PHOSPHORUS APPLICATION AND RHIZOBIA INOCULATION ON GROWTH AND YIELD OF SOYBEAN *(Glycine max L. Merrill)*

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ABSTRACT

An experiment was conducted in the major and minor cropping seasons of 2012 and 2013 under field conditions at Bolgatanga Polytechnic, to study the effect of phosphorus fertilizer and Rhizobia inoculation on growth and yield of soybean using randomized complete block design in three replications. The treatments studied were: Soybean + phosphorus fertilizer + Rhizobia inoculation (+P/+I), Soybean + phosphorus fertilizer only (+P/-I), Soybean inoculated with Rhizobia (-P/+I) and the control-Soybean only (-P/-I). Results indicated that Phosphorus fertilizer application is required for shoot growth, pod and seed yield. Nodulation and root growth were significantly increased by Phosphorus + Rhizobia inoculation (+P/+I), but P fertilizer only did not enhance root growth. Dry matter accumulation was highest between onset of flowering and Podding. Grain yield was again highest for Rhizobia inoculation plus Phosphorus fertilizer (+P/+I) and Phosphorus fertilizer only (+P/-I) recording 7.61 t/ha and 7.30 t/ha respectively whiles Rhizobia inoculation only (-P/+I) and the control (-P/-I) produced the lowest grain yield (4.41 t/ha) and (3.80 t/ha) respectively.

Keywords: *Soybean, inoculation, nodulation, phosphorus, rhizobia, protein energy malnutrition, soil fertility improvement*

INTRODUCTION

Soybeans *(Glycine max L. merr*) is an ancient leguminous crop and originated from China. About 70% of the world`s soybean is produced in the USA and China being the second largest producer. In terms of production, consumption and trade, soybean is the world most important grain legume (Tweneboah, 2000).

Soybean was initially introduced to Ghana in 1909 for export and for animal feed but failed due to poor understanding of its cultivation and utilization; it had been tried a few more times in 1930s, 1950s, 1970s and the 1990s but was unsuccessful due to unsuitable rainfall pattern, soil type and technique of cultivation) (Woomer, 2010).

Soybeans have many industrial uses and importance for human use. The beans has an oil content of about 20%, protein content of 40-45% and carbohydrate of 30 % and total sugar of about 10% (Haas et al., 2006). The bean is also a good source of calcium, phosphorus, copper, potassium, magnesium and thiamine. Van Gerpen and Krahl (2005); Haas *et al*. (2006) stated that soybean oils can be converted to biodiesel and glycerol. In the US, the major biofuel for transportation is soybean biodiesel, which displaces petroleum and diesel (Tweneboah, 2000).

Soybean has a great potential for the alleviation of Protein Energy Malnutrition (PEM) in Ghana because animal sources of proteins are scarce and expensive. In Ghana, soybeans is used in the preparation of soy paste, soy milk, soy custard, soy kebab, soy cheese, soy palaver sauce and many others.

Soybean is produced by small holder farmers in Ghana, under rain fed conditions. Under these subsistence methods, soybean provided averagely yield of 0.8 metric ton per hectare. FAO estimated that Ghana imports 2700 gallons of soybean oil. There is also limited supply of soybean feed for the poultry industry resulting in major constraints in the growth of the industry. This has resulted in significant growth of import of poultry and other protein sources for consumption (MIDA, 2013).

Poor soils has become a problem as a result of poor land management which leads to continuous loss of soil nutrients through the activities of soil erosion, leaching and crop removal. The resultant affected in the decline of crop yield. Ohyama *et al* (2013) stated that soybean seed yield can reach 4.6 t/ha with well managed fields under good climatic and soil conditions. The highest yield of soybean was recorded in Japan at 7.8 t/ha (Ohyama *et al*., 2013). Recently, an amazing high soybean seed yield over 10 t/ha was recorded in 2008 and 2010 by a farmer in Missouri, USA. Therefore the potential productivity of soybean should be higher than we have thought (Ohyama *et al*., 2013).

In view of the above, measures must be taken to improve BNF because when nitrogen is lost from soils; leguminous plants however, are not able to convert atmospheric N into absorbable forms of NH4+ and NO_3- before used by plants.

Natural reserves of soil N are normally low in many African soils and again phosphorus fertilizers which are important elements necessary for plants' growth are also lacking (Hardarson *et al*. 1987). Since the crop has immeasurable potential in Africa at large and Ghana in particular, therefore, the study was conducted with the objectives to study the effects of P fertilizer application on nodulation, shoot growth and yield; to evaluate the effects of Rhizobia inoculation on nodulation and yield.

MATERIAL AND METHOD

The study was conducted in the major and minor cropping seasons of 2012 and repeated in 2013, at the experimental field of the Bolgatanga Polytechnic which resides in Sumbrungu, located 7 km away from Navrongo. It shares boundaries with Kasssena-Nankana district at the north, Bongo district at the East, south to Bolgatanga Municipality and West to Sirigu and lies between latitude 10°45' North and longitude 0°51' West of Ghana**.** The experiment was laid in a Randomized Complete Block Design (RCBD) with three replications.

The land was slashed, ploughed and harrowed, and then finally raked, lined and pegged before planting at the appropriate spacing and seeds were planted in rows on flat field; spaced 5cm x 75cm apart, 3 seeds were planted per hill and were not thinned. Each plot in a block measured 3m x 3m and in each block there were 4 plots. The distance between the blocks was 1 meter making a total of 12 plots.

TREATMENTS: A - Control plot which is planted to soybean only (-P/-I). B - Plot planted to soybean seed which is inoculated (-P/+I). C - Plot planted to soybean and Triple Superphosphate (TSP) is applied and inoculated (+P/+I)**.** D - Plot planted to soybean and TSP is applied (+P/-I)

SOYBEAN INOCULATION WITH RHIZOBIA

One kilogram of soybean seed was weighed and placed in a rubber container and three spoonful of sugar solution was added as an adhesive and ten gram of inoculants was also weighed and added to the soybean seeds. It was then stirred with a spoon until the seeds were evenly coated with the Rhizobia. After coating, the seeds were spread onto a clean surface and air dried under a shade for about an hour. The inoculated air dried seeds were then planted, three seeds per stand.

FERTILIZER APPLICATION

Triple Superphosphate (TSP) fertilizer was applied after two weeks at a rate of 139.4 g per plot in a band, 10cm away from the planting line in a 2cm deep trench and covered with moist soil after application. The application was done after seedlings developed ten to eleven leaves to enable effective use of the fertilizer.

WEED AND PESTS MANAGEMENT

All plots were weeded by hand and using hoe. The Insecticide, Cyperdicot (Cypermethrin); SPCT as an active ingredient, was used, at a recommended rate of 25ml per half knapsack sprayer, to control insects, particularly at the flowering stage.

DATA COLLECTION

Growth Parameters measured included plant height, number of leaves per plant, number of nodules per plant, leaf dry weight per plant**,** stem dry weight, roots dry weight and nodule dry weight.

Yield Parameters measured also included number of pods per plant, number of pods per meter square, number of plants per meter square, number of seeds per pods, 100 seeds weight and mean seed weight.

DATA ANALYSIS

Averages, range, standard deviation and coefficient of variation were computed for the measurement data. Quantitative data was subjected to analysis of variance (ANOVA) using GenStat discovery edition 4.0 (GenStat 5 Committee, 2000). Means were separated by least significant difference at 5%.

Table 1: Average Climatic data for 2012 and 2013 (Upper East Region)

Months	Average Temperature (^{0}c)	Total Rainfall (mm)	Sunshine / hrs	Relative Humidity (%)
January	27.5	0.0	9.5	23.0
February	30.7	0.9	7.9	24.3
March	33.1	0.0	6.5	24.8
April	31.8	99.9	6.7	54.0
May	29.1	154.4	7.4	70.8
June	28.9	79.3	8.1	69.0
July	27.1	241.9	6.3	78.5
August	26.7	128.8	6.8	80.3
September	26.9	235.1	5.8	80.0
October	28.5	47.7	7.7	71.5
November	30.1	0.0	9.9	50.3
December	28.6	0.0	9.5	27.5

Source: Navrongo Meteorological Station, year 2012 and 2013

RESULTS AND DISCUSSION

Table 2: Chemical Properties of Soil at the Experimental Site

Chemical Composition	(0-15 cm) depth
pH	6.1
*Available P (mg/kg)	2.3
Organic Carbon (%)	0.36
Organic matter (%)	0.62
Total N (%)	0.03
Exchangeable Bases (Cmol/kg/Me/100g)	
Ca	3.2
K	0.1
Mg	1.34
Na	0.04

**Available P (mg/kg), from 20.0 mg/kg and above is = Adequate to high*

EMERGENCE AND FLOWERING

Emergence was complete within 10 days after planting; all the treatments recorded 50% emergence at the fourth day. The Rhizobia inoculated treatment significantly increased the number of seedlings that emerged, but there was no significant difference between the rest. This may be attributed to Rhizobia effect which enhanced emergence and germination. All the treatments took 47days to attain 50% flowering. Nevertheless, rhizobia inoculation only recorded the highest number of flowers of 281 followed by the other treatments and this has a relationship with emergence (Table 3).

Table 3: Effect of phosphorus and Rhizobia inoculation on emergence and flowering of soybean

Treatments	50 % Seedling Emergence (4 days)	50% flowering (47 days)
(-P/-I) No Inoculation and No P fertilizer	78.1	238

(-P/+I) Rhizobia inoculation only	109.3	281
(+P/+I) Rhizobia Inoculation + P fertilizer	80.3	264
(+P/-I) P fertilizer Only	76.0	233
Lsd (5%)	**28.7**	**99.1**
CV (%)	**19.4**	**19.1**

NS = Not Significant, P > 0.05

LEAF DRY WEIGHT

In all the treatments, leaf dry weight increased to a peak 56 DAP and started to decline after it owing to onset of senescence (Figure 1). Phosphorus plus Rhizobia inoculation recorded the highest leaf dry weight of 50.7 g/plant and was followed by phosphorus application only which recorded a weight of 36.4 g/plant. Rhizobia inoculation alone also took the same trend, recording a leaf dry weight of 29.5 g/plant. The control (-P/-I) recorded the lowest leaf weight of 20.70 g/plt. The results showed that the phosphorus application plus Rhizobia inoculation produced the greatest leaf dry matter. Field observations also showed that phosphorus and rhizobia inoculation enhanced dark green colour of the leaves. It appeared, therefore that rhizobia inoculation enhances P uptake leading to the development of a larger photosynthetic area. Phosphorus application has been reported to increase shoot growth or leaf growth of soybeans and other leguminous crops (Othman, 1991; Cadish *et al*., 1992).

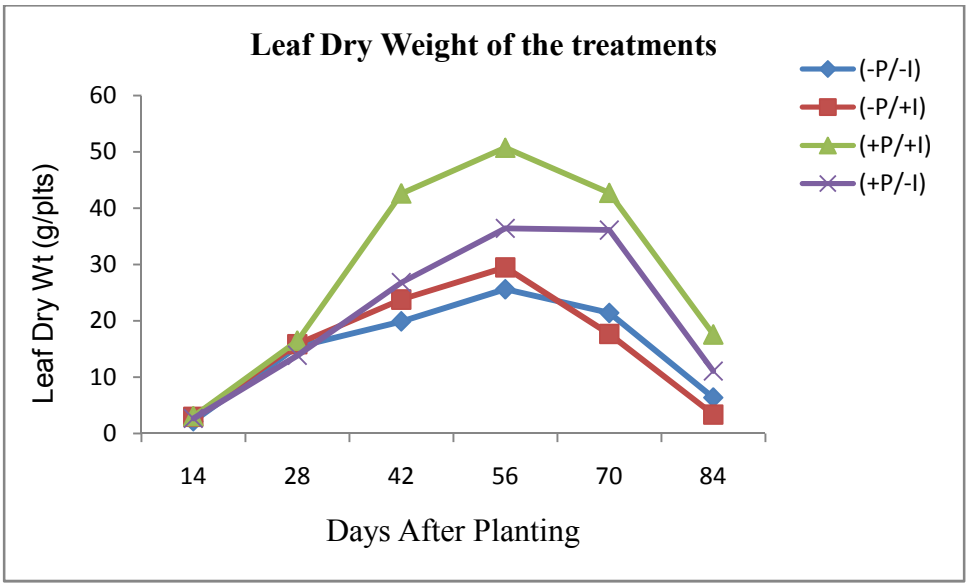

Figure 1: The effect of phosphorus and Rhizobia inoculation on leaf dry weight of soybean.

STEM GROWTH

Stem dry weight also followed the same trend as leaf growth but the peak stem growth was recorded at 70 DAP and at 84 DAP for all the treatments except Rhizobia inoculation only which peaked 56 DAP and remained sturdy until 70 DAP and dropped (Figure 2). Initial stems growths experienced almost the same dry matter accumulation until 28 DAP and then all the treatment begun to split as a result of stem dry weight differences. Phosphorus plus Rhizobia inoculation (+P/+I) recorded the highest dry matter residues to point at 56 DAP (Figure 2). On 56 DAP, there was stability in dry matter accumulation to 70 DAP which marked the end of vegetative growth and commencement of flowering and pods development, therefore dry matter accumulation was more rapid between 28 and 56 DAP, hence the steady weight drop because soybean stems loss their green colour as the crop matures or senescence sets.

The acute deficiency of phosphorus can prevent nodulation in legumes. Phosphorus is required for nodule metabolism and tends to be concentrated in the nodules when the plant is deficient in these nutrients (Cassman et al., 1981).

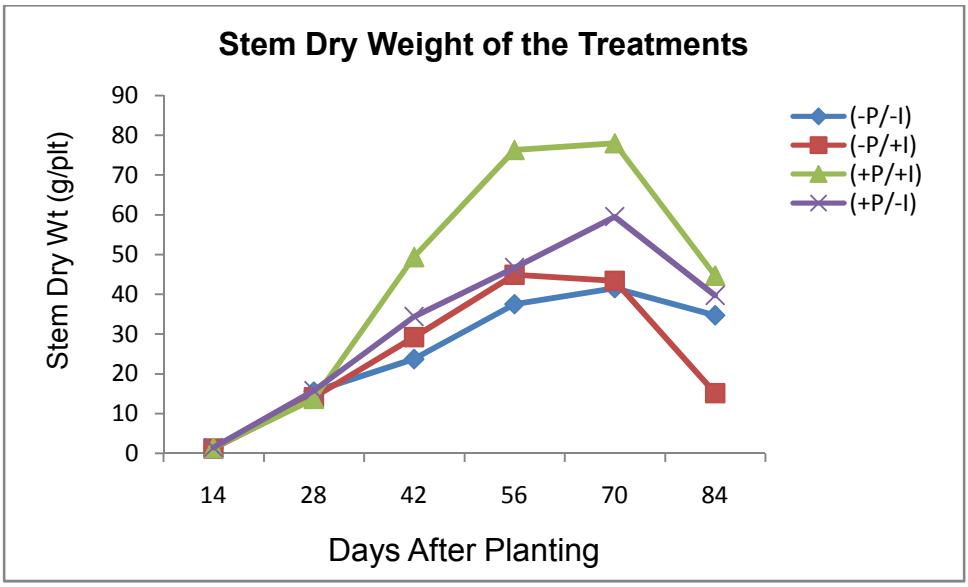

Figure 2: Effect of phosphorus and Rhizobia inoculation on stem growth of soybeans.

ROOT GROWTH

Comparing root growth of the treatments, the Rhizobia plus phosphorus (+P/+I) treatment again produced the highest root dry matter of 69.3 g/plt at 56 DAP. The Rhizobia inoculated treatment alone recorded the next higher value of 61.7 g/plt but at 70 DAP when the (+P/+I) treatment was gradually dropped (Figure 3). The growth may be attributed to increase in cell dry matter accumulation with well-developed tap root during growth of the plant. The control was the next highest, whiles phosphorus application only increased root growth up to 42 DAP and begun to drop. Rhizobia inoculation might have lead to increased root growth of the plant. This finding is in line with Okagun *et al*., (2004) who observed a significant increase in root dry matter yield of inoculated improved soybean over uninoculated improved variety.

The drop in root growth at 84 DAP may be due to senescence. Plant roots get dried in the soil and lose its weight as the crop matures. Therefore, as the plants mature, the more it loses dry matter leading to significant drop in dry weight at different growth stages leading to maturity and senescence.

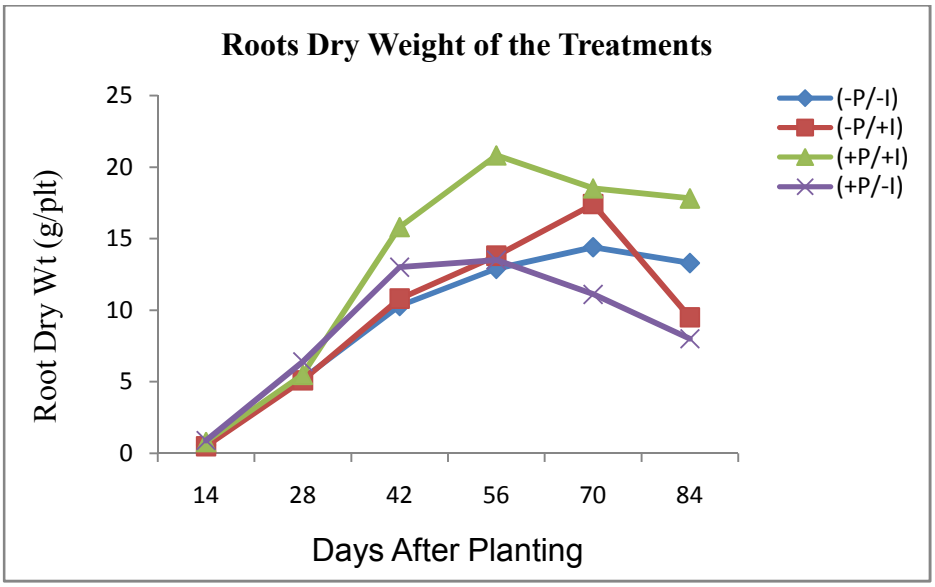

Figure 3: The effect of phosphorus and Rhizobia inoculation on root growth of soybean.

NUMBER OF NODULES

From Figure 4, on 14 days-after-planting, treatment (+P/-I) recorded a slightly sharp increase in number of nodules produced per plant. This may be due to bacteria inoculation having been less effective in nodulating at the second week of growth.

On the other hand the control treatment (-P/-I) recorded the lowest number of nodules showing that the soil did not contain much bacteria to produce more nodules at this stage, but the application of P increased nodulation. On 28 days after planting, phosphorus plus rhizobia inoculation (+P/+I) treatments recorded a tremendous increase in number of nodules produced. Higher values were recorded again for the same treatments at 42 and 56 days after planting (Figure 4), indicating effective utilization of the bacteria and fertilizer.

On 70 days after planting, there was a sharp drop in the number of nodules recorded as a result of the use of more energy to convert protein into food reserves that the plants required, therefore, nodules number degenerated or disappeared indicating the point of maturity. This agrees with the research findings of Fournier *et al*, (2008).

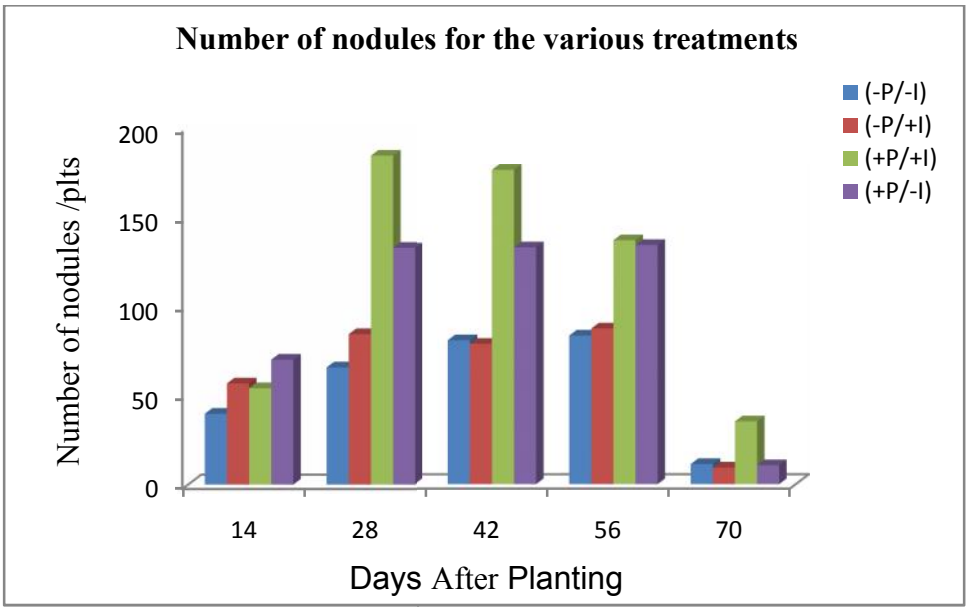

Figure 4: The effect of phosphorus and Rhizobia inoculation on of nodule number of soybean.

NODULE GROWTH

At different growth stages, there was no significant increase in Rhizobia effect for the treatment (-P/-I) and (-P/+I) on nodule growth at 14 DAP, but treatments (+P/+I) and (+P/-I) had attained a slight increase of (0.3g/plt) and (0.29g/plt) respectively (Figure 5). The increase in growth of nodules was equivalent to increased nodule dry weight of the plants. Therefore, on 28 DAP, (+P/+I) had a significant increase in dry weight of 1.3g/plt and treatment (+P/-I) with 0.8g/plt. Nodule growth therefore increased between the pre-flowering and early pod-forming stages. It was necessary to analyse the origin of nodules on the various parts of the root system independently in order to establish clearly the effectiveness of the Rhizobia inoculation in terms of nodulation.

On 28 DAP, treatment (+P/+I) recorded an increase in dry matter residue whiles treatment (+P/-I) recorded the second highest dry weight of nodules produced (Figure 5). Treatments (-P/-I) and (-P/+I) obtained the lowest of 56 DAP, but as the trend continued, there was a slow dropped in weight to about 1.0 g/plts for (+P/+I) and 0.83 weight g/plt for (+P/-I). This may be as a result of plant senescence or ageing because much photosynthetic product is absorbed to forming pods.

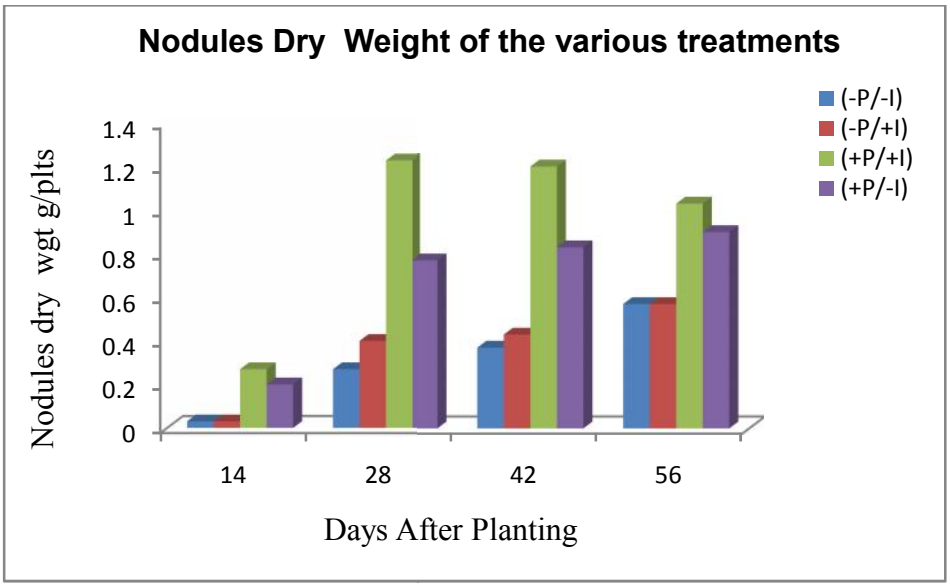

Figure 5: Effect of phosphorus and rhizobia inoculation on nodule growth of soybean.

EFFECT OF PHOSPHORUS AND RHIZOBIA INOCULATION ON POD NUMBER, NUMBER OF SEEDS PER POD, 100 SEED WEIGHT AND SHELLING %.

Phosphorus fertilizer treatment only (+P/-I) and Rhizobia inoculation plus phosphorus (+P/+I) produced significantly more pods (3903.7 Kg/ha) and (3858.3 Kg/ha) than the rhizobia inoculation only which recorded 2301.0 Kg/ha and the control (2130.7 Kg/ha). But there was no significant difference between the phosphorus fertilizer treatment only (+P/-I) and Rhizobia inoculation plus phosphorus (+P/+I). There was again no significant difference between the control and the Rhizobia inoculated treatment only (Table 4). Phosphorus fertilizer application therefore had an influence on pod formation and pod number, and P availability also encourages nodulation; this was the reflection on the high number of pods produced by the rhizobia inoculation plus phosphorus (+P/+I) treatment but rhizobia inoculation alone recorded a lower pod number. However, the treatments did not have any significant effect on number of seeds per pod and 100 seed weight (Table 4). But there were significant effects on shelling percentage with the P fertilizer treatment only.

Treatment (+P/-I) recording the highest shelling percentage of 69% followed by the control. It showed that yield of soybean depended on pod number per unit area. This

228 finding is in line with that of Ohyama *et al*. (2013) who stated that soybean yield depends
229 mostly on pod number per unit area (Cassman *et al*., 1981).
230

231 **Table 4: Effect of phosphorus and rhizobia inoculation on yield components of soybean (Jenguma**
232 **HSCII51) at final harvest**

Treatments	**Pod No./m^2**	**Number of seeds/pod**	**100 Seed wt**	**Shelling %**
(-P/-I) No Inoculation and No P fertilizer	2130.7	2.000	10.67	0.68
(-P/+I) Rhizobia inoculation only	2301.0	1.833	10.83	0.66
(+P/+I) Rhizobia Inoculation + P fertilizer	3858.3	1.967	10.97	0.67
(+P/-I) P fertilizer Only	3903.7	1.900	11.60	0.69
Lsd (5%)	**1278.5**	**NS**	**NS**	**0.02**
CV (%)	**10.5**	**3.4**	**1.8**	**0.9**

233 *NS = Not Significant, P > 0.05*

234

235 **EFFECT OF PHOSPHORUS AND RHIZOBIA INOCULATION ON SEED YIELD**
236 **COMPONENTS OF SOYBEAN.**

237 Seed yield is a function of plants per unit area, the mean number of pods per plant, the
238 mean number of seeds per pod and the mean individual seed weight. Rhizobia
239 inoculation plus P fertilizer (+P/+I) and P fertilizer only (+P/-I) recorded significantly the
240 highest pod dry weight of (1131.1 Kg/ha) and (1054.8 Kg/ha) respectively.
241 Again, there was no significant difference between the control (-P/-I) and the Rhizobia
242 inoculation only (-P/+I) (Table 5).
243

Grain yield was highest for Rhizobia inoculation + P fertilizer (+P/+I) and P fertilizer only (+P/-I), recording 7.61 t/ha and 7.30 t/ha respectively, whiles Rhizobia inoculation only and the control recorded the lowest grain yields of 4.41 t/ha and 3.80 t/ha respectively (Table 4). Ohyama *et al*. (2013), reported that soybean seed yield can reach 4-6 t/ha with well managed field under good climatic and soil conditions. They again stated that the highest yield of soybean (7.8) t/ha were recorded in Japan. The yield obtained in this study was much close to that recorded in Japan and this could be considered as the highest yield reportedly obtained in the current study.

The control and the Rhizobia inoculation (-P/+I) treatments, did not have any significant increase on grain yield; hence phosphorus fertilizer can be said to have a greater influence on grain yield of soybean. This is because the impact of phosphorus fertilizer was significant across board in terms of dry matter produced, pod yield and grain yield. According to the soil analysis results of the experimental site (Table 2), the P content of the soil was very low (2.3 mg/kg) as compared to 20 mg/kg regarded as adequate or the standard.

Adequate phosphorus application and Rhizobia will lead to effective pod dry matter accumulation, and subsequently to higher grain yield.

The yield characteristics are a reflection of shoot dry weight which was increased by P fertilizer application. This may indicate that when shoot growth is limited by P unavailability, pod filling (Shelling %) and total seed yield will be accordingly limited. This shows the importance of P supply in improving the production of soybean in Ghana. The poor yields obtained by farmers may be attributed to P unavailability in most soils particularly during the period of high shoot growth rate (42 to 70 DAP).

Table 5: Effect of phosphorus and Rhizobia inoculation on pod dry weight, grain yield and harvest index of soybean

Treatments	Pod Dry wt. (g/m^2)	Grain Yield (g/m^2)	Grain Yield (t/ha)	Harvest Index
(-P/-I) No Inoculation and No P fertilizer	562.2	379.9	3.80	0.54
(-P/+I) Rhizobia inoculation only	668.7	441.7	4.41	0.52
(+P/+I) Rhizobia Inoculation + P fertilizer	1131.1	763.6	7.61	0.52
(+P/-I) P fertilizer Only	1054.8	727.9	7.30	0.50
Lsd (5%)	**385.2**	**253.6**	**0.30**	**NS**
CV (%)	**6.0**	**6.2**	**6.2**	**3.8**

NS = Not Significant, P > 0.05

CONCLUSION AND IMPLICATIONS OF THE STUDY

Most savannah soils, lack of P; under the low-input agricultural systems, farmers do not apply fertilizers to soybean, they generally use the limited amount of fertilizer available for staple cereal crops like millet, sorghum, and rice and maize. Promiscuous soybean cultivars were proposed as alternatives to the use of inoculants and there seems to be P requirement for plant growth and development. The field experiment results have shown P requirement for shoot growth and yield of soybean.

Based on the study, phosphorus and Rhizobia application to soybean played a very significant role in increasing nodulation, pod formation and development, and a subsequent grain yield. Furthermore, the study has shown that in order to derive full benefit from biological

nitrogen fixation and hence to maximize soybean productivity in these soils, it is necessary to apply Phosphorus as part of a deliberate programme to improve soil fertility levels. It is recommended that farmers apply phosphorus fertilizer to their crop (soybean) so as to increase their harvest and income level affording them, the edge to go into large scale production of the crop. Nevertheless, for proper establishment, soybean seeds could be inoculated with appropriate Rhizobia.

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