Table 18).

Cultivar had no significant influence on mean uriede nitrogen of root bled sap and stem extract (Tables 17 and 18) except on stem extract in 1995 when TGx 1019-2EN had significantly higher uriede nitrogen than SAMSOY-2.

None of the interactions was significant for root bled sap and stem extract (Table 17 and 18).

4.1.12. Nitrate nitrogen (nm)

Nitrate nitrogen of root bled sap and stem extract was not significantly influenced by *Bradyrhizobium* inoculation (Tables 17 and 18).

Table 17: Uriede nitrogen (nm), nitrate nitrogen (nm), alpha amino nitrogen (nm) of root content bled sap of soyabean as influenced by *Bradyrhizobium* inoculation, method of molybdenum application and cultivar at 10 WAS at Samaru in 1995 and 1996.

Treatment	Uriede nitrogen (nm)		Nitrate nitrogen (nm)		Amino nitrogen (nm)	
	1995	1996	1995	1996	1995	1996
Inoculants (I)						
Uninoculated	0.08	1.15	0.01	0.04	0.45	0.62
IRJ 2184A	0.09	1.15	0.01	0.06	0.48	0.63
R.25B	0.12	1.05	0.01	0.03	0.51	0.68
SE±	0.03	0.11	0.08	0.08	0.08	0.07
Molybdenum (M)						
No molybdenum	0.06	1.16	0.01	0.03	0.42	0.52
Seed treatment	0.07	1.09	0.01	0.05	0.57	0.67
Foliar Spray	0.16	1.11	0.01	0.04	0.46	0.75
SE±	0.03	0.11	0.003	0.08	0.08	0.07
Cultivar (C)						
SAMSOY-2	0.09	1.08	0.01b	0.05	0.43	0.65
TGx 1019-2EN	0.10	1.14	0.02a	0.03	0.53	0.64
SE±	0.02	0.09	0.002*	0.07	0.07	0.06
Interactions						
I x C	NS	NS	NS	NS	NS	NS
I x C	NS	NS	NS	NS	NS	NS
M x C	NS	NS	NS	NS	NS	NS
I x M x C	NS	NS	NS	NS	NS	NS

Means with the same letter within the same treatment group, year and stage of growth are are not significantly different at 5% level of probability using Duncan multiple range test.

Abbreviations
* Significant at 5% level of probability
** Significant at 1% level of probability
NS Not Significant
WAS weeks after sowing
SE Standard error.

Table 18: Uriede nitrogen (nm), nitrate nitrogen (nm) content of stem extract of soyabean as influenced by *Bradyrhizobium* inoculation, method of molybdenum application and cultivar at 10 WAS AT Samaru in 1995 and 1996.

Treatment	Uriede nitrogen (nm)		nitrate nitrogen (nm)	
	1995	1996	1995	1996
Inoculant (I)				
Uninoculated	0.16	0.65	0.01	0.02
IRJ 2184A	0.16	0.74	0.01	0.02
R.25B	0.16	0.63	0.01	0.02
SE±	0.002	0.039	0.0004	0.0004
Molybdenum (M)				
No molybdenum	0.13b	0.57b	0.01	0.02a
Seed treatment	0.19a	0.69a	0.01	0.01b
Foliar Spray	0.19a	0.77a	0.01	0.01b
SE±	0.002**	0.039*	0.0004	0.0004**
Cultivar (C)				
SAMSOY-2	0.15b	0.67	0.01b	0.17
TGx 1019-2EN	0.19a	0.68	0.02a	0.17
SE±	0.00**	0.03	0.0003**	0.0003
Interactions				
IxM	NS	NS	NS	NS
IxC	NS	NS	NS	NS
MxC	NS	NS	NS	NS
IxMxC	NS	NS	NS	NS

Means with the same letter within the same treatment group, year and stage of growth are not significantly different at 5% level of probability using Duncan multiple range test.

Abbreviations
* Significant at 5% level of probability
** Significant at 1% level of probability
NS Not Significant
WAS Weeks after sowing
SE Standard error.

Molybdenum had no significant effect on nitrate nitrogen of root bled sap (Table 17). On the other hand, the application of molybdenum as seed treatment and foliar spray significantly decreased nitrate nitrogen of stem extract in 1996 when compared with no molybdenum control (Table 18).

Cultivar effect showed that in 1995 TGx 1019-2EN produced significantly more nitrate nitrogen than SAMSOY-2 in both root and stem extract (Table 17 and 18).

All interactions were not significant for root bled sap and stem extract (Table 17 and 18).

4.1.13 Alpha amino nitrogen (nm)

Alpha amino nitrogen of root bled sap was not significantly influenced by *Bradyrhizobium* inoculation, molybdenum application and cultivar (Table 17).

All interactions were not significant for root bled sap (Table 17).

4.1.14 Uriede nitrogen concentration (mM)

Uriede concentration of root bled sap and stem extract was not significantly influenced by *Bradyrhizobium* inoculation in both years (Tables 19 and 20).

Seed and foliar application of molybdenum significantly increased uriede concentration of root bled sap in 1995 (Table 19) and stem extract in both 1995 and 1996 (Table 20) when compared with no molybdenum control.

Cultivar had no significant effect on uriede concentration of root bled sap (Table 19) and stem extract (Table 20), except in 1995 when TGx 1019-2EN had significantly higher uriede concentration of stem extract compared with SAMSOY-2.

All interactions were not significant for root bled sap and stem extract (Table 19 and 20).

4.1.15. Actual uriede nitrogen concentration (mM)

Actual uriede concentration of root bled sap and stem extract was not significantly inlfuenced by *Bradyrhizobium* inoculation (Tables 19 and 20).

Molybdenum application significantly influenced actual uriede concentration of root bled sap in 1995 only (Table 19) and stem extract in both years (Table 20). In 1995, seed and foliar treatment with molybdenum increased actual uriede of root bled sap by 153% and 189% respectively when compared with no molybdenum control. Similarly, in 1995, seed and foliar applied molybdenum increased uriede concentration of stem extract by 43% and 40% respectively when compared with no molybdenum control. Moreover, seed applied molybdenum increased uriede concentration by 20% when compared to foliar spray molybdenum. In 1996, molybdenum application as seed and foliar treatment increased actual uriede of stem extract by 25% and 35% respectively when compared with no molybdenum control.

Table 19: Uriede concentration (mM) actual uriede concentration (mM) nitrate concentration (mM), alpha amino concentration (mM) of root bled sap of soyabean as influenced by *Bradyrhizobium* inoculation, method of molybdenum application and cultivar at 10 WAS at Samaru in 1995 and 1996.

Treatment	Uriede concentration (mM)		Actual Uriede concentration (mM)		Nitrate concentration (mM)		Alpha amino concentration (mM)	
	1995	1996	1995	1996	1995	1996	1995	1996
Inoculant (I)								
Uninoculated	0.27	3.53	1.08	14.28	0.11	0.38	3.63	6.21
IRJ 2184A	0.31	3.68	1.31	14.10	0.13	0.35	3.71	6.56
R.25B	0.39	3.49	0.96	13.64	0.12	0.28	3.90	7.15
SE±	0.09	0.36	0.30	1.37	0.02	0.05	0.58	0.70
Molybdenum (M)								
No molybdenum	0.19b	3.54	0.78b	14.17	0.09	0.28	3.11	5.91
Seed treatment	0.48a	3.34	1.74a	13.29	0.13	0.34	4.33	6.28
Foliar Spray	0.55a	3.70	1.83a	14.56	0.15	0.39	3.79	7.74
SE±	0.09**	0.36	0.30*	1.37	0.02	0.05	0.58	0.70
Cultivar (C)								
SAMSOY-2	0.30	3.50	1.18	13.73	0.14	0.38	3.59	6.50
TGx 1019-2EN	0.35	3.56	1.06	14.28	0.10	0.29	3.90	6.78
SE±	0.07	0.29	0.25	1.11	0.02	0.04	0.48	0.57
Interactions								
I x M	NS	NS	NS	NS	NS	NS	NS	NS
I x C	NS	NS	NS	NS	NS	NS	NS	NS
M x C	NS	NS	NS	NS	NS	NS	NS	NS
I x M x C	NS	NS	NS	NS	NS	NS	NS	NS

Means with the same letter within the same treatment group, year and stage of growth are not significantly different at 5% level of probability using Duncan multiple range test.

Abbreviations
* Significant at 5% level of probability
** Significant at 1% level of probability
NS Not Significant
WAS *weeks after sowing*
SE Standard error.

Table 20: Uriede concentration (mM) actual uriede concentration (mM) and nitrate concentration (mM) of stem extract (SE) of soyabean as influenced by *Bradyrhizobium* inoculation, method of molybdenum application and cultivar at 10 WAS at Samaru in 1995 and 1996.

Treatment	Uriede Concentration (mm)		Acutual uriede concentration (mm)		Nitrate concentration (nm)	
	1995	1996	1995	1996	1995	1996
Inoculant (I)						
Uninoculated	0.081	0.311	0.350	1.224	0.108	0.159
IRJ 2184A	0.088	0.364	0.351	1.456	0.106	0.150
R.25B	0.088	0.334	0.354	1.339	0.106	0.154
SE±	0.005	0.02	0.002	0.06	0.004	0.004
Molybdenum (M)						
No molybdenum	0.067b	0.281b	0.268c	1.123b	0.101	0.166a
Seed treatment	0.096a	0.350a	0.383a	1.405a	0.110	0.148b
Foliar Spray	0.094a	0.378a	0.375b	1.511a	0.109	0.149b
SE±	0.005**	0.02**	0.002**	0.06**	0.004	0.004**
Cultivar (C)						
SAMSOY-2	0.077b	0.338	0.306b	1.136b	0.101b	0.158
TGx 1019-2EN	0.095a	0.334	0.378a	1.337a	0.113a	0.151
SE±	0.004**	0.01	0.002**	0.05	0.003**	0.003
Interactions						
IxM	NS	NS	NS	NS	NS	NS
IxC	NS	NS	NS	NS	NS	NS
MxC	NS	NS	NS	NS	NS	NS
IxMxC	NS	NS	NS	NS	NS	NS

Means with the same letter within the same treatment group, year and stage of growth are not significantly different at 5% level of significance using Duncan multiple range test.

Abbreviations

* Significant at 5% level of significance
** Significant at 1% level of significance
NS Not Significant
WAS Weeks after sowing
SE Standard error

Cultivar effect was significant only for stem extract in 1995 (Table 20), with TGx 1019-2EN having significantly higher uriede concentration than SAMSOY-2.

All interactions were not significant for root bled sap and stem extract (Table 19 and 20).

4.1.16 Nitrate nitrogen concentration (mM)

Nitrate concentration of root bled sap and stem extract was not significantly influenced by *Bradyrhizobium* inoculation in both years. (Tables 19 and 20).

Molybdenum significantly increased nitrate concentration of stem extract, only in 1996 (Table 20), with seed and foliar applied molybdenum decreasing nitrate concentration by about 12%.

Cultivar effect was significant only for stem extract in 1995 (Table 20), with TGx 1019-2EN having significantly higher nitrate concentration than SAMSOY-2.

All interactions were not significant for root bled sap and stem extract (Table 19 and 20).

4.1.17 Alpha amino nitrogen concentration (mM)

In both years, alpha amino concentration on root bled sap was not significantly influenced by *Bradyrhizobium* inoculation, molybdenum and cultivar (Table 19).

None of the interactions was significant for root bled sap (Table 19).

4.1.18. Total sap nitrogen (mM)

Total sap nitrogen of root bled sap was not significant influenced by *Bradyrhizobium* inoculation (Table 21). However, in 1995, inoculation with stain IRJ 218A and R.25B significantly increased total sap nitrogen of stem extract by 7% when compared to control (Table 22).

Molybdenum application as seed and foliar spray increased total sap nitrogen of root bled sap by 86% and 71% respectively in 1995 when compared with no molybdenum control (Table 21). In 1996, molybdenum had no effect on the total sap nitrogen of root bled sap. For stem extract, seed applied molybdenum increased total sap nitrogen by 32% and 20% in 1995 and 1996 respectively while foliar spray increased total sap nitrogen by 30% and 29% respectively when compared with no molybdenum control (Table 22).

Cultivar effect was not significant for root bled sap (Table 21). For stem extract, cultivar effect was significant only in 1995, with TGx 1019-2EN having significantly higher total sap nitrogen than SAMSOY-2.

None of the interactions was significant for root bled sap and stem extract (Table 21 and 22).

4.1.19. Relative uriede nitrogen (%)

Relative uriede nitrogen of root bled sap and stem extract was not significantly influenced by *Bradyrhizobium* inoculation in both years (Table 21 and 22).

Relative uriede nitrogen of root bled sap was significantly increased by molybdenum application in 1995 only (Table 21). Seed and foliar application molybdenum increased relative uriede nitrogen by 20% and 32% respectively, when compared with no molybdenum control. Similarly, molybdenum applied as seed treatment, significantly increased relative uriede nitrogen of stem extract by 8% and 4% in 1995 and 1996 respectively when compared to no molybdenum control. Foliar spray with molybdenum also resulted in 8% and 5% increase in relative uriede nitrogen of stem extract in 1995 and 1996 respectively (Table 22).

Cultivar had no significant effect on relative uriede nitrogen of root bled sap and stem extract (Tables 21 and 22).

All interactions were not significant for root bled sap and stem extract.

4.1.20 Total Plant nitrogen (kg/ha)

Nitrogen content was not significantly influenced by *Bradyrhizobium* inoculation, cultivar in both years (Table 23).

Molybdenum applied as seed and foliar spray significantly increased nitrogen conlent by 5% and 8% in 1995 and 7% and 9% in 1996 respectively.

None of the interactions was significant (Table 23).

4.1.21 Nitrogen fixed (%)

Bradyrhizobium inoculation had no significant effect on the percentage nitrogen fixed of root bled sap and stem extract (Table 21 and 22). Though strain R.25B increased nitrogen fixation when compared to uninoculated control, the increase was not significant though it was almost significant.

Molybdenum significantly increased the percentage nitrogen fixed of root bled sap only in 1995 (Table 21), with seed and foliar applied molybdenum increasing nitrogen fixed by about 13%, when compared with no molybdenum control. For stem extract, seed and foliar applied molybdenum increased nitrogen fixed by 9% and 6% in 1995 and 1996 respectively (Table 22).

Cultivars did not differ significantly in the percentage nitrogen fixed (Table 21 and 22). All interactions were not significant for root bled sap and stem extract (Table 21 and 22.)

4.1.22 Nitrogen fixed (kg/ha)

Bradyrhizobium inoculation did not significantly increase kilogram nitrogen fixed of root bled sap, stem extract and nitrogen fixed determined by difference method (Table 23). Though strain R.25B increased nitrogen fixed when compared with uninoculated control, the increase was not significant, though it was almost significant.

Table 21: Total sap nitrogen (mM), relative uriede nitrogen (%), nitrogen fixed (%), nitrogen fixed (kg/ha) of root bled sap (RBS) of soyabean as influenced by Bradyrhizobium inoculation, method of molybdenum application and cultivar at 10 WAS Samaru in 1995 and 1996.

Treatment	Total Sap nitrogen (mM)		Relative uriede nitrogen (%)		Nitrogen fixed (%)	
	1995	1996	1995	1996	1995	1996
Inoculant (I)						
Uninoculated	5.17	20.70	51.60	75.20	56.16	71.60
IRJ 2184A	6.19	21.00	53.20	77.00	56.83	72.94
R.25B	6.13	20.00	54.40	80.60	57.50	72.62
SE±	1.00	1.85	3.61	1.82	2.28	1.82
Molybdenum (M)						
No molybdenum	3.98b	19.50	50.12b	67.60	54.82b	66.90
Seed treatment	7.42a	19.70	60.40a	72.69	61.93a	70.25
Foliar Spray	6.80a	22.40	66.20a	73.02	62.20a	70.33
SE±	1.00*	1.85	3.60*	1.82	2.28*	1.82
Cultivar (C)						
SAMSOY-2	5.18	20.01	51.50	76.84	56.16	72.90
TGx 1019-2EN	6.59	21.00	54.40	76.90	57.50	72.94
SE±	0.81	1.51	2.95	1.49	1.80	1.48
Interactions						
IxM	NS	NS	NS	NS	NS	NS
IxC	NS	NS	NS	NS	NS	NS
MxC	NS	NS	NS	NS	NS	NS
IxMxC	NS	NS	NS	NS	NS	NS

Means with the same letter within the same treatment group, year and stage of growth are not significantly different at 5% level of probability using Duncan multiple range test.

Abbreviations
* Significant at 5% level of probability
** Significant at 1% level of probability
NS Not Significant
WAS Weeks after sowing
SE Standard error.

Table 22: Total sap nitrogen (mM), relative uriede nitrogen (%), nitrogen fixed (%), nitrogen fixed (kg/ha) of stem extract (SE) of soyabean as influenced by *Bradyrhizobium* inoculation, method of molybdenum application and cultivar at 10 WAS at Samaru in 1995 and 1996.

Treatment	Total sap nitrogen (mM)		Relative uriede nitrogen (%)		Nitrogen fixed ((Kg/ha)	
	1995	1996	1995	1996	1995	1996
Inoculant (I)						
Uninoculated	0.43b	1.38	64.54	87.79	67.58	81.33
IRJ 2184A	0.46a	1.61	65.96	88.08	68.96	82.52
R.25B	0.46a	1.48	66.29	89.50	69.29	83.22
SE±	0.004**	0.06	0.65	0.58	0.65	0.64
Molybdenum (M)						
No molybdenum	0.37b	1.29b	71.79b	85.88b	64.83b	79.17b
Seed treatment	0.48a	1.55a	77.50a	89.50a	70.50a	84.04a
Foliar Spray	0.48a	1.66a	77.50a	90.29a	70.50a	84.04a
SE±	0.004**	0.06**	0.65**	0.58**	0.65**	0.64**
Cultivar (C)						
SAMSOY-2	0.41b	1.51	75.33	88.81	68.33	82.31
TGx 1019-2EN	0.49a	1.49	75.86	88.31	68.89	82.33
SE±	0.004**	0.05	0.53	0.49	0.53	0.52
Interactions						
IxM	NS	NS	NS	NS	NS	NS
IxC	NS	NS	NS	NS	NS	NS
MxC	NS	NS	NS	NS	NS	NS
IxMxC	NS	NS	NS	NS	NS	NS

Means with the same letter within the same treatment group, year and stage of growth are not probability different at 5% level of significance using Duncan multiple range test.

Abbreviations
* Significant at 5% level of probability
** Significant at 1% level of probability
NS Not Significant
WAS Weeks after sowing
SE Standard error.

Table 23: Nitrogen content (kg/ha), nitrogen fixed (kg/ha) of root bled sap (RBS), stem extract (SE) and by difference method of soyabean as influenced by *Bradyrhizobium* inoculation, method of molybdenum application and cultivar at 10 WAS at Samaru in *1995* and *1996*.

Treatments	Total Plant Nitrogen (kg/ha)		Nitrogen fixed (RBS) (Kg/ha)		Nitrogen Fixed (SE) (Kg/ha)		Nitrogen Fixed (difference method) (Kg/ha)	
	1995	1996	1995	1996	1995	1996	1995	1996
Inoculant (I)								
Uninoculated	81.23	83.72	49.56	63.23	54.53	68.20	38.19	52.72
IRJ 2184A	80.00	83.00	50.19	64.59	55.71	70.32	39.42	57.91
R.25B	82.57	87.61	53.58	67.28	58.70	76.19	41.68	59.87
SE±	1.70	2.32	1.36	2.57	1.40	2.68	1.92	2.75
Molybdenum (M)								
No molybdenum	70.23b	75.44b	42.02b	56.16b	47.93b	59.63b	31.92b	43.41b
Seed treatment	73.49a	81.00a	48.49a	63.52a	56.83a	67.92a	42.25a	51.19a
Foliar Spray	76.00a	82.37a	52.00a	64.32a	57.00a	69.10a	42.63a	55.32a
SE±	1.70**	2.32**	1.36**	2.75**	1.40**	2.68**	1.92**	2.75**
Cultivar (C)								
SAMSOY-2	73.71	78.65	45.00	61.29	50.41	64.43	35.62	55.31
TGx 1019-2EN	73.16	73.03	46.00	56.57	51.35	60.21	35.39	54.77
SE±	1.39	2.33	1.21	1.93	1.13	2.19	1.73	2.33
Interactions								
I x M	NS	NS	NS	NS	NS	NS	NS	NS
I x C	NS	NS	NS	NS	NS	NS	NS	NS
M x C	NS	NS	NS	NS	NS	NS	NS	NS
I x M x C	NS	NS	NS	NS	NS	NS	NS	NS

Means with the same letter within the same treatment group, year and stage of growth are not significantly different at 5% level of probability using Duncan multiple range test.

Abbreviations
* Significant at 5% level of probability
** Significant at and 1% level of probability
NS Not Significant
WAS weeks after sowing
SE Standard Error.

Molybdenum applied as seed and foliar spray significantly increased nitrogen fixed of root by difference method. For root bled sap, seed and foliar applied molybdenum increased nitrogen fixed by 7kg/ha (15%) and 10 kg/ha (23%) respectively in 1995. In 1996, seed and foliar applied molybdenum increased nitrogen fixed by 7kg/ha (13%) and 8kg/ha (15%) respectively. For stem extract, seed and foliar applied molybdenum both increased nitrogen fixed by 10kg/ha (19%) in 1995.

In 1996, seed and foliar applied molybdenum increased nitrogen fixed by 8kg/ha (14%) and 9kg/ha (16%) respectively. Similarly using the difference method seed and foliar applied molybdenum significantly increased nitrogen fixed by 10kg/ha (32%) and 11kg/ha (34%) respectively in 1995. In 1996, seed and foliar applied molybdenum increased nitrogen fixed by 8kg/ha (18%) and 12kg/ha (22%) respectively.

Cultivars did not differ significantly in nitrogen fixed. (Table 23) None of the interactions was significant (Table 23).

4.1.23 Phosphorous content (%)

Bradyrhizobium inoculation, molybdenum application and cultivar had no significant influence on phosphorous content (Table 24). None of the interactions was significant (Table

4.1.24. Days to 50% flowering

Neither *Bradyrhizobium* inoculation nor molybdenum application had significant effect on days to 50% flowering (Table 25).

Cultivar effect showed that TGx 1019-2EN attained 50% flowering earlier than SAMSOY-2 in both years (Table 25).

None of the interactions was significant (Table 25).

4.1.25. Days to physiological maturity

In both years, *Bradyrhizobium* inoculation with IRJ 2184A significantly delayed physiological maturity when compared with the uninoculated control (Table 25).

Molybdenum had no significant effect on days to physiological maturity (Table 25).

Cultivar effect showed that SAMSOY-2 attained physiological maturity earlier than TGx 1019-2EN (Table 25).

None of the interactions was significant (Table 25).

4.1.26. Days to harvest maturity

Bradyrhizobium inoculation and molybdenum application had no significant effect on days to harvest maturity (Table 25).

Cultivar effect showed that SAMSOY-2 attained harvest maturity earlier than TGx 1019-2EN (Table 25).

None of the interactions was significant (Table 25).

Table 24: Phosphorous content (%) of soyabean as influenced by *Bradyrhizobium* inoculation, method ofmolybdenum application and cultivar at 10 WAS at Samaru in 1995 and 1996.

Treatment	Phosphorous Content (%)	
	1995	1996
Inoculants (I)		
Uninoculated	0.25	0.18
IRJ 2184A	0.25	0.18
R.25B	0.26	0.20
SE±	0.005	0.008
Molybdenum (M)		
No molybdenum	0.30	0.22
Seed treatment	0.29	0.19
Foliar Spray	0.29	0.19
SE±	0.005	0.008
Cultivar (C)		
SAMSOY-2	0.23	0.18
TGx 1019-2EN	0.24	0.19
SE±	0.004	0.007
Interactions		
IxM	NS	NS
IxC	NS	NS
MxC	NS	NS
IxMxC	NS	NS

Means with the same letter within the same treatment group, year and stage of growth are not significantly different at 5% level of probability using Duncan multiple range test.

Abbreviations
* Significant at 5% level of probability
** Significant at 1% level of probability
NS Not Significant
WAS Weeks after sowing
SE Standard error.

4.1.27. Grain yield (kg/ha)

Grain yield was not significantly influenced by Bradyrhizobium inoculation in 1995 (Table 26). In 1996, *Bradyrhizobium* strain R.25B significantly increased yield by 15% and 9% when compared to the uninoculated control and IRJ 2184A respectively (Table 26).

In both years, molybdenum increased grain yield significantly (Table 26), when compared with no molybdenum control in 1995, yield increases were 11% and 19% when molybdenum was applied as seed and foliar treatment respectively. Similarly, molybdenum applied as seed treatment and foliar spray both increased grain yield by about 19% in 1996, when compared with no molybdenum control.

Cultivar effect was significant only in 1996, with SAMSOY-2 producing 25% more grains than TGx 1019-2EN (Table 26).

4.1.28. Number of pods per plant

Number of pods per plant was not significantly influenced by *Bradyrhizobium* inoculation in both years (Table 26).

In 1996, seed and foliar treatment with molybdenum significantly increased pod number by 15% when compared with no molybdenum control (Table 26). Whereas in 1995, molybdenum had no effect on this parameter.

Cultivar effect showed that SAMSOY-2 produced 16% and 28% more pods than TGx 1019-2EN in 1995 and 1996 respectively (Table 26).

Inoculant x Cultivars interaction was significant in 1996 (Table 27). Strain R.25B decreased number of pods per plant of SAMSOY-2 significantly when compared with IRJ 2184A, whereas inoculation had no significant effect on TGx 1019-2EN (Table 27).

4.1.29. Weight of pods per plant(g)

Bradyrhizobium inoculation increased pod weight per plant significantly only in 1996 (Table 26). Inoculation with strain R.25B resulted in 35% and 29% increase in pod weight compared with IRJ 2184A and uninoculated control respectively. Strain IRJ 2184A and uninoculated control were at par.

In 1995, seed treatment and foliar spray increased pod weight significantly by 16% and 17% respectively when compared with no molybdenum control (Table 26). In 1996, seed application of molybdenum increased pod weight by 53% and 25% when compared with no molybdenum control and foliar spray molybdenum respectively. Foliar application of molybdenum also increased pod weight significantly by 22% when compared with no molybdenum control.

Cultivar effect showed that pod weight of SAMSOY-2 was significantly higher than that of TGx 1019-2EN in 1996 (Table 26).

None of the interactions was significant (Table 26).

Table 25: Days to 50% flowering, physiological and harvest maturity of soyabean as influenced by *Bradyrhizobium* inoculation, method of molybdenum application and cultivar at Samaru in 1995 and 1996.

Treatments	Days to 50% Flowering		Days to Physiological Maturity		Days to Harvest Maturity	
	1995	1996	1995	1996	1995	1996
Inoculant (I)						
Uninoculated	45.50	47.54	95.56b	99.54b	98.80	101.79
TRJ 2184A	45.83	47.92	97.10a	101.08a	99.77	102.67
R.25B	45.37	47.42	96.70ab	100.67ab	99.95	102.92
SE±	0.44	0.41	0.42*	0.38*	0.35	0.35
Molybdenum (M)						
No molybdenum	48.73	47.83	96.3	100.33	99.32	102.46
Seed treatment	45.82	47.83	96.69	100.71	99.47	102.54
Foliar Spray	45.23	47.21	96.25	100.25	99.29	102.38
SE±	0.44	0.41	0.42	0.38	0.35	0.33
Cultivar (C)						
SAMSOY-2	49.38a	51.47a	95.53b	99.58b	98.73b	101.64b
TGx 1019-2EN	41.64b	43.78b	97.23a	101.28a	100.39a	103.28b
SE±	0.36**	0.30**	0.34**	0.34**	0.28**	0.25*
Interactions						
IxM	NS	NS	NS	NS	NS	NS
IxC	NS	NS	NS	NS	NS	NS
MxC	NS	NS	NS	NS	NS	NS
IxMxC	NS	NS	NS	NS	NS	NS

Means with the same letter within the same treatment group, year and stage of growth, are not significantly different at 5% level of probability using Duncan multiple range test.

Abbreviations
* Significant at 5% level of probability
** Significant at 1% level of probability
NS Not Significant
WAS Weeks after sowing
SE Standard error.

Table 26: Grain yield (kg/ha), number of pods per plant and weight of pods per plant (g) of soyabean as influenced by *Bradyrhizobium* inoculation method of molybdenum application and cultivar at harvest at Samaru in 1995 and 1996.

Treatment	Grain yield (kg/ha)		No. of pods per plant		Weight of pods per plant (g)	
	1995	1996	1995	1996	1995	1996
Inoculants (I)						
Uninoculated	1600	1623b	34.00	28.00	10.05	13.66b
IRJ 2184A	1626	1718b	34.00	30.00	9.78	14.27b
R.25B	1644	1866a	35.00	28.00	10.61	18.41a
SE±	61.31	38.89**	1.54	1.37	0.41	0.82**
Molybdenum (M)						
No molybdenum	1433b	1543b	34.00	26.00b	9.14b	12.38c
Seed treatment	1585a	1834a	34.00	30.00a	10.64a	18.90a
Foliar Spray	1694a	1830a	36.00	30.00a	10.67a	15.06b
SE±	61.31*	38.89**	1.54	1.37*	0.41*	0.82**
Cultivar (C)						
SAMSOY-2	1621	1901a	37.00a	32.00a	10.54	17.02a
TGx 1019-2EN	1528	1571b	32.00b	25.00b	9.75	13.85b
SE±	49.78	31.75**	1.33**	1.12**	0.33	0.67**
Interactions						
IxM	NS	NS	NS	NS	NS	NS
IxC	NS	NS	NS	*	NS	NS
MxC	NS	NS	NS	NS	NS	NS
IxMxC	NS	NS	NS	NS	NS	NS

Means with the same letter within the same treatment group, year and stage of growth, are not significantly different at 5% level of probability using Duncan multiple range test.

Abbreviations
* Significant at 5% level of probability
** Significant at 1% level of probability
NS Not Significant
WAS Weeks after sowing
SE Standard error.

Table 27: Interaction between *Bradyrhizobium* inoculation and cultivar on number of pods per plant of soyabean at harvest in Samaru in 1996.

	Cultivar		
	SAMSOY-2		TGx 1019-2EN
Inoculant (I)			
Uninoculated	32.00ab		23.00c
IRJ 2184A	36.00a		24.00c
R.25B	28.00bc	1.94*	28.bc
$SE_{\pm}$			

Means with the same letter within the same treatment group, year and stage of growth, are not significantly different at 5% level of probability using Duncan multiple range test.

Abbreviations
* Significant at 5% level of probability
** Significant at 1% level of probability
NS Not Significant
WAS Weeks after sowing
SE Standard error.

4.1.30. Number of seeds per plant

Bradyrhizobium inoculation significantly increased number of seeds per plant in 1996 only (Table 28). Strain IRJ 2184A and R.25B significantly increased seed number per plant compared with the uninoculated control.

In both years, molybdenum application significantly increased seed number per plant (Table 28). In 1995, seed and foliar treatment with molybdenum both increased seed number by 22% when compared with no molybdenum control. In 1996, the application of molybdenum as seed and foliar treatment increased seed number significantly by 81% and 27% respectively when compared with no molybdenum control (Table 28). Similarly, seed applied molybdenum increased seed number by 43% when compared with foliar spray.

Cultivar effect was significant only in 1996 with SAMSOY-2 producing 47% more seeds than TGx 1019-2EN (Table 28).

4.1.31 Weight of seeds per plant (g)

Bradyrhizobium inoculation significantly influenced seed weight per plant only in 1996 (Table 28). *Bradyrhizobium* strain R.25B increased seed weight by 29% and 18% when compared with uninoculated and IRJ 2184A respectively.

Table 28: Number of seeds per plant, weight of seeds per plant(g), one hundred seed weight (g) and number of seeds per pod as influenced by *Bradyrhizobium* inoculation, method of *molybdenum* application and cultivar at harvest at Samaru in 1995 and 1996.

Treatments	Number of Seeds Per plant		Weight of Seeds per plant(g)		One hundred seed weight (g)		Number of seeds per pod	
	1995	1996	1995	1996	1995	1996	1995	1996
Inoculants (I)								
Uninoculated	45.00	54.00b	6.00	10.42b	14.36	13.23b	1.00	2.00
IRJ 2184A	46.00	68.00a	5.62	11.42b	13.07	13.97ab	1.00	2.00
R.25B	50.00	74.00a	6.24	13.43a	13.93	14.24a	1.00	2.00
SE±	2.02	4.76*	0.28	0.62**	0.22	0.33*	0.11	0.09
Molybdenum (M)								
No molybdenum	41.00b	48.00c	4.96b	9.95b	13.70	13.42	1.00	2.00
Seed treatment	50.00a	87.00a	6.71a	13.92a	13.60	14.40	1.00	2.00
Foliar Spray	50.00a	62.00b	6.19a	11.39b	14.06	13.91	2.00	2.00
SE±	2.02**	4.76**	0.28**	0.62	0.22	0.33	0.11	0.09
Cultivar (C)								
SAMSOY-2	49.00	78.00a	6.04	12.99a	14.77	14.00	1.00	2.00
TGx 1019-2EN	45.00	53.00b	5.87	10.52b	12.87	13.82	1.00	2.00
SE±	1.65	3.89**	0.22	0.51**	0.18	0.72	0.09	0.08
Interaction								
M x C	NS	NS	NS	NS	NS	NS	NS	NS
I x C	NS	NS	NS	NS	NS	NS	NS	NS
I x M	NS	NS	NS	NS	NS	NS	NS	NS
I x M x C	NS	NS	NS	NS	NS	NS	NS	NS

Means with the same letter within the same treatment group, year and stage of growth are not significantly different at 5% level of probability using Duncan multiple range test.

Abbreviations
* Significant at 5% level of probability
** Significant at 1% level of probability
NS Not Significant
WAS *weeks after sowing*
SE Standard Error.

In 1995, molybdenum application as seed and foliar treatment increased seed weight by 35% and 25% respectively when compared with no molybdenum control (Table 28). However, in 1996 seed treatment with molybdenum increased seed weight by 40% and 22% when compared with no molybdenum control and foliar spray respectively (Table 28).

Cultivar effect was significant only in 1996 (Table 28) with SAMSOY-2 having heavier seeds than TGx 1019-2EN.

4.1.32. One hundred seed weight (g)

Bradyrhizobium inoculation had no significant effect on one hundred seed weight in 1995 (Table 28). However, in 1996, strain R.25B increased one hundred seed weight by 8% when compared with uninoculated control.

Molybdenum and cultivar had no significant influence on one hundred and weight (Table 28).

None of the interactions was significant (Table 28).

4.1.33 Number of seeds per pod

In both years, *Bradyrhizobium* inoculation, molybdenum application and cultivar had no significant influence on the number of seed per pod (Table 28).

None of the interactions was significant (Table 28).

4.1.34. Threshing percentage (%)

Bradyrhizobium inoculation and cultivar did not influence threshing percentage significantly (Table 29).

Molybdenum application was significant only in 1995 (Table 29). Seed treatment with molybdenum increased threshing prcentage significantly when compared with no molybdenum control.

Molybdenum x cultivar interaction was significant in 1996 (Table 30). Foliar spray of molybdenum resulted in decrease of threshing percentage of TGx 1019-2EN when compared to either no molybdenum control and seed treatment molybdenum. Molybdenum had no effect on threshing percentage of TGx 1019- 2EN.

4.1.35. Harvest index (%)

Bradyrhizobium inoculation with both strains influenced harvest index significantly only in 1995, with Strain R.25B and IRJ 2184A decreasing harvest index (Table 29).

Molybdenum and cultivar had no significant effect on harvest index in both years (Table 29).

None of the interactions was significant (Table 29).

4.1.36 Correlation Analysis

Analysis of correlation coefficient between growth characters, yield components and grain yield was done in 1995 and 1996 to determine their relationship (Tables 31 and 32).

There was a significant and positive correlation between grain yield and dry matter accumulation at harvest in 1995 and 1996. In 1996, significant and positive correlation was

There was a significant and positive correlation between grain yield and dry matter accumulation at harvest in 1995 and 1996. In 1996, significant and positive correlation was observed between grain yield and nodule number, nodule dry weight, harvest index and plant height. Moreover, a significant and negative correlation between grain yield and days to 50% flowering was recorded in 1996 (Table 32).

Table 29: Threshing percentage (%) and harvest index (%) of soyabean as influenced by *Bradyrhizobium* inoculation, method of molybdenum application and cultivar at Samaru in 1995 and 1996.

Treatment	Threshing Percentage (%)		Harvest Index (%)	
	1995	1996	1995	1996
Inoculant (I)				
Uninoculated	58.80	79.50	36.92a	31.50
IRJ 2184A	58.50	82.29	31.96b	33.08
R.25B	58.80	76.75	32.33b	31.33
SE±	1.79	1.39	1.12**	0.84
Molybdenum (M)				
No molybdenum	54.30b	79.79	34.75	32.96
Seed treatment	63.10a	78.42	32.71	31.25
Foliar Spray	58.70ab	77.33	30.75	31.71
SE±	1.79*	1.39	1.12	0.84
Cultivar (C)				
SAMSOY-2	60.30	79.22	33.44	31.64
TGx 1019-2EN	57.10	77.81	32.03	32.31
SE±	1.61	1.13	0.92	0.68
Interactions				
IxM	NS	NS	NS	NS
IxC	NS	NS	NS	NS
MxC	NS	xx	NS	NS
IxMxC	NS	NS	NS	NS

Means with the same letter within the same treatment group, year and stage of growth, are not significantly different at 5% level of probability using Duncan multiple range test.

Abbreviations
* Significant at 5% level of probability
** Significant at 1% level of probability
NS Not Significant
WAS Weeks after sowing
SE Standard error.

Table 30: Interaction between method of molybdenum application and cultivar as it influenced threshing percentage (%) of soyabean at Samaru in 1996.

	Cultivar	
	SAMSOY-2	TGx 1019-2EN
Molybdenum(M)		
No molybdenum	79.75a	79.83ab
Seed treatment	75.83bc	81.00ab
Foliar spray	82.08a	72.58b
SE±	1.96**	

Means with the same letter within the same treatment group, year and stage of growth, are not significantly different at 5% level of probability using Duncan multiple range test.

Abbreviations
* Significant at 5% level of probability
** Significant at 1% level of probability
NS Not Significant

WAS Weeks after sowing
SE Standard error.

2.	0.3404**	1.000												
3.	-0.0399	0.0166	1.000											
4.	0.0721	0.1299	0.1920	1.000										
5.	0.1440	0.0336	0.2575*	0.5581**	1.000									
6.	0.2053	0.1630	0.1537	0.7490**	0.7810**	1.000								
7.	0.1725	0.2624*	-0.1818	0.1781	-0.0108	0.2743*	1.000							
8.	0.1990	0.0006	-0.4207**	0.2094	0.5267**	0.4675**	0.2244	1.000						
9.	0.1491	0.0569	-0.4392**	0.1830	0.4171**	0.4184**	0.2787*	0.8549**	1.000					
10.	0.1418	0.0699	0.3314**	0.0789	0.1564	0.0998	-0.3220**	-0.1195	0.2176	1.000				
11.	0.1025	0.1769	0.2973*	0.2009	0.2271	0.1473	-0.1425	-0.0165	0.1241	0.5109**	1.000			
12.	0.0470	0.0775	0.1229	-0.1660	-0.1449	-0.1615	-0.2127	-0.3804**	-0.3616**	0.2833*	0.0528	1.000		
13.	0.1216	-0.0364	-0.1900	0.1083	-0.1101	-0.0315	0.1850	-0.0791	-0.0433	-0.1326	-0.2705*	-0.1908	1.000	
14.	0.1086	-.04641**	0.1809	-0.1706	-0.0900	-0.1366	-0.0998	0.1020	0.1727	0.1263	-0.0810	0.0145	0.067	1.000
15.	-0.0774	0.0736	-0.0100	-0.0797	0.5163**	0.5788**	0.1532	0.4580**	0.4170**	0.0421	-0.0537	-0.0505	-0.1911	-0.0093	1.000
16.	-0.0861	0.0803	-0.3597**	0.1069	-0.2383*	0.0201	0.5532**	0.1786	0.2963*	-0.4971**	-0.1447	-0.3401**	0.04301**	-0.0006	-0.1899	1.000

r (5%) = 0.232

r (1%) =0.302

1.	Grain yield (kg/ha)	7.	Leaf area index (10 WAS)	13.	Days to 50% flowering
2.	Dry matter accumulation at harvest (kg/ha)	8.	Nodule number/plant (10 WAS)	14.	Total Sap nitrogen (SE)
3.	Number of pods/plant	9.	Nodule dry weight/plant (10 WAS)	15.	Relative uriede Nitrogen (SE) (%)
4.	Weight of pods/plant (g)	10.	Harvest Index (%)	16.	Nitrogen fixed (SE (kg/ha))
5	Number of seeds/per plant	11.	Shelling percentage (%)		
6.	100 seed weight (g)	12.	Plant height (cm) (10 WAS)		

* Significance at 5% level of significance

** Significance at 5% and 1% level of significance.

1.	1.000														
2.	0.5966**	1													
3.	-0.0072	0.2467*	1												
4.	0.1758	0.2778*	0.4717	1											
5.	0.0461	0.2563*	0.4583**	0.7192**	1										
6.	0.1329	0.2213	0.3791**	0.8922**	0.7585**	1									
7.	0.0456	0.2023	0.4251**	0.3377**	0.2038	0.2985*	1								
8.	*0.2419**	*0.0780*	*0.0805*	*0.1269*	*0.0256*	*0.0659*	*0.0424*	1							
9.	0.2780*	0.1599	-0.0160	0.0449	-0.0589	-0.1234	0.1140	0.0558	1						
10.	-0.1836	0.2009	0.3471**	0.2570*	0.2150	0.2523*	0.0620	-0.1744	*0.2402**	*1*					
11.	0.3373**	0.1177	0.4028**	0.5636**	0.4939**	0.0115**	0.3742**	-0.0995	0.0425	0.5430**	1				
12.	0.47414**	0.0919	0.0707	-0.1240	-0.1477	-0.0802	0.0593	-0.0858	-0.0440	0.0145	-0.0850	1			
13.	-0.4615**	0.1476	-0.0827	-0.1237	-0.2251	-0.1238	0.1461	-0.0471	-0.0696	-0.1011	-0.0772	0.5378**	1		
14.	0.2073**	0.0165	0.0573	-0.1791	-0.1951	-0.1485	-0.0329	-0.0051	-0.3004*	0.1076	-0.0167	0.0531	0.1169	1	
15.	-0.1836	0.0377	-0.1830	-0.4281**	-0.0908	-0.1462	-0.1417	-0.1258	-0.0977	0.2185	-0.0370	-0.1321	-0.0074	0.521	1
16.	0.1374**	0.3913**	-0.2930*	-0.2252	-0.2771*	-0.2400*	0.0429	0.0511	0.2031	-0.1394	-0.2982*	0.2118	0.3882**	0.0111	-0.0089

r (5%) = 0.232

r (1%) =0.302

1.	Grain yeild (kg/ha)	7.	*Leaf area index (10 WAS)*	13.	Days to 50% flowering
2.	Dry matter accumulation at harvest (kg/ha)	8.	Nodule number/plant (10 WAS)	14.	Total Sap nitrogen (SE)
3.	Number of pods/plant	9.	Nodule dry weight/plant (10 WAS)	15.	*Relative uriede Nitrogen (SE)* (%)
4.	Weight of pods/plant (g)	10.	Harvest Index (%)	16.	Nitrogen fixed (SE (kg/ha))
5	Number of seeds/per plant	11.	Shelling percentage (%)		
6.	100 seed weight (g)	12.	Plant height (cm) (10 WAS)		

* Significance at 5% level of significance

** Significance at 5% and 1% level of significance.

CHAPTER FIVE

5.0 DISCUSSION

5.1. Effect of *Bradyrhizobium* Inoculation

Plant height, grain yield , weight of pods per plant, number of seeds per plant, one hundred seed weight, leaf area and leaf area index were significantly increased by *Bradyrhizobium* inoculation.

Inoculation with strain R.25B increased nitrogen fixation. This probably resulted in a significant increase in leaf area and leaf area index at 10 WAS which was the period of maximum vegetative growth. This may be attributed to the fact that nitrogen is an essential mineral nutrient. It is an important constituent of chlorophyll, protoplasm and is important in protein, nucleic acid and amino acid synthesis (Gasser and Williams, 1980; Samuel, 1980). It therefore stimulated rapid vegetative growth and its presence in appropriate level enhanced vegetative growth.

Strain R.25B significantly increased grain yield by 15% when compared with uninoculated control in 1996 This significant and postive response could be due to increase in nitrogen fixation with increased leaf area and leaf area index. The yield of an agricultural system is dependent on the size and efficiency of its photosynthetic system (Loomis and Williams, 1963). The increased vegetative growth possibly increased the photosynthetic surface available for light interception, thus favouring increased production and

translocation of assimilates to the seed , hence increased grain yield. This may be due to compatibility of strain R.25B with its host soyabean plant, and the fact that this strain was isolated from the nodules of soyabean grown in Zaria. It may therefore be adapted to the climatic and edaphic conditions of Zaria and its environ resulting to its better competitive advantage over the indigenous soil rhizobia. Thus, R.25B probably had high survival rate and persistence in the soil (Giller and Wilson, 1991).

The grain yield increase due to inoculation with strain R.25B was probably as a result of higher pod weight per plant, number and weight of seeds per plant, one hundred seed weight, leaf area and leaf area index. Using soils with no previous history of soyabean, Kang (1975) in the forest zone of Nigeria observed increased grain yield with *Bradyrhizobium* inoculation. Similar findings were reported in other parts of the tropics (Bonnier and Seeger, 1958, Freiere, 1970). Chiezey *et al.* (1992) reported that the number of pods per plant and one hundred seed weight were the most important yield component of soyabean.

In 1995, strain R.25B increased grain yield but this yield was not significant This can be attributed to the fact that planting was done rather late in 1995, 17 July in 1995 as against 18 June in 1996. In addition, lower rate of inoculant was used in 1995 compared to 1996. In 1995, 18 grams peat based inoculant was applied per kilogram of seed. This was increased to 27 grams in 1996.

In contrast, IRJ 2184A which was isolated from the nodules of soyabean at Ibadan did not increase soyabean yield significantly . This may be an indication of more competitive indigenous strains of rhizobia in the soil and/or low survival rate and persistance in the soil. (Giller and Wilson, 1991). Strain IRJ 2184A therefore failed to adapt and compete favourably in the northern Guinea savanna.

5.2. Effect of Method of Molybdenum application

The result of this study showed a significant increase due to molybdenum application as seed treatment and foliar spray on the following parameters: Leaf dry weight, leaf area and leaf area index , dry matter accumulation at 8, 12 WAS and at harvest, plant height at harvest and height to the lowest pod. Also, total sap nitrogen, uriede and actual uriede concentration ,relative uriede nitrogen percentage and kilogram nitrogen fixed. Grain yield, number and weight of pods per plant, number and weight of seeds per plant were also significantly increased by molybdenum application.

Molybdenum application as seed and foliar treatment significantly increased growth characters such as leaf dry weight and leaf area and area index at 10 and 12 WAS. This is in agreement with Nayak *et al.* (1989) on application of 73g/ha molybdenum and Parker and Harris (1962) on application of 224g/ha molybdenum. They observed that molybdenum applied as seed and foliar treatment increased vegetative growth

significantly. The positive and significant response to molybdenum applied as seed treatment and foliar spray in this present study could be due availability of nitrogen to the plant from both locations hence increased nitrogen fixation. Molybdenum is essential for symbiotic nitrogen fixation by legumes (Evans, 1956), because it is a constituent of the nitrogen fixing enzyme nitrogenase (Chatt *et al.*, 1969). This enzyme catalyses the fixation of atmospheric dinitrogen gas to ammonia which is needed by the host plant. It is also an essential element of the enzyme nitrate reductase which reduces nitrate to nitrite in protein synthesis (Evans, 1956). As a result, its presence in appropriate level enhances nitrogen fixation and efficient functioning of the nitrogen fixing bacteria. This increased nitrogen fixation was probably translated to increased vegetative growth in terms of leaf area , leaf area index and dry matter accumulation . Molybdenum increased nitrogen fixation by increasing actual and relative concentration . Uriede producing legumes such as soyabean fixes most of its nitrogen in form of uriede, whilst nitrogen absorbed from the soil is absorbed in form of nitrate and to a lesser extent in form of amide. The proportion of nitrogen in the xylem sap present as uriede has been developed as an index of nitrogen fixation (McClure *et al.*, 1980, Pate *et al.*, 1980).

Seed and foliar applied molybdenum increased grain yield by 11% (152 kg/ha) and 18% (261 kg/ha) respectively in 1995

and by 19% (287 kg/ha) in 1996 . This increase may be as a result of increased leaf area and leaf area index , which increased interception of solar radiation, production of photosyntate and consequent grain yield. Molybdenum increased soyabean yield by increasing the number of pods per plant (Huceng, 1979) weight of pods per plant, number of seeds per plant (Huceng, 1979) and weight of seeds per plant (Parker and Harris, 1962). Similar grain yield increased were observed by Harris *et al* (1965) who found that 27g molybdenum/67kg seed gave yield increases of 20 - 43% on soils of pH of 5.4. Also Parker and Harris (1962) recorded up to 1,123kg/ha (50%) grain yield increase on application of 224g molybdenum as seed treatment when compared to no molybdenum control at pH of 5.1.

Foliar applied molybdenum was found to be as effective as seed treatment. This is because molybdenum was equally available to the plant from both application sites . This finding is in agreement with (Boswell and Anderson, 1969) who reported that foliar sprayed molybdenum was as effective as seed treatment, when applied at the pre-bloom stage.

Plant height at harvest was significantly reduced by only foliar applied molybdenum. Also height to the lowest pod was decreased significantly by both seed and foliar treated molybdenum. This may be an indication of competition between the reproductive and vegetative organs, with the pods having a higher competitive advantage, thus increasing partitioning of assimilates to the pods at the expense of plant height. This may have led to increased grain yield but reduced plant height.

5.3. Effect of Cultivar

The cultivars studied differed in the following parameters: leaf number and weight, leaf area, leaf area index, plant height at harvest, height to the lowest pod, nodule number and weight and branch number. Others are dry matter accumulation, grain yield, number and weight of pods per plant, number and weight of seeds per plant, number of seeds per pod, 50% flowering, physiological and harvest maturity. These differences may be due to variation in genetic make up. Characters are genetically controlled though influenced by environmental factors (Anon, 1985). SAMSOY-2 proved to be superior to TGx 1019-2EN in many characters.

The indeterminate TGx 1019-2EN cultivar consistently produced taller plants and height to the lowest pod than the determinate SAMSOY-2 cultivar. This variation is an indication of their varying genetic make up. Sheelwanter and Pantil (1979) observed that indeterminate cultivar attained greater height than determinate cultivar.

The influence of genotype on leaf number , leaf dry weight , leaf area , leaf area index and branch number was significant. SAMSOY-2 exhibited more vigorous vegetative growth when compared with TGx 1019-2EN. This may be due varying genetic make up and better adaptability of SAMSOY-2 to this ecological zone.

SAMSOY-2 produced significantly higher grain yield by (25%) when compared with TGx-1019-2EN in 1996. Though SAMSOY-2 produced higher grain yield in 1995, the increase was not significant. The yield advantage of SAMSOY-2 over TGx 1019-2EN could be attributed to its higher leaf area (Table 3), branch number (Table 8) number and weight of pods per plant (Table 26), number and weight of seeds per plant (Table 28). Yield advantage of shorter over taller soyabean cultivar had been attributed to higher branch number (Buttery, 1969 & larger seed size and higher leaf area Watson, 1958; Cooper, 1977).

In the present study, the shorter SAMSOY-2 cultivar also exibited more vigorous vegetative growth than TGx 1019-2EN in terms of leaf number , leaf dry weight , leaf area , and leaf area index . This vigorous growth enhanced production of photosynthetic assimilates hence increased grain yield.

The two cultivars did not differ significantly in the amount of nitrogen fixed . This is because the two cultivars are promiscuously nodulating in that they form effective symbiosis with indigenous soil *Bradyrhizobium* (Olufajo *et al.*, 1984).

5.4. Molybdenum x Cultivar Interaction

Foliar spray molybdenum significantly increased dry matter accumulation of SAMSOY- 2 but not TGx 1019-2EN in 1991 when compared with no molybdenum control . This was due to

increased leaf area , leaf area index at 10 and 12 WAS. This may be attributed to significant increase in nitrogen fixation, thus increased nitrogen for vegetative growth.

5.5. Inoculant x Molybdenum Interaction

In both years, inoculant x molybdenum interaction was significant on plant height at harvest . Height of uninoculated plot was not affected by molybdenum in both years. For plots inoculated with *Bradyrhizobium* strain R.25B, foliar applied molybdenum depressed plant height. This could be attributed to increased partitioning of assimilates to the reproductive organs of pods. Competition may have occured between the vegetative and reproductive organs, and the pod having higher competitive advantage may have cause decreased yield.

5.6. Inoculant x cultivar interaction

Rhizobial inoculation with R.25B significantly decreased plant height of TGx 1019-2EN but not SAMSOY-2 in both years(Table 14).This variation in response can be attributed to their varing genetic constitution.

5.7. Correlation Analysis

There was a significant and positive correlation between grain yield and dry matter accumulation in 1995 and 1996. Also in 1996, there was a significant and positive correlation

between grain yield and nodule number, plant dry weight at 10 WAs, plant height at 12 WAS and harvest index.

Similar observations were made by Singh *et al.* (1982), Sahabi (1988) on cowpea, and Black (1956) on soyabean. They attributed the significant and positive association to the fact that increasing plant height exposed the plant to solar radiation, hence higher photosynthetic rate. There was a significant and negative correlation between days to 50% flowering and yield. This could be due to the fact that increasing days to 50% flowering delayed reproductive growth and increased vegetative growth at the expense of reproductive growth, hence less time and photosynthate for grain growth.

CHAPTER SIX

6.0 SUMMARY AND CONCLUSION

Field experiments were conducted at the Institute for Agricultural Research Farm at Samaru during the wet seasons of 1995 and 1996 to investigate the response of two soyabean (*Glycine max* (L.) Merrill) cultivars to *Bradyrhizobium* inoculation and methods of molybdenum application.

Bradyrhizobium strain R.25B isolated from the nodules of soyabean at Zaria, increased nitrogen fixed by 5kg/ha. Leaf area, leaf area index, weight of pods per plant, number of seeds per plant and one hundred seed weight were significantly increased by strain R.25B. IRJ 2184A isolated from the nodules of soyabean plant at Ibadan proved to be ineffective.

Molybdenum applied as either seed or foliar spray significantly increased nitrogen fixation by 15% and 19% respectively. this increase was brought about by significant increase in actual and relative uriede nitrogen content. Also significantly increased by molybdenum application were leaf dry weight , leaf area and leaf area index. Yield Increases of 15% and 19% were obtained with application of molybdenum and seed treatment respectively.

The short indeterminate soyabean cultiver exibited more vigorous vegetative growth in terms of leaf number and weight,leaf area and leaf area index when compared with the taller indeterminate TGX 1019-2EN and proved to be better adapted to the northern Guinea savanna ecological zone. SAMSOY-2 and TGX-1019-2EN fixed 74 and 73 kgN/ha respectively.

Based on the result obtained from the study, it can be concluded that yield benefits can be obtained from use of strain R.25B isolated from soyabean nodule at Zaria. Molybdenum appeared beneficial to soyabean in Nigerian savanna. When applied at the pre-bloom stage, foliar application of molybdenum seems to be equally as effective as seed treatment. In savanna soils soyabean could fix as much as 37 to 118KG N/ha which was equivalent to 24 to 90% of its total nitrogen.

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Appendix 1: Physical and Chemical properties of soil of the experimental site before sowing at Samaru in 1995 and 1996.

	Soil Depth			
	0 - 15cm		15 - 30cm	
Soil Characteristics	1995	1996	1995	1996
Physical Characteristics (g/kg)				
Clay	400	470	430	440
Silt	500	400	450	410
Sand	100	130	120	150
Textural Class	Silt loam	loam	loam	loam
Chemical Characteristics				
pH (1:2.5) in H_2O	5.7	5.6	5.0	5.2
pH (1:2.5) in $CaCl_2$	5.0	5.0	4.8	4.9
Organic carbon (g/kg)	6.0	6.4	5.0	4.8
Total nitrogen (g/kg)	0.6	0.4	0.6	0.3
Available Phosphorus (g/kg)	13.3	12.1	14.3	13.9
Molybdenum (g/kg)	0.52	0.50	0.91	0.83
Exchangeable Bases (cmol/kg)				
Ca	1.72	1.99	1.18	1.27
Mg	0.42	0.71	0.39	0.62
K	0.13	0.10	0.15	0.14
Na	0.15	0.13	0.15	0.10
H + Al	0.16	0.10	0.15	0.10
CEC	5.20	7.00	4.10	6.70

Appendix 2: Total rainfall, maximum and minimum temperature, and relative humidity at ten days interval during the experimental period at Samaru in 1995.

Month	Days	Rainfall (mm)	Max. Temp. (°C)	Min. Temp. (°C)	Relative Humidity (%) a.m	Relative Humidity (%) p.m
	1-10	39.4	-	-	-	-
June						
	11-20	66.4	32	18	87	52
	21-30	19.62	30	19	79	54
July	1-10	36.8	29	22	80	60
	11-20	52.3	30	23	79	70
	21-31	67.8	30	21	82	73
Aug.	1-10	86.3	29	20	82	74
	11-20	73.6	28	20	83	73
	21-31	96.7	28	21	79	81
Sept.	1-10	39.3	30	22	82	79
	11-20	80.3	29	19	82	69
	21-30	39.3	28	21	81	63
Oct.	1-10	32.5	31	20	78	69
	11-20	Nil	33	19	71	44
	21-31	Nil	32	18	53	73

Appendix 3: Total rainfall, maximum and minimum temperature and relative humidity at ten days interval during the experimental period at Samaru in 1996.

Month	Days	Rainfall (mm)	Max. Temp. °C	Min. Temp. °C	Relative Humidity a.m.	Relative Humidity (pm)
June	1-10	91.3	30	18	76	62
	11-20	38.8	31	20	77	66
	21-30	11.8	30	20	80	60
July	1-10	36.7	28	19	78	56
	11-20	47.4	28	18	82	57
	21-31	72.6	27	17	77	68
Aug	1-10	40.6	28	20	80	71
	11-20	77.1	27	20	84	77
	21-31	53.4	28	20	81	70
Sept	1-10	76.6	30	21	83	70
	11-20	37.8	30	19	82	69
	21-30	22.6	30	20	80	76
Oct.	1-10	19.5	32	20	80	60
	11-20	31.7	32	20	77	74
	21-30	Nil	28	17	43	42

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