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Research Article

Comparative Assessment of *Bradyrhizobium japonicum* Inoculant and Phosphorus on Growth and Yield of Soybeans (*Glycine max* L.) Genotypes

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Abstract

Background and Objective: Variability in symbiotic effectiveness of soybean varieties to inoculation and phosphorus supplementation have not been fully explored. The aim of this study was to assess the comparative effects of *Bradyrhizobium japonicum* inoculant and single super phosphate (SSP) on growth and yield parameters of three improved soybeans genotypes. **Materials and Methods:** A field trial was conducted at the Teaching and Research Farm of the University of Agriculture Makurdi, Nigeria during the rainy season in 2017. The experiment was laid out in a split plot design with three soybeans genotypes constituting the main plot and the inoculant and SSP in the subplot. The inoculant and SSP were incorporated into the soil at planting. **Results:** Results showed that under main effects of fertilization types, inoculation with *Bradyrhizobium japonicum*, irrespective of variety gave the highest biomass per plant (13.61 g) and grain yield of 3.44 t ha⁻¹ while nodules and root weights were statistically higher under SSP fertilization. All varieties performed best under P fertilization across all parameters measured, although there was significant variability in P responses by individual varieties. However, the interaction of TGX 1904-6f and inoculation gave the highest grain yield of 4.62 t ha⁻¹, which indicated higher symbiotic effectiveness between this variety and *Bradyrhizobium japonicum*. **Conclusion:** Symbiotic effectiveness, P requirement and yield of soybeans varied among the different varieties and variety interaction with inoculation had the highest yield. Although P remained the major requirement for overall soybean productivity.

Key words: *Bradyrhizobium japonicum*, inoculant, P fertilization, soybean, phosphorus supplementation, fertilization

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Soybean (*Glycine max* L. Merr.) is a source of edible oil (20-25 %) and protein (42-45 %)¹. According to Singh *et al.*², soybean is more protein rich than any of the common vegetable or legume food sources in Africa. Among the essential nutrients needed for soybean growth, N, P and K play a crucial role in improving growth and yield³. While application of N fertilizers is not common for soybean crop especially in Africa, it is assumed that the ability of the soybean plant to fix N₂ to meet its nitrogen demand and maximum crop yield is enough. The fixation of N₂ from symbiosis between rhizobia and legume is a cheaper source of N and legume inoculant is usually an effective agronomic practice for ensuring adequate supply of N for legume-based crops⁴. The use of *Bradyrhizobium* inoculant to maximize biological nitrogen fixation (BNF) to meet the N requirement of soybean has been studied over time. The BNF has become an attractive and economically viable nitrogen input and an important substitute of inorganic fertilization for resource poor farmers and is environmentally friendly⁵.

Phosphorus deficiency is a major nutrient stress for growth and development of grain legumes⁶. Phosphorus is needed in relatively large proportion by legumes for growth and has been reported to promote nodule number, nodule mass, leaf area, biomass and yield in different legumes⁷-⁹. The deficiency of P supply and its availability remains a limitation on biological nitrogen fixation and symbiotic interactions as nodules are an important sink for P. Nodulation and N₂ fixation are strongly influenced by P availability¹⁰. Nitrogen fixing plants have an increased requirement for P over those receiving direct nitrogen fertilization, probability due to its need for nodule development and signal transduction and to P-lipids in the large number of bacterioids¹¹.

Because of the avoidance of environmental problems, human health and more crop production to meet the increasing need of food, integrated nutrient management by the combination of chemical and bio-fertilizers have been proposed¹². Response to inoculation varies among soil types, plant species grown and season grown in different locations. Several studies have reported the response of soybeans to N fertilization and rhizobium inoculation¹³-¹⁶. However, estimating symbiotic effectiveness of different soybean varieties with inoculation and P fertilization and their interactions on a Savanna alfisol is lacking in reported literature. Therefore, the aim of this research was to examine the comparative effect of *Bradyrhizobium japonicum* inoculation and single super phosphate (SSP) on symbiotic effectiveness and productivity of soybeans varieties.

MATERIALS AND METHODS

Experimental site: The experiment was carried out as a field trial at the Teaching and Research Farm, University of Agriculture Makurdi, Nigeria, during the 2017 rainy season between August and December. Makurdi lies between latitude 7°44' N and 7°50' N and between longitude 8°30'E and 8°45'E at an average altitude of 73 m above sea level with a mean annual temperature range between 22 and 32°C and a mean annual rainfall of 1250 mm. The location falls within the southern Guinea Savannah of Nigeria.

Soil sample collection and analysis: Random soil samples were collected from the experimental site and bulked to form a composite. A sub-sample of the composite was obtained and taken to the laboratory of the Department of Soil Science, University of Agriculture Makurdi for analysis of the physical and chemical properties of the soil. Routine soil analysis was carried out using standard procedures.

Experimental layout: A field with no known history of soybean cultivation was selected for the trial. Treatments comprised of three soybeans genotype (TGX 1935-3F, TGX 1951-3F and TGX 1904-6F), *Bradyrhizobium japonicum* inoculant and phosphorus arranged in a split plot design and laid out in a randomized complete block design with three replications on plot sizes of 4 × 3 m. The main plot comprised of the soybeans genotypes while the subplot comprised of the fertilization (Control, inoculant and SSP).

Treatments application and planting: The inoculant and phosphorus were applied at planting. The inoculant was applied at 5 g kg⁻¹ of seeds using 16% gum Arabic as an adhesive as described by the two-step method¹⁷. In the first step, the weighed seeds in a container were uniformly covered with 16% (w/v) gum Arabic, then the container was closed and swirled until the seeds were uniformly coated. In the second step, the inoculant was added to the sticky seeds and the container was closed and swirled slowly until seeds were uniformly covered. The seeds were air dried to enhance adhesion and thereafter planted. The SSP was applied at the rate of 54 kg P₂O₅ ha⁻¹ using side placement at planting.

Planting was done at two seeds per hole at an intra-row spacing of 20 cm and inter-row spacing of 75 cm the plot was kept weed free throughout the growing season.

Data collection: At 50% flowering, five plants were sub-sampled from the border rows from each plot. They were

carefully uprooted using a hand trowel and assessed for nodulation. The nodules were carefully detached and counted. The roots were detached from the shoots and the plant biomass and nodules were sub-sequently oven dried and weighed to give the nodule, root and shoot dry weights. Data was also collected on the following parameters during the growth phase of the plant: Days to first flowering, days to 50% flowering, plant height at flowering, days to first podding, days to 50% podding, days to first maturity, days to 50% maturity and plant height maturity. The mature plants were harvested and the collected pods were allowed to dry and were subsequently threshed. The seeds were dried and weighed obtain the grain weight and subsequently, yield per plot.

Statistical analysis: All data collected were subjected to two way analysis of variance (ANOVA) using the General Linear Model (GLM) and the means of measured parameters were separated using the least significant difference (LSD) at 5% level of probability. Analysis was conducted using SPSS 20.0 software (IBM Corp, Armonk, NY, USA).

RESULTS

Soil analysis: The results of the physical and chemical properties of the experimental soil determined before the establishment of the trial is shown in Table 1. The analytical values showed that the soil texture was sandy loam. The pH of the soil in water (5.9) was rated as moderately acidic. Organic carbon (3.2 g kg⁻¹), total N (0.36 g kg⁻¹) and available P (13 mg kg⁻¹) contents were rated as low. Exchangeable bases (Ca²⁺, Mg²⁺ and Na⁺) were all of the low class while K⁺ was moderate. Effective cation exchange capacity (6.73 cmol kg⁻¹) was low. The values for the available micronutrients (Zn, Cu, Mn and Fe) were classified as high.

Variety effect on productivity: The main effects of variety on nodulation, growth and yield of soybean as shown in Table 2, indicated that there were variations on these parameters across the three varieties. However, the soybean variety TGX 1951-3f gave the highest biomass yield per plot (54.8 g), biomass per plant (14.56 g) and root dry weight per plant (6.04 g), although, its grain yield (2.96 t ha⁻¹) was lower than that of TGX 1904-6f which had the highest grain yield (3.02 t ha⁻¹). The TGX 1904-6f, despite having lower growth parameters compared to the other varieties had the highest yield (3.02 t ha⁻¹).

Table 1: Experimental soil physical and chemical properties

Parameters	Particle size distribution (%)
Sand	56.00
Silt	28.00
Clay	16.00
Textural class	Sandy loam
Soil pH (H ₂ O) 1:2:5	5.90
Total N (g kg ⁻¹)	0.36
Organic C (g kg ⁻¹)	3.20
Total P (mg kg ⁻¹)	521.00
Available P (mg kg ⁻¹)	13.00
Exchangeable bases (cmol kg⁻¹)	
Calcium (Ca)	7.60
Magnesium (Mg)	0.36
Potassium (K)	0.28
Sodium (Na)	0.09
Exchangeable acidity (H+Al) (cmol kg ⁻¹)	0.80
ECEC (cmol kg ⁻¹)	6.73
Micronutrients (mg kg⁻¹)	
Copper (Cu)	3.58
Manganese (Mn)	14.12
Iron (Fe)	51.63
Zinc (Zn)	87.52

Fertilization effect on productivity: Results presented on Table 3 showed the main effects of inoculation and SSP on growth and yield parameters of soybeans. As observed, inoculation with *Bradyrhizobium japonicum*, irrespective of the variety gave the highest biomass per plant (13.61 g) and grain yield of 3.44 t ha⁻¹. However, weight of nodules (1.07 g), biomass yield per plot (50.3 g) and root dry weight per plant (6.59 g) were statistically higher under SSP fertilization relative to other treatments. This showed the importance of P in root and shoots biomass production.

Variety interaction with fertilization types on productivity: Interaction effect of variety, inoculation and SSP on growth and yield of soybeans indicated that irrespective of variety, inoculation showed a consistent trend in improving plant biomass (Table 4). Under SSP fertilization, TGX 1935-3f had plant biomass per plot, root dry weight per plant and grain yield of 45.1 and 7.63 g and 3.42 t ha⁻¹, respectively. Similar parameters for TGX 1951-3f were 76.0 and 8.07 g and 3.08 t ha⁻¹, while TGX 1904-6f had values of 49.9 and 4.07 g and 2.93 t ha⁻¹, respectively. Nodulation was also increased irrespective of variety under SSP fertilization. Despite the good response of the variety TGX 1935-3f and SSP in yield (3.42 t ha⁻¹), the interaction of TGX 1904-6f and inoculation gave the highest grain yield of 4.62 t ha⁻¹, which indicated higher symbiotic effectiveness between this variety and the bacteria, *Bradyrhizobium japonicum* contained in the inoculant. Generally, P was shown to be of greater importance in growth and yield of soybean across the varieties.

Table 2: Variety effect on growth and yield

Varieties	Days to 50% flowering	Days to 50% podding	Plant height (cm)	Plant height at maturity	Nodule weight/plant (g)	No. of leaves/plant	Biomass yield/plot (g)	Biomass weight/plant (g)	Root dry weight/plant (g)	Grain yield (t ha ⁻¹)
TGX 1935-3f	39.67	55.00	54.4	73.11	1.06	46.5	40.0	11.57	5.05	2.71
TGX 1951-3f	41.17	55.67	47.7	66.53	0.79	42.0	54.8	14.56	6.04	2.96
TGX 1904-6f	50.50	55.83	46.8	59.56	0.46	34.2	37.6	10.65	3.93	3.02
LSD	0.31	0.27	6.53	4.34	0.21	3.9	3.02	1.62	0.57	0.72

LSD: Least significant difference

Table 3: Effect of fertilization on growth and yield

Varieties	Days to 50% flowering	Days to 50% podding	Plant height (cm)	Plant height at maturity	Nodule weight/plant (g)	No. of leaves/plant	Biomass yield/plot (g)	Biomass weight/plant (g)	Root dry weight/plant (g)	Grain yield (t ha ⁻¹)
Control	40.89	56.00	47.7	63.75	0.58	41.3	32.6	11.23	3.64	2.44
Inoculum	39.89	55.22	55.7	68.81	0.8	42.3	49.4	13.61	4.79	3.44
SSP	40.56	55.33	45.5	66.64	1.07	39.1	50.3	11.94	6.59	2.81
LSD	0.36	0.31	6.53	4.34	0.2	1.06	1.02	1.63	0.54	0.70

SSP: Single super phosphate, LSD: Least significant difference

Table 4: Interaction effects of variety and fertilization on biomass and yield

Treatments	Nodule weight/plant (g)	Root dry weight/plant (g)	Biomass weight/plot (g)	Grain yield (t ha ⁻¹)
TGX 1935-3f				
Control	0.45	3.26	26.0	1.52
Inoculum	0.83	4.26	48.8	3.18
SSP	1.89	7.63	45.1	3.42
TGX 1951-3f				
Control	0.87	4.70	39.1	3.30
Inoculum	0.89	5.36	49.5	2.50
SSP	0.98	8.07	76.0	3.08
TGX 1904-6f				
Control	0.43	2.97	32.8	2.50
Inoculum	0.69	4.75	50.0	4.62
SSP	0.78	4.07	49.9	2.93
LSD	0.18	2.12	25.1	0.63

SSP: Single super phosphate, LSD: Least significant difference

DISCUSSION

There was distinct variation in the growth and yield parameters recorded among the varieties assessed. This could be as a result of differences in genetic composition of the seeds as they were likely bred for specific traits which might affect their growth and yield attributes. Variations among yield and yield components of different varieties of soybeans have been previously reported by Haq *et al.*¹⁸ and Zhong¹⁹, who observed significant genotypic differences in growth and grain yield parameters. Similar variation in productivity of crop species were also reported additionally in soybean^{20,21}, in groundnut²² and in cowpea²³. Although, Zuffo *et al.*¹⁶ reported similarity of plant growth and productivity in two soybean cultivars with high productive potential. The varieties assessed exhibited different nodulation potentials. The use of SSP recorded the highest nodule weight per plant, biomass yield per plot as well as the root dry weight in the main effects of fertilization type. The importance of P in nodulation has been previously documented²⁴⁻²⁷. There was an observed increase in nodule number and nitrogenase activity

with P application which resulted in an increase in percentage amount of N derived from bacterial fixation^{28,29}. Similar improvement was recorded by Hayat *et al.*³⁰, who reported an increase of up to 32% in amount % of N derived from bacterial fixation due to application of *P. Rhizobium* use phosphorus as an essential ingredient in converting atmospheric N₂ to ammonium (NH₄), a form useable by plants³¹.

Generally, genotype and SSP interaction favoured nodulation, biomass, root dry weight and grain yield. These parameters were highest in the interaction between the variety TGX 1951-3f and SSP. Phosphorus has been reported to promote early root formation and the formation of lateral, fibrous and healthy roots, which play an important role in N₂ fixation, nutrient and water uptake^{32,33}, irrespective of crop variety. Plant biomass increase due to P supply supported the report of Badar *et al.*³⁴, who observed that P application significantly improved total biomass dry weight and root dry weight of groundnut, similar to the observations in this study. The importance of P on biomass production has been widely reported^{7,24,35}.

On the main effect of fertilization, there was a significant increase in growth and yield of soybean with inoculation. This increase could be attributed to the primary aim of rhizobial inoculant which was to increase the number of desirable strains of *Rhizobia* in the rhizosphere to enable nitrogen fixation, thereby ultimately supplying nitrogen which was required for vegetative growth, high biomass production and improved yield³⁶⁻³⁸. Similarly, interaction effect of variety and inoculation showed that the genotype TGX 1904-6f and inoculation gave the highest grain yield indicating higher symbiotic effectiveness of this variety and the introduced bacteria to promote yield, while also indicating genotypic variation to symbiotic effectiveness among the varieties studied. Wani *et al.*³⁶ stated that within grain legume species, genotypic variability affected nitrogenase activity, consequently, influencing symbiosis and productivity. Several studies have reported that application *Rhizobium* inoculation influenced N₂ fixation and yield of legume crops such as in groundnut^{24,34,35}, cowpea³⁹⁻⁴¹ and soybeans⁴²⁻⁴⁵.

It was observed from this study that different varieties of soybean responded to P application in different measures. Thereby, concerns about uniform P recommendations for the same crop varieties could be raised.

CONCLUSION

Inoculation with *Bradyrhizobium japonicum* irrespective of the variety improved soybean productivity, although with notable variability among the varieties. Symbiotic effectiveness, phosphorus requirement and yield of soybeans varied among the different varieties studied and variety interaction with inoculation had higher plant productivity relative to P, although P remained a major requirement for soybean production.

SIGNIFICANCE STATEMENT

This study discovered that despite the general P requirement adopted for soybean cultivation, specific varieties had different P requirements and symbiotic effectiveness with *Bradyrhizobium* strains. This can be beneficial for farmers to avoid under or over application of growth promoting substances to specific varieties. This study will help researchers to uncover the critical areas of plant varieties growth and productivity response to inputs that many researchers were not able to explore. Thus, a new theory that symbiotic effectiveness of soybean and *Bradyrhizobium japonicum* will be influenced by the genetic composition of the improved crop variety which may also alter the P requirement of either the plant or bacteria or both, may be arrived at.

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