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Influence of *Rhizobium* inoculation on root length, symbiotic performance and grain yield of soybean (*Glycine max*) intercropped with sorghum (*Sorghum bicolor*) with P and K nutrition

Jalilatu Ayuba[1]*, Kenneth Opare-Obuobi[2], Askia Musah Mohammed[1], and Ibrahim Yussif Jnr[1]

[1]Faculty of Agriculture and Natural Resources, Tamale Technical University, P. O. Box 3E/R, Tamale, Ghana.
[2]Savanna Agricultural Research Institute, P. O. Box TL52, Nyankpala, Ghana.

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Limited information is available on optimizing potassium (P) and phosphate (K) nutrition with rhizobia in a soybean-sorghum intercropping system. A two-year field experiment was conducted to study the effects of Rhizobium inoculation with P and K on root length, symbiotic performance and yield. The split-split plot designs with 2 × 4 × 7 factorial arrangement with three replicates were used. The main plots had rhizobial inoculation, while the sub plots included four cropping systems namely sole sorghum, sole soybean and two intercropping levels. The sub-subplots were control; 20K; 40K; 26P; 52P; 26P + 20K; 52P + 40K. Plants were sampled at 50% flowering and shoot processed for N fixation. Nitrogen fixation was estimated using total nitrogen difference method where the total nitrogen obtained from non-fixing plants was subtracted from total nitrogen obtained from fixing plants. The results showed that cropping systems, inoculation and P and K fertilization influenced root length, number of nodules, nitrogen fixation and yield in soybean. Intercropping increased the number of nodules relative to sole cropping. P and K fertilization increased nodulation, nitrogen fixation and yield over the control. The use of combined fertilizers at the 20K+26P improved nodulation and N fixation while fertilization of 52P influenced yield.

Key words: Biological nitrogen fixation, inoculation, intercropping, nodulation, phosphorus, potassium.

INTRODUCTION

Nitrogen (N) is an essential element for all organisms, and a constituent of proteins, nucleic acids and other indispensable organic compounds (Robertson and Groffman, 2024). All plants need relatively large amounts of N for proper growth and development (Leghari et al., 2016). Nitrogen is usually incorporated into the soil through addition of industrial nitrogenous fertilizers (Van Groenigen et al., 2015), the decomposition of soil organic

*Corresponding author. E-mail: jalilatuayuba@gmail.com / ajalilatu@tatu.edu.gh. Tel: +233243048954.

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matter and redistribution of organic materials, natural processes of converting atmospheric N_2 through biological nitrogen fixation (BNF). BNF is the term used for a process whereby atmospheric N is reduced to ammonia in the presence of nitrogenase; a process only possible among a selected group of plants (Dashora, 2011; Kermah et al., 2018). Nitrogenase is a biological catalyst found naturally only in certain microorganisms such as the symbiotic *Rhizobium* and *Frankia*, or the free-living *Azospirillum* and *Azotobacter* (Nyoki and Ndakidemi, 2018; Fatima et al., 2019) Symbiotic relationship between plants and microbes have been reported and a group of soil host bacteria have long been used to improve the availability of nitrogen through BNF. These microorganisms stimulate plant growth by a plethora of mechanisms, hence are called Plant Growth Promoting Rhizobacteria (PGPR) (Vejan et al., 2016). Recently, biofertilizers have emerged as a fundamental component for BNF which provides an ecologically sound and economically attractive way of improving nutrient supply in the soil (Saharan and Nehra, 2011). Significant hosts for these microorganisms to achieve BNF are legumes among which includes soybean, cowpea, groundnut, pigeon pea, bambara groundnut, faba bean, common bean etc. Although BNF has long been a component of many farming systems throughout the world, its importance as a primary source of N for agriculture has diminished. This is due to the increasing use of fertilizer-N for the production of food and cash crops (Peoples et al., 1995; Giller et al., 2004).

BNF has been reported as one of the principal sources of N for crop production, as well as organic resources being recycled within the cropping field (Dakora et al., 2015; Mohammed et al., 2018; Kebede, 2021) or concentrated from a larger area, and mineral N fertilizers (Van Groenigen et al., 2015). Of these sources of N, mineral fertilizers have raised a global environmental concern resulting from the large amounts of N entering the global food production system (Van Groenigen et al., 2015; Nyoki and Ndakidemi, 2018). Studies have also shown that excess N has negative effects on water, air, and ecosystem and human health (de Vries, 2021). Apart from environmental effects of mineral N fertilizers, inaccessibility in rural areas and the high cost of input makes it unaffordable by small holder farmers which increase the costs of production. To minimize the harmful effects of excessive N from mineral fertilizers and to reduce the costs of production, researchers and farming communities have struggled to maintain soil fertility levels relying mostly on BNF (Nyoki and Ndakidemi, 2018).

This study focused on soybean, that is, a legume that has gained prominence in Northern Ghana in terms of research and areas of cultivation (production) as a host plant of nitrogen fixing bacteria. Soybean (*Glycine max*) is a legume that grows in the tropical, subtropical and temperate climates. Soybean has become a cash crop which small holder farmers in Ghana rely on for income partly due to an expansion of poultry and animal feed industry (Ntiamoah et al., 2022; Osman et al., 2018). Additionally, soybean's high nitrogen fixing ability makes is suitable for non-N fixing crops grown in succession to benefit from the residual nitrogen released through BNF (Kebede, 2021). However, for effective nitrogen fixation by *Rhizobium* bacteria during BNF, there must be favourable conditions similar to those necessary for the growth of the host plant. Key among these conditions necessary for plant growth includes the availability of macro nutrient such as N, P and K. Phosphorus (P) has been reported to influence symbiotic N_2-fixation in leguminous plants by many researchers (Hussain, 2017; Zhong et al., 2023). Severe deficiency of this nutrient in the soil can affect the growth of the host plant and symbiotic N_2 fixation (Kafeel et al., 2023) Furthermore, N_2-fixation has higher P requirements for optimal functioning than the host plant requires for its growth and nitrate assimilation (Dakora, 2000; Wang et al., 2021). The process of photosynthesis also requires potassium (K) which is essential in maintenance and balance of the electrical charges at ATP production site (Sardans and Peñuelas, 2021), hence making K an essential element in nitrogen fixation. Translocation of photosynthates (carbohydrate) to storage organs which serves as sinks (fruits or roots) can be largely due to K nutrition (Mengel, 2016). Under the storage organs such as root nodules, carbohydrate produced by the host plant is used by nitrogen fixing bacteria as source energy to fix atmospheric nitrogen (Nyoki and Ndakidemi, 2018). Given the important role P and K nutrients plays on crop production, there is currently limited information regarding the combined effects of P and K and rhizobia inoculation on root length, nodulation and nitrogen fixation in soybean intercropped with sorghum. Thus, this current study aimed to determine the effects of rhizobia inoculation supplemented with P and K on root length, nodulation, nitrogen fixation and grain yield in soybean intercropped with sorghum.

MATERIALS AND METHODS

Soil chemical properties

Before planting in each cropping year, soil was sampled randomly at nine points at the experimental site, pooled, and analyzed for pH (H_2O), organic C (using Walkley Black method), total N (using dry combustion method), P (using P-Brayl), K, Na, Ca, Mg, S, and cation exchange capacity (CEC) (using ammonium acetate method) (Table 1).

Experimental design and treatments

The field experiments were carried out at the research fields of the CSIR - Savanna Agricultural Research Institute (CSIR - SARI) in Nyankpala during the 2021 and 2022 cropping seasons. The experiment was laid out in split-split plot design with 2 × 4 × 7 factorial arrangement in three replicates. The plot size measured 3

Table 1. Chemical properties of soils collected from study site in 2021 and 2022 cropping seasons.

Cropping season	pH (H_2O)	O.C	Total N	P	Na	K	Ca	Mg	CEC
		%	%		mg kg^{-1}				Cmol kg^{-1}
2021	5.45*	0.49	0.06	2.0	19.4	42.85	320.6	82.11	2.84
2022	5.61*	0.45	0.04	1.7	21.25	43.55	322.4	86.45	3.21

*Significant at $p \leq 0.05$.

× 3 cm. The main plots had two rhizobia inoculation treatments, while the sub plots comprised: sole sorghum (75 × 60 cm); sole soybean (75 × 40 cm); sorghum - soybean (intercropping system) at a spacing of 75 × 60 and 75 × 20 cm, sorghum and soybean respectively; and the last cropping system was sorghum - soybean (intercropping system) at a spacing of 75 × 60 and 75 × 40 cm, sorghum and soybean respectively. Sub-subplots were fertilized with the following fertilizer levels (kg ha^{-1}): control; 20 K; 40 K; 26 P; 52 P; 26 P + 20 K; 52 P + 40 K. Fertilizer types used were (35 kg P_2O_5) for P and (30 kg K_2O /ha) for K

Soybean plant harvest and sample preparation

At 52 days after planting (50% flowering), 5 healthy soybean plants from the middle row were sampled for the quantification of nitrogen fixation. The five soybean plants were carefully dug out from the soil without any damage to their root system. The roots were then decapitated from the shoots and were carefully washed. Nodules were detached from the roots, counted and recorded. The length of each root was also measured and recorded. Nodules were then oven dried at 60°C for 48 h and weighed. The shoot of the plants was also oven dried at 70°C for 48 h (Belane et al., 2014), weighed and ground into fine powder (pass through a 2 mm sieve) for determination of nitrogen fixation.

Measurement of Nitrogen fixation

Nitrogen fixation was estimated using the Total Nitrogen Difference (TND) method where the total nitrogen obtained from non-fixing plants (sorghum) was subtracted from total nitrogen obtained from fixing plants (soybean) (Unkovich et al., 2008):

$$N - fixed = total\ N\ in\ legume - \ total\ N\ in\ reference\ crop$$

$$\text{Total N} - \text{fixed (kg/ha)} = \frac{\text{Dry matter weight (kg/ha) X plant \%N}}{100}$$

Grain yield and yield components

At full maturity, two rows of soybean (one left strip without sampling during the 50% flowering stage) were harvested in each plot, avoiding the border rows. All plants were air-dried for at least 15 days and then threshed manually to measure the yield per unit area. In the harvested strip, 10 consecutive soybean plants were selected for measuring the pod and seed number per unit area, and seed weight and aboveground biomass. Seed size was calculated by 300-seeds weight from each treatment. The harvest index (HI) was the ratio between seed weight and aboveground biomass of 10 plants of soybean.

Statistical analysis

Data collected was analysed using GenStat statistical software version 12. The statistical analysis was performed using analysis of variance (ANOVA) in factorial arrangement. The fisher's least significance difference (L.S.D.) was used to compare treatment means at $p = 0.05$ level of significance.

RESULTS

Root length

In this study, *Rhizobium* inoculation with "*Legumefix*" (a commercial inoculant) positively influenced the root length of the soybean plants in both cropping seasons (2021 and 2022). Rhizobium-inoculated soybeans grew longer roots compared with the uninoculated ones. The results showed that cropping systems had no significant effects on the root length of soybeans in both cropping seasons. In the 2021 cropping season, the root length of *Rhizobium*-inoculated plants was 22.86 cm, while in 2022, the root length increased to 23.11 cm. The root length of uninoculated plants was 19.12 and 19.45 cm, respectively. In both cropping seasons, significant differences were observed in the effect of P and K fertilization on the root length of the soybean plants (Table 2).

Number of nodules

Irrespective of the cropping year/season, the results of this study showed that cropping systems significantly affected the number of nodules per plant. Number of nodules ranged from 24.23 to 10 per plant for 2021 season and from 31 nodules per plant to 16.05 nodules per plant for 2022 cropping season (Table 2). Intercropped soybean generally produced more rootnodules than the sole soybean cropping system. Again, *Rhizobium* inoculation markedly increased the number of nodules over uninoculated soybean with a percentage increase of 71.42 and 69.19% relative to uninoculated controls in 2021 and 2022 cropping seasons respectively. Similarly, P and K fertilization increased the number of nodules over the control in both cropping seasons. In this study, the highest mean

Table 2. Effects of cropping systems, *Rhizobium* inoculation supplemented with P and K on soybean root length and number of nodules in 2021 and 2022.

Cropping system		Root length (cm)		Number of nodules	
		2021	2022	2021	2022
Treatments	SSoy	22.11±0.54[a]	22.75±0.62 [a]	10±1.13[bc]	16.05±1.88[bc]
	Sorg+Soy (A)	21.10±0.53[a]	22.65±0.58 [a]	21.89±2.18[b]	27.9±2.13[b]
	Sorg+Soy (B)	22.34±0.61[a]	22.97±0.61 [a]	24.23±2.45[a]	31.55±3.34[a]
Rhizobia	Inoculated	22.86±0.53[a]	23.11±0.63[a]	30.54±3.46[a]	39.44±3.87[a]
	uninoculated	19.12±0.31[b]	19.45±0.40[b]	12.22±1.01[b]	17.56±2.15[b]
Fertilization	Control	21.81±0.61[a]	22.47±0.6[b]	18.1±1.03[ab]	23.56±1.66[ab]
	20K	20.75±0.60[a]	32.28±0.56[a]	19.24±1.11[ab]	26.23±1.73[a]
	40K	20.85±0.54[a]	22.04±0.58[b]	20.86±1.14[ab]	27.56±3.33[a]
	26P	22.41±0.55[a]	24.47±0.63[b]	21.13±1.31[a]	29.82±3.76[a]
	52P	22.19±0.56[a]	21.89±0.61[bc]	22.65±2.22[a]	30.02±3.67[a]
	20K+26P	20.99±0.52[a]	23.01±0.74[b]	24.7±2.58[a]	31.91±3.56[a]
	40K+52P	21.29±0.56[a]	22.51±0.75[b]	22.97±2.67[a]	30.39±3.71[a]
3-way ANOVA F-stats	CroSyt	3.215[ns]	0.164[ns]	2.019*	2.891*
	Rhiz	11.023**	4.935*	3.335***	2.902***
	Fert	1.462[ns]	2.011**	3.262*	2.432*
	CroSyt*Rhiz	1.542[ns]	1.206[ns]	1.643[ns]	0.98[ns]
	CroSyt*Fert	1.336[ns]	1.333[ns]	1.582[ns]	0.76[ns]
	Rhiz*Fert	1.967[ns]	1.031[ns]	2.811*	1.72[ns]
	CroSyt*Rhiz*Fert	1.068[ns]	1.254[ns]	2.451[ns]	1.59[ns]

CroSyt: Cropping systems; Fert: Fertilizers; Rhiz: *Rhizobium*; SSoy: Sole soybean; Sorg+Soy (A): Sorghum/soybean intercropped at a spacing of 75 x 60 and 75 x 20 cm, sorghum and soybean respectively; Sorg+Soy (B): Sorghum/soybean intercropped at a spacing of 75 x 60 and 75 x 40 cm, sorghum and soybean respectively; Values presented are means ± SE; *, **, and ***: significant at p ≤ 0.05, p ≤ 0.01, and p ≤ 0.001 respectively, ns = not significant, SE = standard error. Means followed by dissimilar letter(s) in a column are significantly different from each other at p=0.05 according to Fischer least significance difference (LSD).

number of nodules (24.7 and 31.91) were recorded in 20 K+26 P (kg ha^{-1}) for the 2021 and 2022 cropping seasons respectively, while the lowest mean number of nodules (18.1 and 23.56 per plant) were recorded for 2021 and 2022 cropping season respectively (Table 2).

Interactive effects of *Rhizobium* inoculation and P and K fertilization on number of nodules in 2022 cropping season

The results of this study revealed that, there were significant interactions between *Rhizobium* inoculation and fertilizers in the 2022 cropping year. *Rhizobium* inoculation with "*Legumefix*" inoculant interacted well with fertilizers leading to an increased number of nodules. Contrarily, uninoculated soybean plants produced fewer nodules that were relatively smaller in sizes (though nodule size was not measured). The fertilizer level of 20K and 40K+52P (kg ha^{-1}) produced higher number of nodules when compared with the other fertilizer doses. However, the plots treated with 52P resulted in a high number of nodules over all other treatments followed by 20K+26P (kg ha^{-1}) (Figure 1).

Symbiotic nitrogen fixation

Similar to root length, the results of this study showed that cropping systems had no significant effects statistically on nitrogen fixation for both cropping seasons even though, nitrogen fixation values for intercropped soybean were higher than values of sole cropped soybean (Table 3). Inoculating the soybean plants showed significant effect on nitrogen fixation over uninoculated treatments. There was an N-fixed increase of 65.4 and 64.7 kg ha^{-1} in 2021 and 2022 respectively. In both cropping seasons, P and K fertilization significantly improved nitrogen fixation over the control. Nitrogen fixation was greatest among soybean plants fertilized with 52 P (kg ha^{-1}) whereas the lowest N_2 fixation values were recorded in plants of the control plots for the two cropping seasons (Table 3).

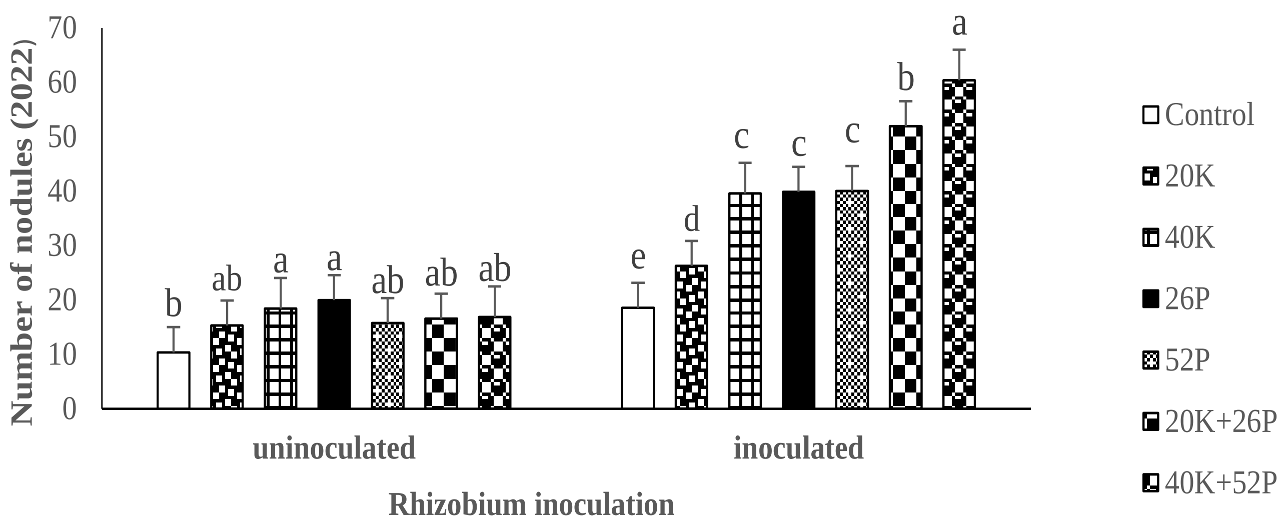

Figure 1. Interactive effects of rhizobia inoculation and P and K fertilization on the number of nodules in 2022 cropping season.

Grain yield

Soybean grain yield was statistically similar across the cropping system but varied markedly between inoculated plants and uninoculated plants. *Rhizobium* inoculation increased grain yield by 549.72 kg ha^{-1} in 2021 and by 620.97 kg ha^{-1} in 2022 cropping season. Similarly to nitrogen fixation, soybean plants fertilized with 52 P (kg ha^{-1}) produced the highest grain yield of 2004.35 (kg ha^{-1}). Lower grain yield was recorded among plants with no fertilization (control) (Table 3). inoculant, P and K fertilizers improved the root length, number of nodules, nitrogen fixation and grain yield. Even though, statistically cropping systems and fertilizers did not show significant effects on root length for the two-cropping season. Nonetheless, roots length was significantly improved with *Rhizobium* inoculation. The improved root length in *Rhizobium* inoculated plots could have been caused by nitrogen fixation, which eventually resulted into available nitrogen for plant growth (Nyoki and Ndakidemi 2018). Moreover, the increased root length in the *Rhizobium* inoculated plots could have been elicited by PGPR which functions through the production of plant hormones such as auxins, and cytokinins (Hayat et al., 2010; Jaiswal et al., 2021). The number of nodules was significantly increased with cropping systems, *Rhizobium* inoculation and fertilization with P and K. The intercropped plots produced more nodules compared with sole grown soybean. The increased number of nodules in intercropped soybean could be caused by enhancement with flavonoids found in root exudates of both soybean and sorghum. Sorghum is recognized for its ability to produce flavonoids (Awika, 2017) as well as large amounts of exudates. These flavonoids have properties reported to serve as chemical signals to support *rhizobium*–legume and arbuscular mycorrhizal symbioses. Again, the root exudates play a crucial role in shaping the composition of rhizosphere microbiomes,

Interactive effects of *Rhizobium* inoculation and P and K nutrients on nitrogen fixation and grain yield in 2022 cropping season

The results of this study showed a significant interaction between *Rhizobium* inoculation and P and K fertilizers for the second (2022) cropping season on nitrogen fixation. The combination of fertilizers and *Rhizobium* showed a good performance in nitrogen fixation where the best combination was observed in plants which received 52 kg of phosphorus and 20 K + 26 P (kg ha^{-1}). Inoculation alone without fertilizers resulted in lower nitrogen fixation compared with the *Rhizobium* plus P and K fertilizers (Figure 2).

Compared with *Rhizobium* inoculated, uninoculated soybean plants recorded a lower amount of fixed nitrogen with fertilizer application. Grain yield of soybean was generally higher in inoculated plants than in the uninoculated controls. Highest grain yield of 2004.35 kg ha^{-1} was recorded on plants fertilized with 52 kg of phosphorus and 26 P (kg ha^{-1}) with *Rhizobium* inoculation (Figure 3).

DISCUSSION

The results of this study showed that cropping systems, *Rhizobium* inoculation with "*Legumefix*" commercial and the roles of root microbiomes in mitigating abiotic stresses and rhizosphere microbial assemblage is

Table 3. Effects of cropping systems, *Rhizobium* inoculation supplemented with P and K on soybean nitrogen fixation and grain yield in 2021 and 2022 cropping seasons.

Cropping system		N-fixed (kg/ha)		Grain yield (kg/ha)	
		2021 season	2022 season	2021 season	2022 season
Treatments	SSoy	84.37±6.98^{ab}	127.25±9.63^{a}	1891.93±123.61^{a}	1961.29±133.8^{a}
	Sorg+Soy (A)	87.41±8.34^{a}	129.32±9.45^{a}	1894±122.1^{a}	1963.36±130.23^{a}
	Sorg+Soy (B)	90.35±10.22^{a}	130.63±8.32^{a}	1895.32±132.45^{a}	1964.67±134.22^{a}
Rhizobia	Inoculated	123.61±10.21^{a}	156.62±7.47^{a}	1921.32±135.38^{a}	1990.66±130.57^{a}
	uninoculated	58.21±4.24^{b}	91.92±6.9^{b}	1371.6±100.56^{b}	1369.96±122.65^{b}
Fertilization	Control	60.32±8.41^{c}	75.62±9.25^{c}	1840.34.133.45^{ab}	1909.34±136.1^{b}
	20K	80.77±10.25^{b}	120.5±7.72^{b}	1885.16±134.78^{ab}	1954.54±142.17^{ab}
	40K	80.28±10.46^{b}	120.05±9.05^{b}	1884.73±120.21^{ab}	1954.09±144.36^{ab}
	26P	91.98±10.84^{ab}	144.22±14.84^{ab}	1908.9±135.64^{a}	1978.26±137.56^{a}
	52P	110.55±10.9^{a}	170.31±14.43^{a}	1934.99±143.34^{a}	2004.35±154.25^{a}
	20K+26P	91.12±10.21^{ab}	144.76±13.58^{ab}	1909.44±136.23^{a}	1978.8±139.34^{a}
	40K+52P	97.81±10.44^{ab}	142.56±13.65^{ab}	1907.44±136.44^{a}	1976.67±141.4^{a}
3-way ANOVA F-stats	CroSyt	1.34^{ns}	0.421^{ns}	63.643^{ns}	1.302^{ns}
	Rhiz	56.89***	63.241***	171.718***	189.341***
	Fert	4.61***	6.442***	7.22***	7.865***
	CroSyt*Rhiz	0.77^{ns}	2.41^{ns}	2.698^{ns}	5.352^{ns}
	CroSyt*Fert	1.82^{ns}	1.19^{ns}	1.311^{ns}	2.291^{ns}
	Rhiz*Fert	3.45**	1.34^{ns}	3.564**	3.532^{ns}
	CroSyt*Rhiz*Fert	2.05^{ns}	1.41^{ns}	1.821^{ns}	2.044^{ns}

CroSyst: Cropping systems; SSoy: Sole soybean; Sorg+Soy (A): Sorghum/soybean intercropped at a spacing of 75 × 60 and 75 × 20 cm, sorghum and soybean respectively; Sorg+Soy (B): Sorghum/soybean intercropped at a spacing of 75 × 60 and 75 × 40 cm, sorghum and soybean respectively; Values presented are means ± SE; **, ***: significant at p ≤ 0.01, p ≤ 0.001 respectively, ns = not significant, SE = standard error. Means followed by dissimilar letter(s) in a column are significantly different from each other at p=0.05 according to Fischer least significance difference (LSD).

increasingly being highlighted (Cloutier et al., 2021). The increase in the number of nodules in intercropped legumes over the sole cropped one is in context with similar findings by Abusuwar et al. (2011), Musa et al. (2012), Egesa et al. (2016) among others. The concept behind these findings is that root exudates contain flavonoids which are signal molecules acting as nod gene inducers for the nodules forming symbiotic *Rhizobium*, hence increased number of nodules over the pure stand legumes (Liu et al., 2017). Apart from intercropping, P and K fertilization also increased number of nodules over the control.

Also, it was found from this study that, control plots (plots that received no P or K fertilization) produced fewer nodule numbers as compared with any level of P and K whether singly or applied in combination. The combined application was greater in eliciting root nodule formation compared to a single nutrient application. These results emphasize the importance of P and K nutrition for nodules formation, which finally enhance nitrogen fixation in soybean. Other studies have similarly reported an increase in nodule number under P sufficient treatments relative to P deficient (Chmelíková and Hejcman, 2014). Potassium has been reported to contribute to root growth consequently improving the number and size of root nodules (Li et al., 2021). This study determined the effects of cropping systems, *Rhizobium* inoculation and P and K fertilization on nitrogen fixation through the N difference method. Apart from for the cropping systems, *Rhizobium* inoculation and P and K fertilization increased nitrogen fixation irrespective of cropping seasons. The increased nitrogen fixation in *Rhizobium* inoculated plots is an indication of effective legume-microbes symbiosis in which legumes serve as sources of carbon to the bacteria and in turn the bacteria fix atmospheric nitrogen for the host plant through symbiosis. Similar to our findings, several studies have reported an increase in nitrogen fixation following *Rhizobium* inoculation in legumes (Gyogluu et al., 2016; Mohammed et al., 2018; Ngwenya et al., 2024). Phosphorus and potassium markedly enhanced BNF in this study relative to unfertilized treatments. Several studies (Martins et al., 2017; Mitran et al., 2018; Lazali and Drevon, 2021) have reported increased nitrogen fixation in different legumes following

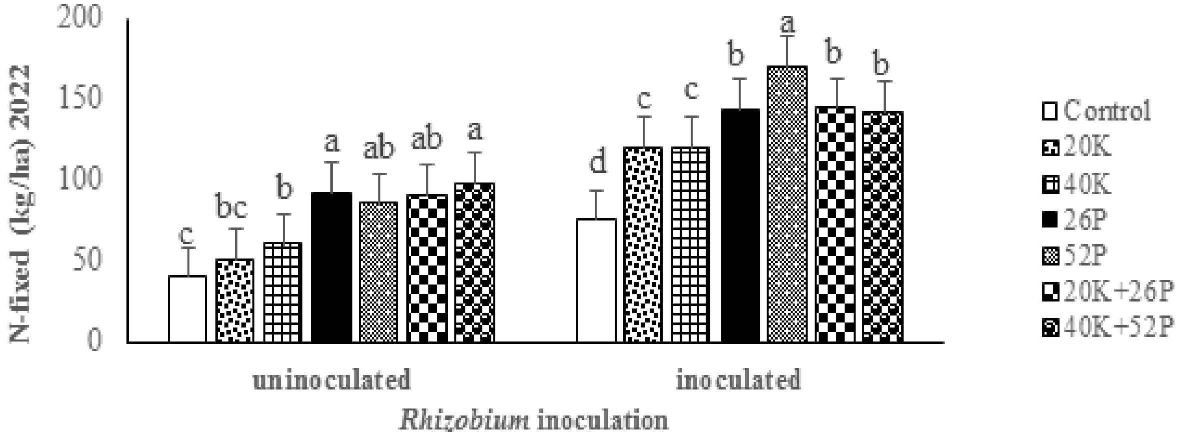

Figure 2. Interactive effects of rhizobia inoculation and P and K fertilization on nitrogen fixation in 2022.

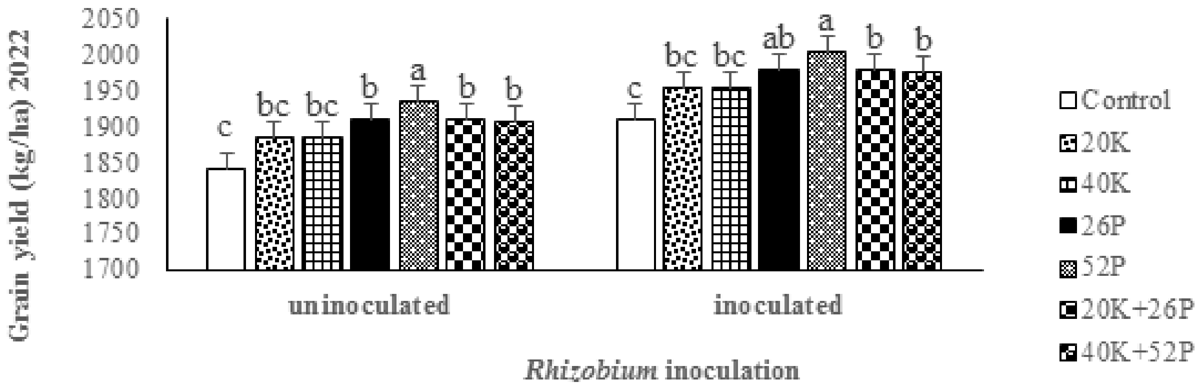

Figure 3. Interactive effects of rhizobia inoculation and P and K fertilization on grain yield of soybean in 2022.

P fertilization and that P deficient reduced nitrogen fixation. Potassium also has been similarly reported to increase nitrogen fixation in different legumes (Nyoki and Ndakidemi, 2018).

Results of this study also showed that grain yield was significantly improved by *Rhizobium* inoculation with P and K fertilization. The highest rate of phosphorous (52 P kg ha^{-1}) produced the maximum soybean grain yield. Several studies states that photosynthesis increases with P application; Wang et al. (2018) reported that the activity of Rubisco, which is a key enzyme of photosynthesis, and the protein concentration of leaves were higher in a high P application than in a low P application. Furthermore, Singh and Reddy (2015) also reported that Rubisco activity, RUBP regeneration, and maximum quantum yield related to the photochemical system were decreased under a P-deficient condition. Dinitrogen fixation activity was also increased by P application (Khan et al., 2020; Taliman et al., 2019) which translates into higher grain yield.

Conclusion

The findings of this study have demonstrated that the cropping system, *"Legumefix"* *Rhizobium* inoculation with P and K fertilization at different levels has influenced measured parameters such as root length, nodulation, nitrogen fixation and grain yield in soybean. In this study, intercropping significantly increased the number of nodules relative to sole soybean. It is interesting to note that the inoculation of soybean significantly increased the soybean root length, number of nodules per plant, nitrogen fixation and grain yield relative to uninoculated controls. Furthermore, even though P and K fertilization did not influence cropping systems, their application significantly increased the number of nodules per plant, nitrogen fixation and grain yield over the control. It is worth mentioning that the best combination of fertilizers which increased the number of nodules in this study was 20 K + 26 P (kg ha^{-1}). Consistently in both cropping seasons, symbiotic nitrogen fixation was greatly influenced by the application of P, K and *Rhizobium* inoculation. There was also a significant interaction of *Rhizobium* inoculation and fertilizers on grain yield of soybean in both cropping seasons. These results reiterate the importance of these mineral elements in enhancing nitrogen fixation, plant growth and development of crops for better grain yield. Hence, the study recommends the use of combined fertilizers 20 K + 26 P (kg ha^{-1}) with *Rhizobium* inoculation.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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