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EFFECT OF DIFFERENT LEVELS OF PHOSPHORUS ON NODULATION AND NITROGEN FIXATION IN BAMBARRA GROUNDNUT (*Vigna subterranea* (L.) Verdc.)

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ABSTRACT

A greenhouse experiment was setup at Kogi State University, Nigeria to evaluate the influence of different levels of phosphorus on nodulation and nitrogen fixation in Bambara groundnut. Soil sample at 0 – 15 cm depth were collected from Teaching and Research Farm of Kogi State University. The dried soil was weighed in 10 kg portions into ten litre plastic bucket. To each labeled pot was added the respective phosphorus levels and 20 ppm Urea. Each treatment was replicated 3 times and then arranged in Completely Randomized Design. The shoots were harvested at fifty percent flowering stage and the buckets were gently turned over to remove roots and nodules. Results indicated that there was significant difference among the means of the various treatment levels in the total nitrogen content and mean height of the plant. In all the parameters measured, the treatments were better than the control except for total nitrogen of soil after harvest. So that nitrogen fixation could benefit with the application of phosphorus.

Keywords: Bambara groundnuts, Nitrogen fixation, Nodulation, Urea, Phosphorus.

INTRODUCTION

Bambarra groundnut (*Vigna subterranea (L.) Verdc.)* is a pulse crop of immense potential in enhancing food security, especially in drought – prone agricultural systems. Its drought tolerance makes it ideal for production by resource-poor farmers, especially in communal resettlement areas. It can grow well in communal areas where pest and disease control are not given serious attention (Vusumuzi, 1992). The crop features prominently in the traditional farming systems all over this country as an intercrop of cereals and other crops. Also, it is planted as sole crop in Jos, Yola and Ogoja in Cross River State (Hepper, 1970; Giller and Wilson, 1991). Even though, majority of the farmers produce Bambarra groundnut primarily for food, they may sell part of their produce when they have produced excess of their food needs or when they are pressed for money to pay taxes, loans or school fees (Linnanmann, 1988)

As one of the two most drought–tolerant legumes – groundnut (*Arachis hypogea*) being the second and despite the numerous local uses the crop is put to and the numerous advantages derived from research work on Bambarra groundnut, complete research information, especially with regard to Nigeria in general and Kogi State in particular, is scanty because not much work has been done on the crop.

Nitrogen fixation either biological or abiotic is the natural process by which nitrogen in the atmosphere is converted to ammonia. This process is essential for plant life because fixed nitrogen is required to biosynthesize the basic blocks of life e.g nucleosides for DNA and RNA and amino acids for protein. Biological nitrogen fixation occurs when atmospheric nitrogen is converted to ammonia by an enzyme called Nitrogenase.

Symbiotic nitrogen fixation has been estimated to contribute approximately half of the amount of nitrogen (N) applied in inorganic N fertilizers (Smil, 2005) and it may represent an ecological alternative to inorganic N fertilization in several areas in the world.

Some food and fodder legumes are known for nitrogen fixing ability and their establishment with nitrogen (N) fertilizers enhances nodulation and hence fixation of the atmospheric nitrogen making it available to subsequent crops. Among the major nutrient, requirement for nitrogen exceeds any other and rarely do soils in the tropics have enough of this nutrient (N) to produce high, sustainable yields (Mkandiwire and Sibuga, 2002). This lack of adequate amount of nitrogen of most soil puts limitation on the farmers' goals of increasing yield productions.

Phosphorus is the most important basic ingredient in the successful establishment of forage legumes (Hague and Jutzi, 1984). Phosphorus is generally essential to plant growth and is an active ingredient in protoplasm. It stimulates early growth and root formation, hastens maturity, promotes seeds production, gives stability of the stem and contributes to the general hardiness of plants.

Phosphorus deficiency is one of the major fertility problems limiting grain legumes production in tropical soils (Fox and Kang, 1977). In legumes, phosphorus is reported to stimulate root and plant growth and initiation of nodules, affects the extent of nodulation in terms of numbers and mass and influences the duration and general efficiency of the *Rhizobium* legume symbiotic system (Andrew, 1977; Robinson *et al.,* 1981). Moreover, the vital role played by phosphorus is reaction involving energy transfer and more specifically ATP in nitrogenase activity suggests that plants dependent on symbiotic nitrogen for growth may require more phosphorus than those dependent on combined nitrogen (Cassman *et al*. 1981, Israel, 1987; Singleton *et al*., 1985).

Scientific information on nodulation and nitrogen fixation by bambara groundnut are rare, if not completely absent in Kogi State, Southern Guinea Savanna of Nigeria. For these reasons the study was carried out to evaluate the effect of different levels of phosphorus fertilizer application on nodulation and nitrogen fixation in Bambarra groundnut (*Vigna substerranea (L.) Verdc.*).

MATERIALS AND METHODS

Description of Project Site

The project was carried out at Faculty of Agriculture, Kogi State University, Anyigba, Kogi State. (lat. 7^{O} 06'N and long. 6^{O} 43'E) located within the South Eastern Guinea Savanna agro-ecology of Nigeria (Kowal and Knabe, 1972).

The site lies within the warm humid climate of the middle belt zone of Nigeria with clear, distinctive dry and wet season. The average temperature is 27^{O}C uniformly high throughout the year. Annual temperature does not exceed 38^{O}C and mean annual rainfall is 1260 mm with peaks in the month of July and September.

Soil Sampling

Soil samples were collected at Kogi State University Teaching and Research Farm (TRF) at a depth of between 0 – 15 cm. The soil was sieved through a 2mm sieve to remove stones, gravels, and debris. Sieved soil samples (10 kg each) were weighed into labeled 10 L plastic pots. Sub–samples were taken prior to planting for laboratory analyses of pH, organic carbon, total nitrogen, phosphorus, exchangeable bases, CEC and particle size.

Treatment and Experimental Design

The treatment consisted of two types of inorganic fertilizer – Dipotassium phosphate (K_2HPO_4) as a phosphorus source and Urea [$(NH_2)_2CO$] as starter nitrogen source. The grain legume used in the study was Bambara groundnut (*Vigna subterranea(L.) verdc.*). The experiment was laid out in Completely Randomized Design (CRD) with 3 replications at different levels of dipotassium phosphate and the same level of urea. Dipotassium phosphate was mixed with soil samples at levels P_0 (0 kgP/ha), P_{20} (20 kgP/ha), P_{40} (40 kgP/ha), P_{50} (50 kgP/ha), P_{60} (60 kgP/ha), P_{70} (70 kgP/ha), P_{80} (80 kgP/ha) and P_{100} (100 kgP/ha), while urea was added uniformly to all treatments at 20 ppm. Four seeds of Bambara groundnut were planted in each pot. The seeds germinated six to seven days after planting. Two weeks after planting, the seedlings were thinned to two plants per pot.

Physico-Chemical Analysis of Soil

Soil particle size analysis was done according to Bouyocous (1962). Organic carbon content was determined using Walkey-Black (1934) chromic acid digestion procedure. Soil pH was determined electronically using glass electrode pH meter (Mclean, 1982). Available phosphorus was determined by Bray and Kurtz (1945) extraction procedure. Exchangeable bases (Ca, Mg, Na, K) were extracted in NH_4OAC buffered at pH 7.0 (Thomas, 1982). Cation exchange capacity (CEC) was determined by summation of exchangeable bases or cations. The N content of soil and plant tissue samples was determined after digestion of the samples with conc. H_2SO_4 in the presence of Kjeldhal catalyst (Bremner and Mulvancy, 1982). The percentage N was calculated thus:

$$\%N = M \times T \times 0.014 \times \frac{V_1}{V_2} \times \frac{100}{Wt}$$

Where; M=Molarity of acid
T=Control of titre value
V_1= Final Volume of Digest
V_2 =Volume of Digest used in Distillation
Wt= Mass of plant digested

Harvesting, Plant Tissue and Data Analysis

At around the time when about 50% of the plant had flowered (seven weeks after planting), the plants were gently uprooted, and shoots were carefully cut at ground level. Roots were carefully removed from soil and washed gently with water. Nodules were collected from the roots, counted and weighed. The fresh weights of the plant were also taken. Plant shoot, root and nodules were oven – dried at 70^{o}C for 36 hours for dry weight determination. Litter was collected as part of the shoot biomass. Plant shoots were ground in a small mortar, passed through a 60 – mesh sieve and later analyzed for total nitrogen concentration using Kjeldahl method. Data collected were analyzed using SPSS and the Least Significant Difference (LSD) test at 5% probability level was used to compare the treatment means.

RESULTS AND DISCUSSION:

Soil Analysis

The various physical and chemical properties of the soil used are shown in Table 1. The pH of the soil as obtained from the composite sample was 6.0. This falls within the moderate acid range (5.6 – 6.0) of pH which is optimum for most crops (the optimum pH range for most plants is between 6 and 7.5). This pH value would ensure the optimal performance of rhizobial organism in the soil. The percentage organic carbon content was 1.27.

The nitrogen content of the soil used was 0.06% which falls within the low range of Soil Fertility Classes for Nigerian soils (Sobulo and Adepetu, 1987; Adepetu, 1990). This result is within the range (0.05 – 1.64%) for tropical soils (Birch and Friend, 1956).

The concentration of available phosphorus in the soil was 15.92 ppm. This falls within the high range of Soil Fertility Classes for Nigerian soils (Sobulo and Adepetu, 1987; Adepetu, 1990). Observations from experiments have shown that requirement of phosphorus for nodulation and nodule activity is much greater than for the host (de Mooy *et al.,* 1973). However, toxicity of phosphorus can lead to reduced nodule number and reduced nodule activity in nitrogen fixation (Fletcher and Kurt, 1964). deMooy and Pesek (1966) showed that phosphate improved the legume ability to respond to the appropriate microorganism.

The result from the determination of exchangeable cations showed that K concentration was 2.53 Cmol/kg soil, Mg 1.79 Cmol/kg soil, Ca 4.09 Cmol/kg soil and Na 0.92 Cmol/kg soil. The effects of Ca and Mg on N_2 fixation are just the neutralizing effect on the soil and this can benefit the growth and survival of the rhizobia. Calcium has been implicated in nodule formation and N_2 fixation.

This result of the Mechanical Analysis (particle size distribution) reveals that percentages of sand is 88.24%, clay 8.48% and silt 3.28%. The textural class of the soil is Loamy Sand.

Table 1: Some physico- chemical characteristics of the soil used in this study

Properties	Value determined
pH 1: 1 Soil	6.0
Organic carbon (%)	1.27
Available phosphorus (ppm)	15.92
Total Nitrogen (%)	0.06
Exchangeable cation (Cmol/kg)	
K^+	2.53
Na^+	0.92
Ca^{++}	4.09
Mg^{++}	1.79
Particle size (%)	
Sand	88.24
Silt	3.28
Clay	8.48
Textural class	Loamy Sand

Soil and Plant Analysis after Harvest

Analysis of variance was carried out on total nitrogen content, fresh nodules count, fresh weight of nodules, fresh weight of shoot, fresh weigh of root, dry weight of nodules, dry weight of shoot, dry weight of root and plant height.

Table 2 shows the influence of different levels of inorganic P on the mean percentage total nitrogen in soil. Statistical analysis showed that there was no significant difference between the means of various treatment levels compared to the control following the application of inorganic P (K_2HPO_4).

In terms of soil total nitrogen content, the highest mean was found when 40 kgP/ha (0.43g) was applied. At 60 kgP/ha, it was 0.10 g which was not different from the level at 70 kgP/ha (see Table 2). At 20 kgP/ha, it was 0.07 g which was not different from the level at 100 kgP/ha. At 50 kgP/ha, it was 0.12 g and at 80 kgP/ha, it was 0.09 g. These results were not significantly different ($P\leq 0.05$) from the control and within treatments.

These results indicate that with the increase in the level of P applied, the percentage total nitrogen in soil also increased inconsistently, from 0.09 g in the control to 0.43 g at 40 kgP/ha. Thereafter there was a decline which indicated that there was no excretion of N from the roots into the soil and no significant root decay had taken place during the period of the experiment.

Table 2 also shows the influence of different levels of inorganic P on the mean percentage total nitrogen content in Bambara groundnut. Statistical analysis shows that there was significant difference between the means of various treatment levels compared to the control following the application of inorganic P.

In terms of tissue total nitrogen content, the highest mean was found when 100 kgP/ha (5.18 g) was applied. At 80 kgP/ha, it was 4.91 g. At 70 kgP/ha, it was 4.80 g. At 60 kgP/ha, it was 4.63 g. At 50 kg P/ha, it was 4.25 g. At 40 kgP/ha, it was 4.05 g. At 20 kgP/ha, it was 3.94 g. These results were significantly different ($P\leq0.05$) from the control and within treatments.

These results indicate that with the increase in the level of P applied, the percentage total nitrogen in Bambara groundnut also increased, from 3.78 g in the control to 5.18 g at the highest level of 100 kgP/ha. The application of P stimulates higher nitrogen accumulation in the tissue probably as a result of nitrogen fixation.

Table 2 also shows the influence of different levels of inorganic P on the number of nodules in Bambara groundnut.

Statistical analysis shows that there was no difference in the numbers of the nodules in Bambara groundnut at various treatment levels compared to the control following the application of inorganic P.

In terms of mean value of nodule numbers, the highest mean was found when 70 kgP/ha (101.3) was applied. At 100 kgP/ha, it was 94.5. At 40 kgP/ha, it was 94.8. At 60 kgP/ha, it was 96.2. At 20 kgP/ha, it was 92.8. At 50 kgP/ha, it was 88.0. At 80 kgP/ha, it was 87.2. Total N concentration does not correlate with nodule count. This might indicate that some of the nodules might not be effective in fixing N_2. These results were not significantly different ($P\leq0.05$) from the control and within treatments.

These results indicated that with the increase in the level of P applied, the number of nodules of Bambara groundnut also increased, from 86.7 in the control to 101.3 at 70 kgP/ha. Thereafter there was a decline. The lack of significance may not be surprising. Israel (1987) indicated that nodule activity is more important than nodule number in nitrogen fixation. It is possible to have a large amount of ineffective nodules which do not contribute to nodule functioning.

Table 2 also shows the influence of different levels of inorganic P on the fresh weights of nodules in Bambara groundnut.

Statistical analysis shows that there was no significant difference found among the mean fresh weight of nodules at various treatment levels compared to the control following the application of inorganic P.

In terms of mean fresh weight of nodules, the highest mean was found when 100 kgP/ha (1.47 g) was applied. At 40 kgP/ha, it was 1.46 g. At 20 kgP/ha, it was 1.23 g. At 60 kgP/ha, it was 1.15 g. At 80 kgP/ha, it was 1.13 g. At 70 kgP/ha, it was 1.12 g. At 50 kgP/ha, it was 1.03 g. These results were not significantly different ($P \le 0.05$) from the control and within treatments.

These results indicate that with the increase in the level of P applied, the mean fresh weight of nodules in Bambara groundnut also increased but not significantly from 1.00 g in the control to 1.47 g at the highest level of 100 kgP/ha. Though there was no steady increase, application of P boosted the growth and weight of fresh nodules. The lack of significance may be due to the high P level in the soil used.

Table 2 also shows the influence of difference levels of inorganic P on the fresh weight of shoot in Bambara groundnut.

Statistical analysis shows that there was no significant difference in the mean fresh weight of shoot at various treatment levels compared to the control following the application of inorganic P.

In terms of mean fresh weight of shoot, the highest mean was found when 40 kgP/ha (52.98 g) was applied. At 100 kgP/ha, it was 551.45 g. At 50 kgP/ha, it was 48.93 g. At 70 kgP/ha, it was 48.04 g. At 20 kgP/ha, it was 46.82 g. At 60 kgP/ha, it was 46.28 g. These results were not significantly different ($P \le 0.05$) from the control and within treatments.

These results indicate that with the increase in the level of P applied, the mean fresh weight of shoot in Bambara groundnut also increased although inconsistently from 45.60 g in the control to 52.98 g at 40 kgP/ha. Thereafter there was a decline. Though the decline was not steady, application of P above 40kgP/ha indicated that the plant shoot fresh weight was not boosted by higher P applied probably due to the initial high level of P in the soil used.

Table 2. Influence of different levels of inorganic P(kg/ha) on soil and in Bambara groundnut.

Different P Level	Total nitrogen in soil (g)	Tissue total nitrogen (g)	Number of nodules	Fresh weight of nodules (g)	Fresh weight of shoot (g)
P_{20}	0.07	3.94	92.8	1.23	46.98
P_{40}	0.43	4.05	94.8	1.46	52.98
P_{50}	0.12	4.25	88.0	1.03	48.93
P_{60}	0.10	4.63	96.2	1.15	46.28
P_{70}	0.10	4.80	101.3	1.12	48.04
P_{80}	0.09	4.91	87.2	1.13	46.82
P_{100}	0.07	5.18	94.5	1.47	51.45
P_0	0.09	3.78	86.7	1.00	45.60
Average	0.13	4.44	92.67	1.20	48.39
LSD	0.21ns	0.27*	8.9ns	0.47ns	7.39ns
CV (%)	12	11	5	14	5

* = significant; ns: not significant

Table 3 shows the influence of different levels of inorganic P on the mean fresh weight of root of Bambara groundnut.

Statistical analysis shows that there was no significant difference in the mean fresh weight of root of Bambara groundnut at various levels of treatment compared to the control following the application of inorganic P.

In terms of mean fresh weight of root, the highest mean was found when 40 kgP/ha (6.24 g) was applied. At 100 kgP/ha, it was 5.83 g. At 80 kgP/ha, it was 5.43 g. At 60 kgP/ha, it was 4.62 g. At 70 kgP/ha, it was 4.10 g. At 20 kgP/ha, it was 4.07 g. At 50 kgP/ha, it was 3.93 g. The increase in fresh weight of shoot followed a similar increase in fresh weight of root at 40 kgP/ha. These results were not significantly different ($P \le 0.05$) from the control and within treatments.

These results indicate that with the increase in the level of P applied, the mean fresh weight of root of Bambara groundnut also increased, from 3.90 g in the control to 6.24 g at 40 kgP/ha. Thereafter there was a decline. Though the decline was not steady, application of P above 40 kgP/ha signifies that the plant root fresh weight were not boosted by higher P applied probably due to the initial high level of P in the soil used.

Table 3 also shows the influence of different levels of inorganic P on the mean dry weight of nodules in Bambara groundnut.

Statistical analysis shows that there was no significant difference in the mean dry weight of nodules of Bambara groundnut at various levels of treatment compared to the control following the application of inorganic P.

In terms of mean dry weight of nodules, the highest mean was found when 70 kgP/ha (0.30 g) and 100 kgP/ha (0.30 g) was applied. At 20 kgP/ha, it was 0.27 g which was not different from 50 kgP/ha. At 40 kgP/ha, it was 0.24 g. At 60 kgP/ha, it was 0.23 g which was not different from 80 kgP/ha. These results were not significantly different (P≤ 0.05) from the control and within treatments.

These results indicate that with the increase in the level of P applied, the mean dry weight of nodules of Bambara groundnut also increased, from 0.22 g in the control to 0.30 g at 70 kgP/ha and at the highest level of 100 kgP/ha. Though there was no steady increase, application of P boosted the weight of dry nodules. The lack of significance might be due to the initial high P level in the soil used.

Table 3 also shows the influence of different levels of inorganic P in the mean dry weight of shoot in Bambara groundnut.

Statistical analysis shows that there was no significant difference in the mean dry weight of shoot of Bambara groundnut at various levels of treatment compared to the control following the application of inorganic P.

In terms of mean dry weight of shoot, the highest mean was found when 40 kgP/ha (15.24 g) was applied. At 100 kgP/ha, it was 12.73 g. At 60 kgP/ha, it was 12.28 g. At 80 kgP/ha, it was 11.52 g. At 20 kgP/ha, it was 10.73 g. At 70 kgP/ha, it was 10.70 g. These results were not significantly different (P≤ 0.05) from the control and within treatments.

These results indicate that with the increase in the level of applied P, the mean dry weight of shoot of Bambara groundnut also increased, from 10.52 g in the control to 15.24 g at 40 kgP/ha. Thereafter there was a decline. Though the decline was not steady, application of P above 40 kgP/ha indicated that the plant shoot dry weight was not boosted by higher P applied probably due to the initial high level of P in the soil used.

Table 3 also shows the influence of different levels of inorganic P in the mean dry weight of root in Bambara groundnut.

Statistical analysis shows that there was no significant difference in the mean dry weight of root of Bambara groundnut at various levels of treatment compared to the control following the application of inorganic P.

In terms of mean dry weight of root, the highest mean was found when 100 kgP/ha (1.47 g) was applied. At 40 kgP/ha, it was 1.28 g. At 80 kgP/ha, it was 1.12 g. At 60 kgP/ha, it was 1.08 g. At 70 kgP/ha, it was 1.06 g. At 50 kgP/ha, it was 1.00 g. At 20 kgP/ha, it was 0.98 g. These results were not significantly different (P≤ 0.05) from the control and within treatments.

These results indicate that with the increase in the level of P applied, the mean dry weight of root of Bambara groundnut also increased, from 0.95 g in the control to 1.47 g at the highest level of 100 kgP/ha. Though there was no steady increase, application of P boosted the weight of dry root. The lack of significance could be due to the initial high P level in the soil used.

Table 3 also shows the influence of different levels of inorganic P on the mean height of plant (cm).

Statistical analysis shows that there was no significant difference in the mean height of Bambara groundnut at various levels of treatment compared to the control following the application of inorganic P.

In terms of mean height, the highest mean height was found when 40 kgP/ha (22.38 cm) was applied. At 100 kgP/ha, it was 22.35 cm. At 20 kgP/ha, it was 22.22 cm. At 60 kgP/ha, it was 21.34 cm. At 50 kgP/ha, it was 20.83 cm. At 80 kgP/ha, it was 20.77 cm. At 70 kgP/ha, it was 20.75 cm. These results were not significantly different (P≤ 0.05) from the control and within treatments.

These results indicate that with the increase in the level of P applied, the mean height of Bambara groundnut also increased, from 20.09 cm in the control to 22.38 cm at 40 kgP/ha. Thereafter there was a decline. Though the decline was not steady, application of P above 40 kgP/ha indicated that the plant height was not boosted by higher P applied probably due to the initial high level of P in the soil used.

Table 3. Influence of different levels of inorganic P(kg/ha) in Bambara groundnut.

Different P Level	Fresh weight of root (g)	Dry weight of nodules (g)	Dry weight of shoot (g)	Dry weight of root (g)	Height (cm)
P_{20}	4.07	0.27	10.73	0.98	22.22
P_{40}	6.24	0.24	15.24	1.28	22.38
P_{50}	3.93	0.27	10.53	1.00	20.83
P_{60}	4.62	0.23	12.28	1.08	21.34
P_{70}	4.10	0.30	10.70	1.06	20.73
P_{80}	5.43	0.23	11.52	1.12	20.77
P_{100}	5.83	0.30	12.73	1.47	22.35
P_0	3.90	0.22	10.52	0.95	22.35
Average	4.77	0.25	11.78	1.12	21.34
LSD	2.36ns	0.08ns	4.73ns	0.53ns	1.60*
CV (%)	18	10	13	14	4

* = significant; ns: not significant

CONCLUSION

The experiment was conducted to evaluate the effect of different levels of P on nodulation and nitrogen fixation in Bambara groundnut.

The result showed that there was significant difference among the means of various treatment levels in the total nitrogen content (20 kgP/ha, 40 kgP/ha, 70 kgP/ha, 80 kgP/ha and control) and height (40 kgP/ha and control) of the plant.

In other parameters measured, the results showed that there was no significant difference among the means of various treatment levels and control. This could be due to the initial high concentration of available phosphorus in the soil used. Thus, further increase in the level of P above 100 kgP/ha could lead to P toxicity. In all parameters measured, the treatment was better than the control – except for total nitrogen of soil after harvest.

From this study, nitrogen fixation could benefit from application of phosphorus. However, it would appear that if the soil is already high in available P, the result might not be as dramatic as if the soil were deficient in available P. Before P application could be recommended in growing Bambara groundnut, proper soil test should be conducted to determine the level of available P in the soil.

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