Yields and economic benefits of soybean (*Glycine max* L.) as affected by *Bradyrhizobium japonicum* inoculation and phosphorus supplementation

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Abstract

A field and glasshouse experiment was conducted at Tanzania Coffee Research Institute (TaCRI) and Selian Agricultural Research Institute (SARI) to study the effect of *Bradyrhizobium japonicum* inoculation supplemented with Phosphorus - Triple Super Phosphate (TSP) on economic returns and yields and yields components of soybean. The treatments consisted of selected strain of *B. japonicum* (with & without), phosphorus at the levels of 0, 20, 40 and 80kgP.ha^{-1}. Both treatments were replicated four times in split plot design. The following yield components were measured; number of branches per plant, number pods per plant, number of seeds per pod and number of nodules per plant. Other yield parameters taken were 100-seed weight (g), seed yields (kg.ha^{-1}) and economic analysis of using inoculants alone and in combination with phosphorus. The results showed that, the inoculated treatment was significantly higher than non-inoculated treatment. Phosphorus supplementation significantly affected 100-seed weight (g) and seed yields (kg.ha^{-1}). Significant increase due to phosphorus supplementation was also observed on number of pods per plant and number of nodules per plant. Interactive effect between inoculation and phosphorus supplementation was observed on the number of nodules per plant. From economic analysis, rhizobial inoculation alone was the most profitable package than all other tested options.

Key words: seed yield (kg) per ha, hundred seed weight (g), number of branches per plant, number of pods per plant, number of nodules per plant, number of seeds per pod, marginal net of return, marginal rate of return.

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Introduction

The nutrient supply in crop production is one of the key components to higher yields (Gehl, et al., 2005). Increased crop yields due to mineral nutrient supply in the developed world are widely documented (Giller et al., 1998). However, Africa is reported to have the lowest use of fertilizer in the world. The per capita consumption of fertilizer in Tanzania is standing at 8kg.ha $^{-1}$ as compared with 52kg.ha $^{-1}$ for South Africa and Zimbabwe and 27kg.ha $^{-1}$ for Malawi (Walter, 2007). Nitrogen (N) is the most limiting nutrient for crop yields, and N fertilizers is an expensive input in agriculture costing more than US$45 billion per year globally (Gyaneshwar et al., 2002). In legumes, nitrogen (N) is more useful because it is the main component of amino acid as well as protein (Hussain et al., 2011). It is documented that the soybean-*Bradyrhizobium* symbiosis can fix up to 300 kg N ha^{-1} under good conditions (Smith and Hume, 1987). The need for artificial N fertilizers can be substituted by N_2 fixation resulting in an economy estimates in US$ 3 billion per crop season (Nicolás et al., 2006). In annual basis, the costs of production are usually reduced due to biological nitrogen fixation (BNF). Silva and Uchida, (2000) demonstrate that field trials have shown the N captured by crops due to the use of rhizobia inoculants costing about $3.00/ha was equivalent to artificial N fertilizer costing $87.00. Shahid et al. (2009) reported that seed production in soybean can increase by 70-75% when the proper bacterial strains were used to inoculate soybean seeds. The higher nodulation due to inoculation resulted in higher nitrogen fixation by *rhizobium* and eventually the number of pods per plant which bring about higher grain yields as a whole (Singh et al., 2011). In other studies, Ibrahim et al. (2011) reported increase in yield and yield component of legumes by inoculating the seeds with specific strain of rhizobia.

Phosphorus is the second most vital plant nutrient apart from N, but for legumes, it presumes primary significance, which plays important role in root proliferation. Singh et al. (2008) report that the yield of legumes is greatly influenced by application of phosphorus and biofertilizer. Phosphorus is crucial in the production of protein, phospholipids and phytin in legume grains (Rahman et al., 2008). Its application also plays a vital role in increasing soybean yield through its effect on the plant itself. With the world-wide emphasis on sustainable agricultural systems, increase in legume production will come mostly from supplementing the crops with phosphorus (in deficient environments). Ndakidemi et al. (2006) reported that combination of beneficial soil bacteria and phosphorus in legume plants significantly increase the marginal rate of return and the grain yield compared with the single use of phosphorus or beneficial bacteria. This was due to the fact that seed inoculation with proper *Rhizobium* strain together with minor amounts of phosphorus at early growth stage stimulated the root nodulation and increase biological nitrogen fixation eventually improving yield components such as number of branches per plant, number pods per plants, number seeds per pod and seed weight (Dahmardeh et al., 2010; Morad et al., 2013).

In view of increasing price of artificial fertilizers, it seems that the cost of nutrients will be increasing in most cropping systems. Evidently, legumes will remain the backbone of farming system in farmers residing in poor areas due to their capacity to fix nitrogen. Research efforts should be directed in assessing the optimum combinations between organic and inorganic fertilizers that will offer immediate economic returns to the resource poor farmers who cannot afford the full package of inorganic fertilizers. The aim of this study was to assess the economic benefits of *rhizobium* inoculation and phosphorus supplementation by targeting on final grain yield of soybean (*Glycine max*) grown in northern Tanzania.

Material and Methods

Description of site location

Glasshouse and field experiments were conducted at Selian Agricultural Research Institute (SARI) and Tanzania Coffee Research Institute (TaCRI) at Northern part of Tanzania. The coordinates of SARI lies at Latitude $3^{\circ}21^{'}50.08^{''}$ and Longitude $36^{\circ}38^{'}06.29^{''}$E at an elevation of 1390masl with mean annual rainfall of 870mm.The mean maximum temperature ranges from

22°C to 28°C whiles the mean minimum temperature ranges from 12°C to 15°C respectively. Mean while, TaCRI lies at Latitude 3°13'59.59"S and Longitude 37°14'54"E, at an elevation of 1268masl with means annual rainfall of 1200mm.The mean maximum temperature is about 21.7°C and the mean minimum temperature is about 13.6°C with relative humidity of about 94%. The field experiment was carried out during the long rain season while for glasshouse was conducted under controlled conditions during 2013 cropping season correspondingly.

Experimental design

Experimental design follows a split plot design in completely randomized blocks with 4 replications per treatment. Treatments comprise of strain of *rhizobium* (with and without), 4 levels of phosphorus 0, 20, 40, and 80kgP.ha^{1-}). Plot size measured 3m x 4m with inter- row spacing of 0.2m and intra- row spacing of 0.5m. Each replication was interspaced by small terraces of 1m apart to avoid contamination. Density of plant population was 200,000 plants per hectare. Land was prepared by ploughing and harrowing before planting. The *Bradyrhizobium japonicum* strain USDA 110 (Batch number 23011302, S) were obtained from *MEA* Company Nairobi-Kenya, sold under license from the University of Nairobi. Soybean seeds (Soya 2 variety) were obtained from the breeder based at Uyole Agricultural Research Institute-Mbeya-Tanzania. Before sowing, the soybean seeds were thoroughly mixed with *Rhizobium* inoculants to supply (10^9cells/gseed), following procedures stipulated by products manufacturer. To avoid contamination, the un inoculated seeds was planted first followed with the inoculated seeds. Three seeds were planted and thinned to two plants after full plant establishment. Weeding and other agronomic practices were done manually using hoe at different growth stage of the soybean plant.

Determination of yields and yield components of the soybean plant

Number of branches.Plant^{-1} and Number of pods.Plant^{-1}was assessed by sampling10 plants from two side middle rows before the guard rows and the average worked out. The same apply to glasshouse, where both two plants were assessed for the number of branches and number of pods per plant respectively. Seeds.Pods^{-1}was taken from 10 representative plants at physiological maturity and pods was taken and then counted finally the mean value computed. Nodule.Plant^{-1}, were assessed by randomly sampling 10 plants at random from two middle rows before the guard rows and then counted, finally the average worked out. Furthermore, the same task were also carried out at glasshouse of which, both the two plants were taken and the nodule number counted. Grain yields were evaluated by randomly sampling two middle rows from the net plot threshed and then adjusted to constant moisture by air drying and weighs them. The 100-seedweight was assessed by randomly counting 100 threshed seeds and weighs them.

Estimation of economic benefits

Soybean economic benefits were computed using economic analysis, which is MNR(Marginal net return) = Y(grain yield of legume crop (kg/ha) × P(selling price of crop at harvest (US$/kg) – TVC (total variable cost or cost of inputs related to the treatment such as fertilizer, seeds, labour etc in US$/ha). In summary, MNR=$Y \times P$ – TVC. Costs of inputs and labour charges for various farm operations are shown in Table 1. The following formula was used to calculate the marginal rate of return (MRR) for each treatments as follows, MRR= MNR/TVC, whereby, MRR=Marginal rate of return.

Table 1. Costs of inputs and labour charges for various farm operations

Inputs	**Total Cost (US$/ha)**	**Activity**	**Total Cost (US$/ha)**
Soybean seeds (Soya 2)	18.55	Land preparation	49.47
Inoculants	14.87	Planting	46.38
Triple super phosphates (TSP)		Weeding (2 times)	43.29
0	-	Pesticide application (2 times)	30.92
20	42.67	Harvesting	30.92
40	85.34		
80	170.69		
Pesticides	61.84		

Statistical analysis

A 2-way ANOVA was used to analyze data collected. The analysis was done using STATISTICA software program 2010. Fisher's least significant difference was used to compare treatment means at p= 0.05 (Steel and Torrie, 1980).

Results

Effects of *Rhizobium* inoculation and Phosphorus supplementation on the Number of Branches.Plant^{-1} in *G. max*.L

Results in Table 2 shows that *Bradyrhizobium japonicum* inoculation significantly increased the number of branches per plant in the field experiment by 21% relative to the control. In glasshouse and field experiment, phosphorus supply showed significant increase in the number of branches by 68, 77, 106% and 36, 38, 40% by supplementing with 20, 40 and 80kgP.ha^{-1} respectively over the control treatment.

Effects of *Rhizobium* inoculation and Phosphorus supplementation on the Number of Pods.Plant^{-1} in *G. max*.L

Table 2 show that inoculated treatments significantly increased the number of pods per plant by 17 and 135% in glasshouse and field experiment respectively relative to non-inoculated treatment. In glasshouse, phosphorus supply showed significant increase in the number of pods per plant by 15, 44 and 53% by supplementing 20, 40 and 80kgP.ha^{-1}. Furthermore, in field experiment, phosphorus significantly increased the number of pods per plant by 46, 38 and 44% by supply 20, 40 and 80kgP.ha^{-1}respectively over the control treatment.

Effects of *Rhizobium* inoculation and Phosphorus supplementation on the Number of Nodules.Plant^{-1} in *G. max*.L

In Table 2, results shows increase in nodule number through rhizobia inoculation in both glasshouse and field experiment. In glasshouse and field experiment, phosphorus supply showed significant increase in the number of nodules by 45, 66, 69% and 25, 36, 47% by supplementing with 20, 40 and 80kgP.ha^{-1} respectively relative to the control treatment.

Effects of *Rhizobium* inoculation and Phosphorus supplementation on 100 seed weight (g) in *G. max.*L

Table 2 results signify increase in 100-seed weight in field experimentation by *Bradyrhizobium japonicum* inoculation by 91% above the control treatment. Phosphorus supply also showed significant increase in 100-seed weight by 5, 9 and 18% by supplementing 20, 40 and 80kgP.ha^{-1} relative to the control treatment.

Effects of *Rhizobium* inoculation and Phosphorus supplementation on seed yield per hectare in *G. max.*L

Results in Table 2 demonstrate that *Bradyrhizobium japonicum* inoculation significantly increased the seed yields by 192 % over un-inoculated control. Furthermore, phosphorus supplementation shows significant increase by 12, 34 and 45% respectively over the control treatment.

Interactive effects of *Rhizobium* and Phosphorus supplementation on the number of nodules per plant in *G.max.*L

Results presented in Figure 1 shows significant interaction between rhizobial inoculation and phosphorus supplementation on the number of nodules per plant for field experiment. In the inoculated treatments, the highest number of nodules was recorded at 80kgP.ha^{-1} relative to other phosphorus levels. However, for un-inoculated treatment, no significant increase was observed.

Table 2. Effects of *Bradyrhizobium japonicum* and phosphorus supplementation on the number of branches per plants, number of pods per plants, number of seeds per pods, number of nodules per plant, 100-seed weight (g) and seeds yields kg/ha.

	Glasshouse				Field					
Treatments	No. Branch.Plant^{-1}	No. Pods.Plant^{-1}	No. Seeds.Pod^{-1}	No. Nodules.Plant^{-1}	No. Branch.Plant^{-1}	No. Pods.Plant^{-1}	No. Seeds.Pod^{-1}	No.Nodules .Plant^{-1}	100-seed weight (g)	Seed yield (kg.ha^{-1})
Bradyrhizobium										
-R	6.3±0.5a	14.0±0.9b	2.1±0.1a	24.4±3.1b	41.7±1.4b	11.8±0.9b	2.1±1.3a	2.9±0.9b	10.97±0.4b	467.3±31.0b
+R	6.4±0.4a	16.4±0.8a	2.3±0.1a	50.9±2.3a	50.5±2.4a	27.6±1.0a	2.3±0.1a	35.8±2.6a	21.00±0.3a	1364.8±47.3a
Phosphorus(kgha^{-1})										
0	3.9±0.3c	11.9±0.7b	2.3±0.2a	26.0±4.7b	35.9±1.7b	14.9±2.9b	2.1±0.1a	11.0±4.2c	14.82±1.9c	745.7±147.6d
20	6.5±0.4b	13.6±0.8b	2.3±0.2a	37.6±5.9a	48.8±1.9a	21.8±3.0a	2.3±0.2a	18.5±5.9b	15.55±1.9c	837.5±168.2c
40	6.9±0.4a	17.1±1.0a	2.3±0.2a	43.1±7.0a	49.5±3.0a	20.5±3.0a	2.1±0.1a	22.0±7.0b	16.14±2.0b	997.5±185.4b
80	8.0±0.4a	18.1±1.1a	2.0±0.0a	43.9±5.5a	50.3±3.2a	21.5±3.4a	2.1±0.1a	25.8±8.2a	17.43±1.8a	1083.7±185.5a
2-way ANOVA(F-Statistic)										
Br	0.09ns	7.91**	0.75ns	72.34***	22.69***	333.96***	0.14ns	688.01***	780.25***	999.17***
P	18.28***	12.05***	0.75ns	6.99**	13.67***	13.96***	0.14ns	25.11***	9.44***	28.92***
Br * P	0.91ns	0.49ns	0.75ns	0.16ns	1.79ns	0.46ns	1.29ns	13.56***	0.62ns	2.84ns

-R: Without *Bradyrhizobium*: +R: With *Bradyrhizobium*: Values presented are means ± SE. **, *** =significant at P≤0.01, P≤0.001 respectively, ns = not significant. Means followed by similar letter in a given column are not significantly difference from each other at *p*=0.05.

Table 3.Effects of *Bradyrhizobium japonicum* and phosphorus supplementation on profitability and marginal rate of return (MRR) of soybean

Treatments	MNR (US$/ha)	% increase over control	TVC (US$/ha)	MRR (US$/ha)
Bradyrhizobium				
-R	223.45±27.99b	-	356.05	0.62±0.07b
+R	1321.45±44.87a	636.17±84.99a	370.92	3.60±0.09a
Phosphorus (kg.Pha^{-1})				
0	635.80±180.18b	-	288.81	2.16±0.60ab
20	706.98±205.81b	22.28±9.08a	331.48	3.00±0.60ab
40	862.76±227.05a	71.95±41.96a	374.15	2.28±0.59a
80	884.26±227.19a	77.95±44.16a	459.50	1.90±0.48b

-R: Without *Bradyrhizobium*: +R: With *Bradyrhizobium*. Means followed by similar letter in a given column are not significantly difference from each other at *p*=0.05.

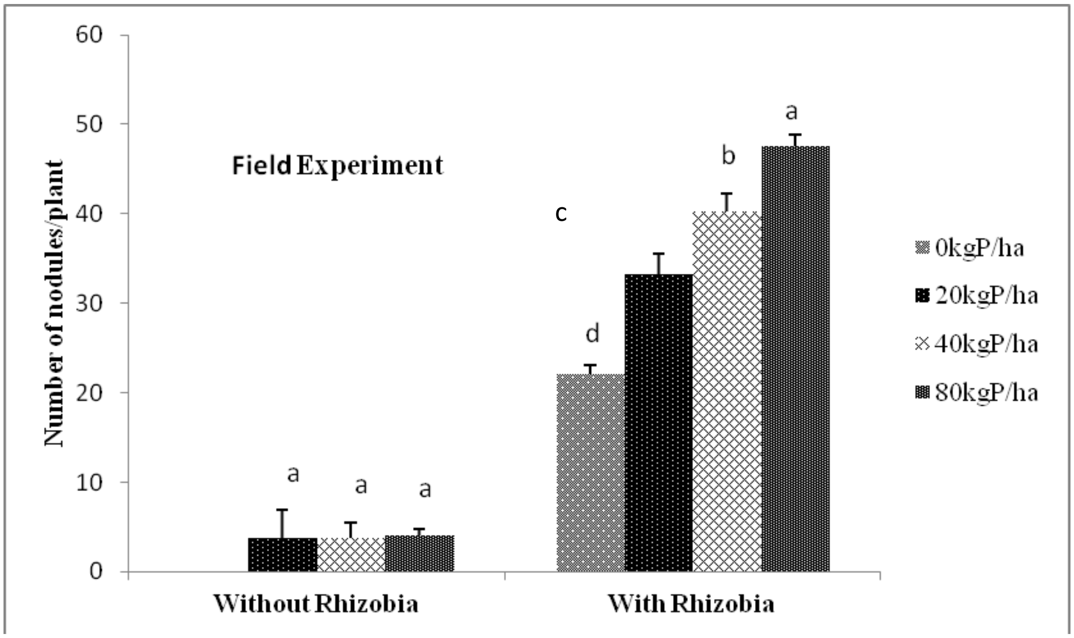

Figure 1. Interactive effects of *Bradyrhizobium japonicum* and phosphorus (kg.ha^{-1}) on nodule number per plant. 0P= 0kgP.ha^{-1}, 20P=20kgP.ha^{-1}, 40P= 40kgP.ha^{-1}, 80P=80kgP.ha^{1}. Bars followed by similar letter are not significantly different at *p*=0.05.

Discussion

The appropriate *Bradyrhizobium japonicum* strain and phosphorus supplementation inform of Triple Super Phosphate (TSP) were used in this study in order to find out a sustainable combination for optimizing higher economic returns for small scale farmers growing soybean in northern Tanzania. In this study, inoculation with *B. japonicum* significantly increased seed yields (kg.ha^{-1}) and 100-seed weight (g.plant^{-1}) of *G.max*.L relative to the control treatment (Table 2). The positive results from this study might be attributed by the efficiency of the *B. japonicum* strain which stimulated growth of the assessed parameters. Furthermore, higher grain yield obtained from this study from the plots treated with *B. japonicum* indicate that the biofertilizers technology is an efficient supplier of N in the tested legume and it can replace the inorganic N fertilizers. Similar to our study, significant effect of rhizobia inoculation on legume yield and yield components has also been documented by (Zhang et al., 2000;Okereke et al., 2001;Egamberdiyeva et al., 2004;Hayat et al., 2004; Kazemi et al., 2005;Fatima et al., 2006;Ndakidemi et al., 2006; Abbas et al., 2008; Shahid et al., 2009; Ibrahim et al., 2011;Shiri-Janagard et al., 2012;EI-Shaarawi et al., 2011;Abdul-Jabbar and Saud, 2012;Solomon et al., 2012).

Supplying phosphorus in this study resulted into positive effect on final seed yield, hundred seed weight, and number of nodule.plant^{-1}, number of pods.plant^{-1} and number of branches.plant^{-1} (Table 2). The positive results obtained mighty be contributed by essential role of phosphorus in legumes which support early root formation and development of lateral, fibrous and healthy roots. Furthermore, phosphorus is essential component in seed formation as it plays huge role in protein synthesis, phospholipids and phytin (Rahman et al., 2008) all of which are important in the growth of the plant. Amongst the different phosphorus levels tested, the highest phosphorus level gave relatively good results in almost all parameters measured in both glasshouse and field experiments (Table 2). These results are in agreement with the findings of (Israel, 1987; Sa and Israel, 1991; Olivera et al., 2004; Richardson and Barea, 2009; Shahid et al., 2009; Tahir et al., 2009; and Bekere and Hailemariam, 2012) that report improved growth, yield and yield components in legumes by supplementing with phosphorus.

Inoculation and phosphorus supplementation has become an alternative means of supplying important nutrients in the studied legume for its higher yields. From this study, rhizobial inoculation and phosphorus supplementation was a better option for resource poor farmers who cannot afford to purchase the expensive commercial fertilizers and demonstrated reduced cost of production. The provision of inoculants and phosphorus in the present study, increase grain yield and dollar profits per hectare relative to the control treatment which eventually resulted into a significantly greater marginal rate of returns (Table 3). Similar findings were also documented by Ndakidemi et al. (2006). However, the N_2- fixing technology in Africa is constrained by un-accessibility of inoculants and the lack of infrastructure for their production, storage and distribution in the rural areas.

In conclusion, seed inoculation with *Bradyrhizobium japonicum* together with phosphorus supplementation performed well over control treatment with regard to yields and yield component and marginal net of return. The results obtained from this study strongly demonstrate the benefits of using biofertilizers as a substitute to expensive inorganic nitrogen fertilizers in soybean production in northern Tanzania.

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