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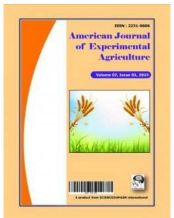

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# Contributions of *Rhizobium* Inoculants and Phosphorus Fertilizer to Biological Nitrogen Fixation, Growth and Grain Yield of Three Soybean Varieties on a Fluvic Luvisol

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***Authors' contributions***

*This work was carried out in collaboration among all the authors. Authors ALAA and AO designed the study, wrote the protocol and author ALAA wrote the first draft of the manuscript. Authors AO and RCA reviewed the experimental design while authors BDKA, AO and RCA reviewed the first draft of the manuscript. Authors ALAA and AO managed the analyses of the study, all the authors identified the plants and author ALAA performed the statistical analyses. Author BDKA critiqued and wrote the final manuscript. All authors read and approved of the final manuscript.*

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## ABSTRACT

An experiment to identify an efficient strategy to optimize biological nitrogen fixation (BNF) in three soybean varieties {Jenguma (TGx1448-2E), Anidaso and Quarshie (TGx1445-2E)} was conducted in the experimental field of the CSIR-Savanna Agricultural Research Institute, Nyankpala, Northern Region, Ghana. The experiment had a split-split plot design with three replications and interactions tested were inoculation rate (0, 50 and 100% inoculation) (main plot), soybean variety (sub-plot) and phosphorus rate (0, 22.5 and 45.0 kg $P_2O_5$/ha) (sub-sub plot) using maize as a reference crop. The soybean was sown at two seeds per hill at a spacing of 50 cm x 10 cm. The results showed

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that inoculating soybean with the commercial inoculant Legumefix (*Bradyrhizobium* strain 532c) had no effect on plant height, nodule number, nodule dry weight, shoot dry weight, pod number, grain yield and 100-seed weight. However, the soybean varieties showed significant differences in pod number, 100-seed weight and phosphorus uptake efficiency (PUE). The amount of $N_2$ fixed (measured by Total Nitrogen Difference method) ranged between 52.3-71.0 kg N/ha. Phosphorus applications of 22.5 and 45.0 kg $P_2O_5$/ha increased grain yield by 35.4 and 33.9%, respectively and also increased $N_2$ fixation by 49.39 and 69.82%, respectively over the unfertilized control. The interactions among these treatments did not significantly influence the parameters measured except PUE for which there were significant differences among the soybean varieties and the phosphorus rates. Inoculation did not therefore increase nodulation and BNF of the three soybean varieties but phosphorus application increased the growth and grain yield of the soybeans.

*Keywords: Inoculation rates; soybean varieties; phosphorus rates; BNF; grain yield.*

## 1. INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is a legume plant belonging to the botanical family Leguminosae. It is an economically important leguminous crop worldwide and also the most important legume in Ghana [1]. According to [2], soybean is more protein-rich than any of the common vegetable or legume food sources in Africa. It is a source of edible oil (20-25%) with 42-45% protein content [3]. Soybean is a promising pulse crop proposed for the alleviation of the acute shortage of protein and oil worldwide [4]. It used as a good source of unsaturated fatty acids, minerals (Ca and P) and vitamins A, B, C and D [1].

Despite these good characteristics, its growth and productivity is affected mainly by nitrogen even though this element is abundant element on the earth and about 78% of the earth's atmosphere is nitrogen gas. Despite the abundance of nitrogen in the atmosphere, plants are unable to use it directly because it is present in an inert form ($N_2$) and the nitrogen in the soil is lost through microbial denitrification, soil erosion, leaching, chemical volatilization, removal of nitrogen- containing crop residues from the land. Nitrogen is therefore the most limiting plant nutrient for crop production in West Africa [5]. Most legumes, however, through symbiosis with *Rhizobia* have the ability to reduce $N_2$ through biological nitrogen fixation (BNF) into a plant-usable form.

Currently, BNF contribution on majority of smallholder farms rarely exceeds 5 kg N/ha/year with nitrogen-fixing legumes in Ghana [6]. A measure of more than 240 kg N/ha of fixed $N_2$ in soybean in southern Africa on small holder farms with associated grain yield of more than 3.5 t/ha has been recorded [7]. This shows that the potential rates of BNF in soybean are not only limited by the efficiency of legume-*Rhizobium* symbiosis but environmental and management factors could also limit the efficiency. These factors may include temperature, rainfall, soil or fertilizer nutrients like nitrogen and phosphorus, soil pH, etc. Phosphorus is an essential ingredient for *Rhizobium* bacteria to carry out BNF processes. Inadequate P restricts root growth, the process of photosynthesis, translocation of sugars and other such functions which directly influence N fixation by legume plants.

The general objective of this study was therefore to identify efficient strategies that will optimize biological nitrogen fixation in soybean for increased crop yield. The specific objectives were: (i) to evaluate the effect of *Rhizobium* inoculation and P fertilizer application rate on nodulation and BNF of three soybean varieties. (ii) to investigate the effect of *Rhizobium* inoculation and P application on growth and yield of three soybean varieties. (iii) to determine the effect of *Rhizobium* inoculation and P application on P uptake efficiency of three soybean varieties.

## 2. MATERIALS AND METHODS

### 2.1 Study Area

The study was carried out on the experimental field of the Savanna Agricultural Research Institute at Nyankpala in the Northern Region of Ghana from June to December 2012. Nyankpala is located the interior Guinea Savanna agro-ecological zone of Ghana about 16 km west of Tamale and lies on latitude N 09º 24’ 15.9” and longitude W 01º 00’ 12.1’. The climate is relatively dry with a mono-modal rainfall pattern. Average ambient temperatures are high year round (about 28ºC) but the months of December and January are characterized by minimum temperatures that may fall to 13ºC at night while March and April may experience temperatures of 40ºC in the early afternoon. The mean annual rainfall of the area is 1000 mm with a total of 1022 mm. The soil at the trial site is a Fluvic luvisol which is classified as the Tingoli series and the vegetation type is a tree Savannah.

### 2.2 Soil Sampling, Preparation and Analysis

Soil samples were taken before the field was ploughed with a tractor. Five soil cores were taken at the depth of 0–15 cm from each replicate plot and bulked to obtain a composite samples Soil samples were air-dried and sieved through a 2-mm mesh before subjecting them to chemical and physical analyses using standard laboratory procedures. Particle size analysis was done by using the hydrometer method [8]. Fifty gram was weighed from the sampled soil and used in determining the particle size. Soil pH was measured in the supernatant suspension of 1 (10 g): 2.5 (25 ml) soils and water mixture using a pH meter. 0.5 g was weighed from sample soil and used in determining soil organic carbon. Soil organic carbon was determined by the method of [9]. Whilst total nitrogen of the soil was determined by the Kjeldahl method [10] and available phosphorus was determined by the Bray 1 method [11]. One gram each was weighed from sampled soil and used in determining the total nitrogen and available phosphorus. Cation Exchange Capacity (CEC) was determined by leaching the ten gram of sampled soil with neutral 1M ammonium acetate [12].

### 2.3 Soil Characteristics

The physical and chemical analyses revealed that the experimental site is loamy-sand in texture and neutral in reaction (pH 5.5-7.0) with a very low organic carbon content (<20 g/kg) (Table 1) Total nitrogen and exchangeable cations were less than 1 g/kg and 5 cmol$^{(+)}$/kg), respectively with effective cation exchange capacity and available P being less than 5 cmol$^{(+)}$/kg) and 10 mg/kg soil, respectively according to [13].

### 2.4 Experimental Design and Treatments

The experiment was designed in a split-split plot with three replications and treatments tested were inoculation rate (0, 50 and 100% inoculation) as the main plot, soybean variety {Jenguma (TGx-1448-2E), Anidaso, Quarshie (TGx-1445-2E)} as the sub-plot and phosphorus rate (0, 22.5 and 45 kg $P_2O_5$/ha) as the sub-sub plot. The inoculants was a commercial peat-based one of *Bradyrhizobium japonicum* strain 532c.The test soybeans are medium-maturing (95-110 days) with 45 days to 50% flowering and have a potential grain yield of 1.8-2.5 t/ha.

**Table 1. Some physical and chemical soil properties of the study area**

| Parameter | Test value |
|---|---|
| Sand (g/kg) | 855.00 |
| Silt (g/kg) | 83.00 |
| Clay (g/kg) | 62.00 |
| Texture | Loamy-sand |
| pH (1:2.5; soil:$H_2O$) | 6.70 |
| Organic carbon (g/kg) | 11.00 |
| Total N (g/kg) | 0.50 |
| Available P (mg/kg) | 2.20 |
| **Exchangeable cations (cmol$^{(+)}$/kg):** | |
| Ca | 1.50 |
| Mg | 0.70 |
| K | 0.08 |
| Na | 0.06 |
| Exchangeable acidity (cmol$^{(+)}$/kg) | 0.73 |
| Effective cation exchange capacity (cmol$^{(+)}$/kg) | 3.07 |

The plot size was 3 m x 4 m and spacing between plots and blocks were 1 m and 2 m, respectively. For the purpose of assessing BNF, a non-fixing reference crop (maize) was added to the treatment combinations. Phosphorus was applied as triple super phosphate to the test crops at the rate of either 22.5 or 45 kg $P_2O_5$/ha but not to the reference crop. However, muriate of potash (MoP) was applied to all experimental plots at 30 kg K/ha. The fertilizers were deposited in holes 5 cm away from the plants and covered with the soil at two weeks after sowing.

### 2.5 Seeds Inoculation

The peat based inoculants were added to the soybean seeds in a container after moistening the seeds. The inoculants and the seeds were mixed thoroughly until the seeds were adequately coated with the inoculants and allowed to air-dry on a sheet of canvas in the shade for a few minutes after which they were planted on the ridges. The treatment with 100% inoculation rate received 5 g of inoculants per kg of seed whilst the half recommended inoculation rate (50%) was done by mixing one kilogram of the seeds with 2.5 g of inoculants.

### 2.6 Data Collection

Plant height, shoot dry weight, nodule dry weight and nodule number per plant were recorded from ten representative plants at eight weeks after planting (8 WAP). Plant height was taken from the ground level to the apex of the plant with a graduated pole and the average was calculated for each plot. After the root systems of the 10 plants were cut and gently washed on a 2-mm mesh sieve under a jet of tap water, the nodules were detached, counted and oven-dried at 65ºC for 48 h. Pods on these ten plants were removed and counted to obtain the pod load (i.e. pod number/plant). The shoots of five plants sub-sampled from the ten plants harvested were also oven-dried at 65ºC for 48 h and the weights recorded.

After threshing the pods harvested in the harvest area of each treatment plot, the grains were sufficiently sun-dried on a concrete platform and weighed on an electronic balance. Hundred seeds from each treatment were randomly picked and weighed. This was replicated three times and the average 100-seed weight determined.

### 2.7 Estimation of $N_2$ Fixed

The technique used to estimate N fixation was the Total Nitrogen Difference (TND) method. This was done by comparing total nitrogen of the legume with that of a non-legume [14]. The amount of N fixed was calculated by subtracting total nitrogen of the reference crop (maize) from that of the legume (soybean), and the difference value is assumed as N derived by BNF ($N_2$ fixed).

Thus, $N_2$ fixed =

Total N in legume -Total N in reference crop

where Total N in plants =

$$\frac{\left(\text{Dry matter weight } \left(\frac{kg}{ha}\right) \times \text{ \% N in plants}\right)}{100}$$

Then shoot nitrogen content was analyzed using the Kjeldahl procedure [12].

### 2.8 Phosphorus Uptake Efficiency

Phosphorus uptake efficiency (PUE) was estimated using an equation by [15].

$$PUE = 100 \times \left[\text{P in fertilized plants - P in control plants}\left(\frac{kg}{ha}\right)\right] \Big/ \text{P applied}\left(\frac{\text{kg P2O5}}{ha}\right)$$

**Table 2. Effects of inoculation rate, variety and phosphorus rate on plant height and shoot dry weight of soybean**

| Treatment | Plant height (cm) | Shoot dry weight (kg/ha) |
|---|---|---|
| **Inoculation rate (%)** | | |
| 0 | 69.99 | 5604 |
| 50 | 69.79 | 4984 |
| 100 | 69.24 | 5089 |
| Pr (I) | 0.826 | 0.269 |
| Lsd (0.05) | 3.26 | 956.7 |
| **Variety** | | |
| Jenguma | 69.34 | 5198 |
| Quarshie | 69.13 | 5647 |
| Anidaso | 70.55 | 4833 |
| Pr (V) | 0.443 | 0.595 |
| Lsd (0.05) | 2.53 | 1704.8 |
| **Phosphorus rate (kg $P_2O_5$/ha)** | | |
| 0 | 67.38 | 3331 |
| 22.5 | 69.29 | 5171 |
| 45 | 72.35 | 7176 |
| Pr (P) | <0.001 | <0.001 |
| Lsd (0.05) | 1.05 | 646.5 |
| Pr (I x V) | 0.950 | 0.762 |
| Pr (I x P) | 0.851 | 0.835 |
| Pr (V x P) | 0.304 | 0.184 |
| Pr (V x I x P) | 0.371 | 0.383 |
| CV (%) | 6.94 | 22.4 |

## 2.9 Statistical Analysis

All data collected were subjected to statistical analysis using Genstat Discovery Edition 10. Nodule count was transformed before the analysis. Analysis of variance (ANOVA) was done to determine differences in means among treatments. All treatment means were compared using the Least Significant Difference (LSD) at 5% level of significance.

# 3. RESULTS AND DISCUSSION

## 3.1 Plant Height

Application of inoculants, soybean variety and their interactions had no significant (P>0.05) effect on plant height at 8 WAP unlike phosphorus application which significantly (p<0.05) affected it (Table 2). Phosphorus applications of 22.5 and 45 kg $P_2O_5$/ha significantly increase plant height by 2.76% and 6.87%, respectively over the control treatment (0 kg $P_2O_5$/ha).This finding is in agreement with reports of [16] that phosphorus application of 60, 120 and 180 mg P/kg significantly influenced soybean height under controlled environments.

## 3.2 Shoot Dry Weight

Shoot dry weight (biomass) at mid-flowering was not significantly influenced by inoculation rate, variety and their interactions (Table 2). Similar shoot dry weights produced by the test soybean varieties at mid-flowering under similar conditions of growth shows that they have equal growth and biomass production potentials as stated by [17]. Contrarily, phosphorus application of 22.5 and 45 kg $P_2O_5$/ha significantly enhanced biomass production in the soybean crops by 35.6 and 53.6%, respectively as compared to the no P treatment with the 45 kg $P_2O_5$/ha rate yielding higher biomass than the lower P rate (Table 2). This result is in agreement with [18] who concluded that omission of P from optimum nutrition of soybean dramatically reduced shoot dry matter yield of soybean.

## 3.3 Nodule Number and Dry Weight

There was no significant (*P*>0.05) effect on nodule number and dry weight of soybean due to application of inoculants, variety and their interactions (Table 3). The lack of nodulation response to *Rhizobium* inoculation might suggest that N was not a limiting nutrient at this site.

**Table 3. Effects of inoculation rate, variety and phosphorus rate on nodule number and dry weight of soybean**

| Treatment | Nodule number (no/plant) | Nodule dry weight (mg/plant) |
|---|---|---|
| **Inoculation rate (%)** | | |
| 0 | 25.75 | 2233 |
| 50 | 24.87 | 1904 |
| 100 | 24.97 | 2049 |
| Pr (I) | 0.971 | 0.67 |
| Lsd (0.05) | 10.96 | 978.2 |
| **Variety** | | |
| Jenguma | 24.08 | 2237 |
| Quarshie | 30.49 | 2289 |
| Anidaso | 21.03 | 1659 |
| Pr (V) | 0.095 | 0.109 |
| Lsd (0.05) | 8.76 | 658.4 |
| **Phosphorus rate (kg $P_2O_5$/ha)** | | |
| 0 | 17.24 | 686 |
| 22.5 | 24.23 | 2304 |
| 45 | 34.13 | 3196 |
| Pr (P) | <.001 | <.001 |
| Lsd (0.05) | 2.67 | 1186.4 |
| Pr (I x V) | 0.797 | 0.944 |
| Pr (I x P) | 0.895 | 0.417 |
| Pr (V x P) | 0.860 | 0.451 |
| Pr (V x I x P) | 0.549 | 0.250 |
| CV (%) | 19.00 | 23.4 |

Other nutrients such low phosphorus and molybdenum could affect inoculation response and also, native *Rhizobia* can prevent the *Rhizobia* introduced in the inoculants from forming nodules on the crop. It could also be possible that in this study the native *Rhizobian* might have also fixed as much nitrogen as the plant needs making inoculation unnecessary. Contrarily, significant positive effects of high rates of inoculation (high numbers of rhizobia per seed) have been demonstrated by [19] and [20]. With the application of P, however, there was a significant ($P<0.05$) enhancement in nodulation of soybean and the degree of enhancement was dependent on the rate of P applied (Table 3). Phosphorus application of 22.5 and 45 kg $P_2O_5$/ha significantly increase number of nodules by 11.85 and 21.71%, respectively over the control treatment. This significant positive effect of phosphorus on nodulation underlines the influence phosphorus has on nodule development through its basic functions as an energy source [23].

## 4. YIELD AND YIELD COMPONENTS

### 4.1 Pod Number

Inoculation rate and the interactions had no significant ($P>0.05$) effect on pod number though inoculated treatments (50 and 100% inoculation) apparently had more pods than non-inoculated ones whereas phosphorus rate and varietal differences significantly ($p<0.05$) affected the number of pods (Table 4). Application of 22.5 and 45 kg $P_2O_5$/ ha increased pod number by 33.39 and 47.87%, respectively over the control treatment and this result was supported by the findings of [21] that higher numbers of pod were produced when higher doses of phosphorus were applied.

The observation with inoculation contradicts the findings of [22] that seed inoculation of soybean produced more pods per plant than un-inoculated control. Bhuiyan et al. [24] and Bouquet [25] also reported that pod number of mung bean and soybean were significantly increased by inoculating with *Bradyrhizobium.* The variety Quarshie produced mean pod number of 66.1 which was statistically ($P<0.05$) higher than the mean pod number of Anidaso but statistically similar to that of Jenguma. Genetic factors of the soybean varieties may have contributed to the significant differences in their pod loads which may be affirmed by the assertion of [26] that genotype selection is one of the most important factors for increasing pod yield in soybean.

### 4.2 Grain Yield and Grain Size

Inoculant had no significant ($P>0.05$) influence on both grain yield and seed size (100-seed weight) (Table 4). The lack of grain yield response to inoculation can be attributed to the fact that inoculation did not increase nodulation (Table 3) and $N_2$ fixation (Table 5) and this may indicate an abundance of effective native soybean rhizobia at this location. Other factors may include cultivar and strain interaction and drought. Chemining et al. [27] reported that drought affects symbiosis between host and rhizobia and this influences Rhizobial survival in the soil, the host or the process of nodulation and grain yield. Lack of significant effect on yield improvement by inoculation has also been reported by other workers [28,29]. Varietal differences were observed to be significant ($p<0.05$) for seed size only (Table 4) and the seeds of inoculated Jenguma were statistically bigger (12.57 g/100 seeds) than the seed size (10.8 g/100 seeds) of Anidaso but statistically similar to that of Quarshie (12.6 g/100 seeds). Seed weight has been noted to affect seedling vigour and it was reported that genotypes with heavier seeds produced more vigorous seedlings and the relationship between seed weight and seedling vigor was more pronounced in the earlier growth stage [30]. Similar to this result, Solomon et al. [30] reported significant difference ($p\leq0.01$) in 100-seed weight by the main effect of variety. Phosphorus rate significantly ($p<0.05$) increased both grain yield and 100-seed weight (Table 4). Phosphorus applications at 22.5 $P_2O_5$ /ha and 45 kg $P_2O_5$/ha induced significantly similar responses with respect to grain yield and seed size but both were significantly higher than that of the control (0 kg $P_2O_5$/ha). Soybean has a relatively high requirement for phosphorus and yield and seed quality can be enhanced by phosphorus fertilizer in soils testing low in phosphorus [31]. Malik et al. [23] Also reported that seed yield of soybean increased with phosphorus fertilizer applications of 20, 40 and 60 kg $P_2O_5$/ha.

### 4.3 $N_2$ Fixation

The amount of $N_2$ fixed in soybean was not significantly ($p>0.05$) affected by inoculation, variety and the interactions (Table 5). The failure of inoculation to enhance the amount of $N_2$ fixed might be due to the inability of the inoculation to increase nodulation in soybean though [18] has reported of an increased $N_2$ fixation in soybean due to increased nodulation. This observation is contrary to the report that the amount of $N_2$ fixed in *Phaseolus vulgaris* L. was increased significantly with *Rhizobium* inoculation in all organs (roots, shoots, pods and whole plant) both in glasshouse and field experiments compared with the un-inoculated control treatment [32].

**Table 4. Effects of inoculation rate, variety and phosphorus rate on pod number, grain yield and 100-seed weight of soybean**

| Treatment | Pod number (No/plant) | Grain yield (kg/ha) | 100-seed weight (g) |
|---|---|---|---|
| **Inoculation rate (%)** | | | |
| 0 | 55.6 | 1545 | 12.1 |
| 50 | 57.9 | 1646 | 11.8 |
| 100 | 59.3 | 1815 | 12.1 |
| P(I) | 0.31 | 0.26 | 0.39 |
| Lsd (0.05) | 5.83 | 385.60 | 0.13 |
| **Variety** | | | |
| Jenguma | 60.5 | 1881 | 12.7 |
| Quarshie | 66.1 | 1560 | 12.6 |
| Anidaso | 46.2 | 1566 | 10.8 |
| P (I) | 0.018 | 0.078 | <.001 |
| Lsd (0.05) | 13.3 | 318.10 | 0.58 |
| **Phosphorus rate (kg $P_2O_5$/ha)** | | | |
| 0 | 39.1 | 1233 | 11.5 |
| 22.5 | 58.7 | 1909 | 12.2 |
| 45 | 75.0 | 1864 | 12.36 |
| P (P) | <.001 | <.001 | 0.002 |
| Lsd (0.05) | 7.32 | 258.80 | 0.49 |
| P (I x V) | 0.874 | 0.265 | 0.993 |
| P (I x P) | 0.916 | 0.901 | 0.195 |
| P (V x P) | 0.337 | 0.267 | 0.653 |
| P (I x V x P) | 0.595 | 0.402 | 0.908 |
| CV (%) | 23.0 | 28.10 | 7.40 |

**Table 5. Effects of inoculation rate, variety and phosphorus rate on Amount of $N_2$ fixed and Phosphorus uptake efficiency of soybean**

| Treatment | Amount of $N_2$ fixed (kg/ha) | P uptake efficiency (%) |
|---|---|---|
| **Inoculation rate (%)** | | |
| 0 | 59.40 | 16.67 |
| 50 | 59.40 | 21.11 |
| 100 | 63.60 | 17.78 |
| P(I) | 0.132 | 0.503 |
| Lsd (0.05) | 5.08 | 10.02 |
| **Variety** | | |
| Jenguma | 59.10 | 23.70 |
| Quarshie | 71.00 | 13.33 |
| Anidaso | 52.33 | 18.52 |
| P (I) | 0.228 | 0.003 |
| Lsd (0.05) | 22.58 | 5.04 |
| **Phosphorus rate (kg $P_2O_5$/ha)** | | |
| 0 | 29.00 | ----- |
| 22.5 | 57.30 | 33.33 |
| 45 | 96.10 | 22.22 |
| P(P) | <0.001 | <0.001 |
| Lsd (0.05) | 9.74 | 2.74 |
| P(I x V) | 0.697 | 0.537 |
| P(I x P) | 0.711 | 0.202 |
| P(V x P) | 0.238 | <0.001 |
| P(I x V x P) | 0.919 | 0.697 |
| CV (%) | 29.00 | 26.80 |

The non-significant difference of variety on the amount of N2 fixed supports the facts that soybean maturity date affects the amount of $N_2$ fixed in soybean. $N_2$ fixation has been reported to increase with increasing crop duration [33] because longer growth duration allows for a longer period of $N_2$-fixation in the nodules. Increased crop duration in the field means a longer period of nodule activity. The soybean varieties used in this study have the same maturity period (they are medium-maturing varieties). This might explain why the difference in the amount of $N_2$ fixed was not significant among the three varieties. However, the amount of $N_2$ fixed at the experimental site (52.1 to 71 kg N / ha) in the soybean varieties studied was within the range of 15-162 kg N/ha estimated for soybean by [34] and [35] in the tropics. This is higher than the 41-50 kg N / ha reported by [36] but lower than the 61-109 kg N/ha obtained by [37]. Unlike with the varieties, phosphorus application significantly enhanced $N_2$ fixation at both rates of 22.5 and 45 kg $P_2O_5$ / ha resulting in increases of 49.39 and 71.90% of fixed nitrogen, respectively over the control (Table 5).This indicates that P deficiency which limits plant growth can also seriously limit symbiotic $N_2$ fixation as the latter has been noted to have a higher P requirement for optimal functioning than either plant growth or nitrate assimilation [37].

### 4.4 Phosphorus Uptake Efficiency

Whereas application of the commercial inoculants had no significant effect on Phosphorus uptake efficiency (PUE), remarkable differences in PUE were observed among the three soybean varieties used as well as the P levels applied (Table 5). Jenguma was the most efficient in taking up the fertilizer P applied followed by Anidaso and Quarshie. The differences in the PUEs of the test legume varieties may suggest that differences in PUE could occur among plant species or genotypes due to differences in the abilities of their root systems to acquire P from the soil and accumulate it in the shoots [38]. Soybean varieties are known to differ in their ability to grow under low-P conditions and the more P-efficient varieties may have internal and/or external mechanisms that allow greater soil P extraction and grain yield [39]. In this work, the higher grain yield of Jenguma compared to the other two varieties (Table 4) can be attributed to its significantly higher PUE (Table 5). This genetic PUE superiority of Jeguma may, however, be adversely affected if more P than the optimum required by the variety is applied since the higher rate of P in this study led to a significant reduction in PUE (Table 5). The lower PUE observed with the higher P application rate in this investigation is similar to the findings of [16] who reported that P uptake efficiency decreased with the rate of applied P in un-inoculated plots but increased in inoculated plots at NIFA.

Phosphorus uptake efficiency was significantly affected by the interaction of variety and phosphorus rate (Fig. 1).

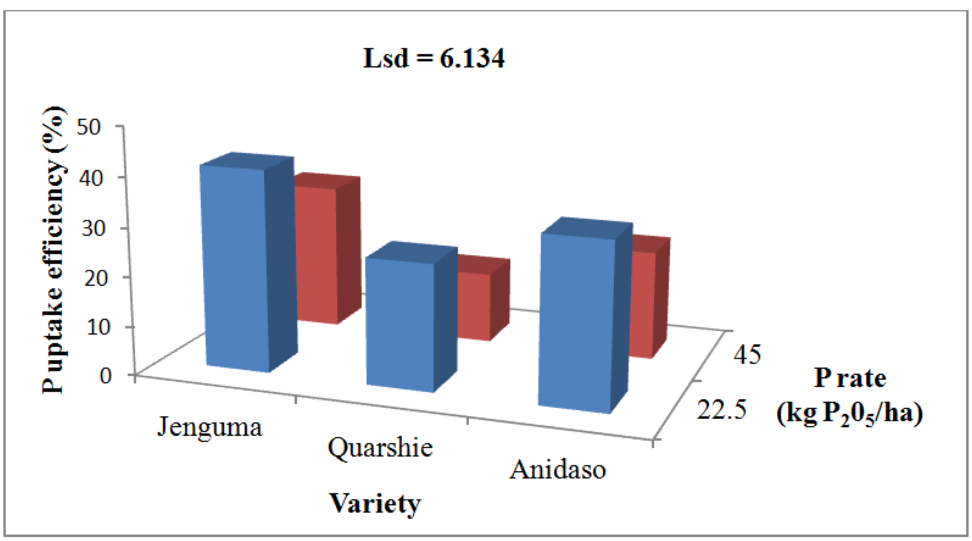


**Fig. 1. Interaction effect of variety and phosphorus application rate on Phosphorus uptake efficiency of soybean**

Jenguma had statistically higher mean phosphorus uptake efficiency (41.11%) than Quarshie and Anidaso when phosphorus was applied. Among the interactions tested, the application of P and variety proved to be the best combination with respect to phosphorus uptake efficiency. This observation contradicts the work [40] who found no significant interaction of genotype and phosphorus (G ∗P) on P uptake efficiency among Moroccan faba bean varieties.

## 5. CONCLUSION

In this study, inoculation of soybean with the commercial inoculants Legume fix at the rates of 2.5 and 5 g/kg seed had no significant effect on plant height, nodulation, shoot dry weight, pod number, pod yield, grain yield, grain size, P uptake efficiency and $N_2$ fixation. The combined application of P fertilizer and inoculation also had no significant effect on nodulation and BNF of the test soybean varieties. Inoculation of soybean with Legume fix on Fluvicluvisol therefore did not favour nodulation and BNF of the three soybean varieties. The soybean varieties were similar in height, nodule formation and development, biomass and grain yields and $N_2$ fixationbut differed significantly with respect to pod load, seed size and phosphorus uptake efficiencyin the order Jenguma> Quarshie> Anidaso. The application of phosphorus fertilizer, however, significantly increased all the parameters measured but it was observed that supplying soybean with 45 kg $P_2O_5$/ha increased growth and grain yield more than applying 22.5 kg $P_2O_5$/ha though the latter was more efficiently used by the crop. It is therefore concluded that P application increases growth and yield of the three soybean varieties and care should be taken to apply the P in the right amount.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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