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RESEARCH ARTICLE

Rhizobia populations and Soybean yield response to Legumefix inoculant and isolated effective rhizobia strains from Uyole, Kilosa and Mkuyuni soils in Tanzania

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ABSTRACT

A study on the yield response of soybeans (*Glycine max* L. Merrill) to the Legumefix inoculant and the enumeration of rhizobia from soybean-growing soils was conducted in the laboratory and the screen house of the Department of Soil Science at Sokoine University of Agriculture. Laboratory analysis revealed that the soils were moderately fertile, with loamy textures, and hence suitable for soybean production. Low rhizobial populations in the tested soils hinder the soybean crop from reaching its full yield potential. Rhizobial populations were 450, 200, and 680 CFU g^{-1} soil in Uyole, Kilosa, and Mkuyuni, respectively. The pot experiment was conducted in the screen house, involving five treatments: Control, Legumefix inoculant, 30 kg P ha^{-1}, 30 kg P ha^{-1} + Legumefix, and 30 kg P ha^{-1} + 60 kg N ha^{-1}, in a CRD with three replications. Results revealed that all treatments produced significant ($P \leq 0.05$) differences in soybean nodulation and yield across the three soils. Soybean-growing soils should be tested for the presence and population levels of indigenous rhizobia to determine whether inoculation with introduced rhizobial strains is needed. It is important to develop inoculants based on native rhizobial populations and make them available for use in indigenous soils.

Keywords: enumeration, inoculation, isolation, nodulation, rhizobia, soybean, yield

INTRODUCTION

Soybean-nodulating bacteria (rhizobia) are widely distributed across regions worldwide, and their genetic diversity reflects variations in geography, climate, soil characteristics, and host plant diversity (Nakei et al., 2022; Wekesa et al., 2022). Despite their widespread occurrence, many soils cultivated with legumes do not contain sufficient populations of highly effective rhizobia to support optimal biological nitrogen fixation. In sub-Saharan Africa, populations of soybean nodulating rhizobia are often low or absent. Where they occur, their abundance, diversity, and symbiotic effectiveness vary considerably across locations due to differences in soil properties, environmental conditions, and land-use history (Gitonga et al., 2021; Nakei et al., 2022).

Soybean (*Glycine max* L.) is not indigenous to Africa. Consequently, many African soils lack abundant and effective *Bradyrhizobium* strains for soybean nodulation. As a result, inoculation with effective strains such as *Bradyrhizobium japonicum or Bradyrhizobium diazoefficiens* is often necessary to achieve effective nodulation, biological nitrogen fixation, and optimum grain yield (Jaiswal & Dakora, 2019; Kyei-Boahen et al., 2023; Jarecki et al., 2024). Although earlier studies suggested that indigenous rhizobial populations exceeding approximately 1,000 cells g^{-1} soil are generally adequate for successful soybean nodulation, recent research emphasizes that the effectiveness and competitiveness of rhizobial strains together with soil fertility, environmental conditions, and soybean genotype are equally important determinants of nodulation and nitrogen fixation (Lindström & Mousavi, 2020; Nakei et al., 2023).

Consequently, soybean inoculation is recommended in locations where compatible indigenous rhizobia are absent, present at low population levels, or are ineffective. Inoculation enhances nodulation, biological nitrogen fixation, nitrogen uptake, and grain yield, particularly in soils with no previous history of soybean cultivation (Chibeba et al., 2023; Savala et al., 2022). Furthermore, inoculation is generally recommended when soybeans are introduced into a field for the first time because compatible soybean-nodulating rhizobia are often absent or insufficient to establish an effective symbiosis (Savala et al., 2022; Nakei et al., 2023).

Therefore, this study was conducted to evaluate soybean yield response to inoculation with the Legumefix inoculant and to determine the abundance of indigenous soybean nodulating rhizobia in the study soils. The findings will provide baseline information on native rhizobial populations and their potential utilization in developing effective inoculants and improving sustainable soybean production.

MATERIALS AND METHODS

Description of the study area

The experiments were conducted in the laboratory and the screen house of the Department of Soil Science at Sokoine University of Agriculture (SUA), Morogoro, located at 06°50' S, 37°38' E, and at an altitude of 526 m. a.s.l. The study area experiences a bimodal rainfall pattern, ranging between 500 and 800 mm per year, characterized by two rainfall peaks, with a distinct dry season separating the short and long rains (Magang & Lou, 2024). The short rain season runs from October to December, while the long rain season runs from March to May (TMA, 2022). In general, the climate of Morogoro District is sub-humid tropical.

Soil sampling and analysis

Soil samples from Uyole, Mkuyuni, and Kilosa were collected to a depth of 0 – 30 cm and then brought to the SUA Department of Soil Science Laboratory for analysis of both physical and chemical parameters. The physical and chemical properties of the soil samples determined included soil pH, particle-size distribution, cation exchange capacity, exchangeable bases, total nitrogen, organic carbon, extractable phosphorus, and extractable micronutrients.

Enumeration of indigenous rhizobia

Three soil samples from Uyole (Mbeya Municipal, Mbeya Region), Kilosa (Kilosa District, Morogoro Region), and Mkuyuni (Morogoro Rural, Morogoro Region) were used to prepare dilutions for plant inoculation. For each soil, serial dilutions were done by weighing 10 g of soil into 90 ml of sterile distilled water. After mixing the water and soil to make suspensions, 1 ml of the suspension (10-1) was drawn and diluted with 9 ml of water to make the second dilution (10-2). One ml was drawn from 10-2 into another 9 ml to make the 10-3 dilution, and finally, a 10-4 dilution was made. For each soil, the last three dilutions (10-2, 10-3, and 10-4) were used to inoculate soybean seedlings in modified Leonard jars, with three replications arranged in a completely randomized design (CRD). To enumerate indigenous rhizobia in experimental soils, the most probable number (MPN) plant-infection technique, as detailed by Senthilkumar et al. (2020), was used. To calculate the most probable number of organisms in the original sample, *p1,* which represented the number of positive tubes in the most concentrated dilution in which the greatest number of tubes is positive, was selected. After letting *p2* and *p3* represent the numbers of positive tubes in the next two highest dilutions, the obtained dilutions were tabulated using MPN tables, and the MPN value for the original sample was determined (Senthilkumar et al., 2020; Nakei et al., 2022).

Pot experiment

The pot experiment was conducted in the screen house at the Department of Soil Science, SUA main campus, located at 06° 51' S and 37° 39' E. Soils from Uyole, Mkuyuni, and Kilosa were used in the pot experiment. The soils were weighed in separate pots, and the pots were arranged by soil source. The arrangement of pots followed a Completely Randomized Design (CRD) with three replications and five treatments. For these three soils, each had 15 pots; hence, the whole experiment had 45 pots.

The five treatments applied were: Control (0 kg P, 0 kg N, and no inoculant) (T1), Inoculation with Legumefix alone (T2), 30 kg P alone (T3), 30 kg P + Inoculation with Legumefix (T4), and 30 kg P + 60 kg N (T5) as a reference treatment. The source of P was potassium dihydrogen orthophosphate (KH2PO4), and that of N was Ammonium Sulfate ((NH4)2SO4). All these treatments were applied during planting.

Preparation of planting materials

The Uyole Soya 1 was used in this study. The seeds were selected and cleaned in water to remove storage chemicals and then surface sterilized in 3% Hydrogen peroxide (H2O2) for two minutes, then in alcohol (70% ethanol) for two minutes again. Seeds were subsequently rinsed five times with distilled water to remove excess hydrogen peroxide and alcohol. After sterilization, the seeds to be inoculated were separated from the rest, and the Legumefix inoculant was applied. About 2.5 g of Legumefix was applied to 250 g of soybean seeds, and the mixture was mixed well to ensure effective coating of the seeds with the inoculant before planting.

Treatment application, planting, and management of pot experiments

Fertilizer treatments were applied in the potted soils 2 days before planting by evenly spreading the fertilizers in solution form on the respective potted soils, followed by thorough mixing. The pots were irrigated to field capacity, and planting was done 2 days later. To avoid contamination, non-inoculated seeds were planted first, followed by inoculated seeds. The soil moisture in the pots was maintained at field capacity by timely application of distilled water to replenish moisture lost through evapotranspiration. Six to eight seeds were planted per pot, then thinned to four plants, after which two were uprooted at 50% flowering for the determination of nodule number and shoot biomass. Two plants were retained to maturity, where pod numbers and grain yield were determined.

Isolation of rhizobia from nodules of soybean plants harvested from a potted-soil experiment

The Yeast Mannitol Agar medium was prepared by dissolving 10 g of mannitol, 0.5 g of K2HPO4, 0.2 g of MgSO4, 0.1 g of NaCl, 2 g of yeast extract, and 10 g of agar into 1000 ml of distilled water in 0.5 L screw cap bottles. The medium was well mixed and sterilized by autoclaving at 1.05 kg/ cm2 (15 pounds per square inch (psi)) and 121°C for 15 minutes. The sterile medium was left to cool in a water bath maintained at 50°C, ready for plating. Petri dishes and pipettes were sterilized in an oven at 170°C for 24 hours prior to use.

Fresh nodules from harvested plants were selected from each pot containing non-inoculated soil. Large, healthy nodules were selected and stored in silica gel nodule storage vials, awaiting preparation of rhizobia isolation medium. Once the media were prepared, the nodules were removed from the silica gel vials and placed in vials containing sterile distilled water. They absorbed moisture and regained their previous fresh sizes. The nodules were then surface-sterilized with 97% alcohol for 2 minutes, rinsed with sterile distilled water, and placed in 20% H2O2 for 3 minutes, followed by 6 rinses with sterile distilled water.

The nodules were crushed in a drop of sterile distilled water in a Petri dish with a sterilized forceps. The nodule contents were streaked with an inoculation wire loop onto yeast-mannitol agar (YMA) plates fortified with Congo red dye (Kebede et al., 2022; Adjei-Amankwah et al., 2022) and incubated at 28 °C. The Petri dishes were sealed and labeled accordingly. The forceps and loops were flamed before streaking the contents from different nodules to avoid cross-contamination. The bench of the laminar flow hood was also surface-sterilized with 70% alcohol before rhizobia isolation.

Data collection

For the pot experiment, collected data included nodule numbers, nodule dry weights, shoot dry matter, and shoot total N. For the enumeration experiment, nodule counts were recorded for MPN calculation. Soil analysis data were also collected for assessing the fertility status of the studied soils.

Data analysis

Data from the pot experiment were analyzed using GenStat software. Normality was first assessed with the Shapiro-Wilk test, followed by analysis of variance (ANOVA) to evaluate soybean yield responses to inoculation and fertilization. Treatment means were separated using Duncan's New Multiple Range Test (DMRT) at the 5% significance level (De Souza et al., 2023).

RESULTS

The physical, chemical and biological properties of the composite soil samples from the study areas

A selection of the physical, chemical, and biological properties of the three composite soil samples from Uyole, Kilosa, and Mkuyuni is presented in Table 1. Observations from the MPN plant infection technique are presented in Table 2.

Table 1. Chemical and physical properties of the composite soil samples of Uyole, Kilosa and Mkuyuni

Chemical and physical properties parameters	Values			Rating		
	Uyole	Kilosa	Mkuyuni	Uyole	Kilosa	Mkuyuni
Sand, %	52.24	72.24	76.24			
Silt, %	22.64	5.64	5.64	Sandy clay loam	Sandy clay loam	Sandy loam
Clay, %	25.12	22.12	18.12			
pH (H_2O)	6.25	6.73	6.63	Slightly acid	Very slightly acid	NeutraL
Organic carbon, %	1.57	1.67	2.17	Medium	Medium	Medium
Organic matter (%)	2.70	2.87	3.73	Medium	Medium	Medium
Total nitrogen, %	0.12	0.11	0.15	Low	Low	Low
Bray 1 P, mg/kg	10.30	10	4.43	Medium	Medium	Low
Exchangeable Ca, cmolc (+)/kg	11.40	15.5	17.39	Very high	Very high	Very high
Exchangeable Mg, cmolc (+)/ kg	1.10	2.5	45.04	High	Very high	Very high
Exchangeable Na, cmolc (+)/ kg	0.70	0.2	0.18	Medium	Low	Low
Exchangeable K, cmolc (+)/ kg	3.09	1.03	0.9	Very high	Very high	High
CEC, cmolc (+)/ kg	26.00	18.8	34.3	High	Medium	High
Base saturation (%)	63	76.14	63.94	High	High	High
DTPA extractable Fe, mg/kg	52.89	39.48	18.93	Very high	Very high	Very high
DTPA extractable Mn, mg/kg	90.05	33.63	45.04	Very high	Very high	Very high
DTPA extractable Zn, mg/kg	8.13	0.84	1.87	High	Low	High
DTPA extractable Cu, mg/kg	3.68	1.55	1.12	High	High	High
Available S	6.5	12.46	3.98	Low	Low	Very low
C:N ratio	12.76	15.45	14.45	Good quality	Moderate quality	Moderate quality

Table 2. Populations of soybean rhizobial cells in the soils from Uyole, Kilosa and Mkuyuni

Soil	Number of bacteria (cells g^{-1} soil)
Uyole soil	4.5 X 10^2
Kilosa soil	2.0 X 10^2
Mkuyuni soil	6.8 X 10^2

Growth performance of soybean to rhizobial inoculation and fertilizer P and N application to the soils from Uyole, Kilosa and Mkuyuni

Results showed that rhizobial inoculation and fertilizer applications significantly influenced soybean nodulation parameters in Uyole soil (Table 3). Nodule number per plant differed significantly ($p<0.05$) among treatments, ranging from 1 to 26 nodules per plant^{-1}; the combination of 30 kg P ha^{-1} and 60 kg N ha^{-1} produced the fewest (1), while 30 kg P ha^{-1} alone produced the most (26). Legumefix inoculant (25) yielded the most, with the control (19) and 30 kg P ha^{-1} + inoculant (21) intermediate. Nodule dry weight followed a similar pattern, varying from 1.0 to 78.0 mg plant^{-1}, with the highest in 30 kg P ha^{-1} alone (78.0) and the lowest in the N-fertilized treatment (1.0); other treatments ranged from 8.0 to 50.0 mg plant^{-1}. Shoot total nitrogen (TN) content showed slight variation (1.81–2.23%), with the lowest value in the 30 kg P ha^{-1} + inoculant treatment (1.81%).

Table 3. Effects of rhizobial inoculation and fertilizer P and N on soybean nodulation and nitrogen fixation in Uyole soil

Treatment	Nodule number plant^{-1}	Nodule dry weight, mg plant^{-1}	Shoot TN (%)
Control	19^b	8.0^b	2.01^a
30 kg P ha^{-1}	26^c	78.0^b	2.16^a
30 kg P ha^{-1} + 60 kg N ha^{-1}	1^a	1.0^a	2.23^a
Inoculant (Legumefix)	25^c	50.0^b	2.10^a
30 kg P ha^{-1} + Inoculant	21^b	44.0^b	1.81^a
CV %	12	42.5	10.5

Note: TN = Total nitrogen; means with the same letter in a column are not significantly (*P≤0.05*) different using DMRT; CV = Coefficient of variation; N = Nitrogen; P = Phosphorus; P=Phosphorus; ha= hectare; kg = kilogram

Effects of rhizobial inoculation and fertilizer P and N on soybean nodulation and nitrogen fixation in Kilosa soil

Rhizobial inoculation and P and N fertilizers affected soybean nodulation and nitrogen fixation parameters in Kilosa soil (see Table 4). Nodule number per plant ranged from 2 to 15 nodules plant^{-1}, with the highest value (15) in the 30 kg P ha^{-1} + Legumefix inoculant treatment and the lowest (2) in 30 kg P ha^{-1} + 60 kg N ha^{-1}, while other treatments were intermediate. Nodule dry weight varied from 20 to 80 mg plant^{-1}, with the highest (80) recorded in Legumefix inoculant alone. At the same time, the lowest (20) was observed in the control and N-fertilized treatments with 30 kg P ha^{-1} and in the combined P + inoculant intermediate treatment. Shoot total nitrogen (TN) content ranged from 1.42% to 2.22%, with 30 kg P ha^{-1} alone having the lowest (1.42%) and the control having the highest (2.22%); the remaining treatments were similar, ranging from 1.96% to 2.18%.

Table 4. Effects of rhizobial inoculation and fertilizer P and N on soybean nodulation and nitrogen fixation in Kilosa soil

Treatment	Nodule number plant^{-1}	Nodule dry weight, mg plant^{-1}	Shoot TN (%)
Control	4^{ab}	20^{a}	2.22^{b}
30 kg P ha^{-1}	4^{ab}	30^{ab}	1.42^{a}
30 kg P ha^{-1} + 60 kg N ha^{-1}	2^{a}	20^{a}	2.18^{b}
Inoculant (Legumefix)	10^{ab}	80^{b}	2.01^{b}
30 kg P ha^{-1} + Inoculant	15^{b}	50^{ab}	1.96^{b}
CV %	75.6	74.9	12.6

Note: TN = Total nitrogen, means with same letter in a column are not significantly (*P≤0.05*) different using DMRT, CV=Coefficient of variation, N=Nitrogen, P=Phosphorus, ha= hectare, kg=kilogram

Effects of rhizobial inoculation and fertilizer P and N on soybean nodulation and nitrogen fixation in Mkuyuni soil

Rhizobial inoculation significantly ($P < 0.05$) enhanced nodulation in soybean grown in Mkuyuni soil (Table 5). The Legumefix inoculant alone produced the highest nodule number per plant (20), followed by 30 kg P ha^{-1}+ inoculant (17), while 30 kg P ha^{-1}+ 60 kg N ha^{-1} produced none (0). Nodule dry weight (mg plant^{-1}) followed suit, with the inoculant alone at 0.13 mg plant^{-1}, 30 kg P ha^{-1}+ inoculant at 0.09 mg plant^{-1}, and no production from 30 kg P ha^{-1} + 60 kg N ha^{-1} (0 mg plant^{-1}), while the control and P alone yielded 0.03 mg and 0.04 mg plant^{-1}, respectively. Shoot total nitrogen (TN) content increased similarly with inoculant alone (1.81%) or with P (1.79%), exceeding the control (1.27%) and P alone (0.91%), though 30 kg P ha^{-1} + 60 kg N ha^{-1} suppressed nodulation (0 nodules plant^{-1} and 0 mg plant^{-1}) but boosted TN to 2.22%.

Table 5. Effects of rhizobial inoculation and fertilizer P and N on soybean nodulation and nitrogen fixation in Mkuyuni soil

Treatment	Nodule number plant^{-1}	Nodule dry weight, mg plant^{-1}	Shoot TN (%)
Control	7^{ab}	0.03^{a}	1.27^{ab}
30 kg P ha^{-1}	3^{a}	0.04^{ab}	0.91^{a}
30 kg P ha^{-1} + 60 kg N ha^{-1}	0^{a}	0.00^{a}	2.22^{c}
Inoculant (Legumefix)	20^{c}	0.13^{c}	1.81^{bc}
30 kg P ha^{-1} + Inoculant	16^{bc}	0.09^{bc}	1.79^{bc}
CV %	58.2	42.5	19.1

Note: means with the same letter in a column are not significantly (*P≤0.05*) different using DMRT

Plant color scores across locations

Plant color scores (1-3 scale) differed significantly among treatments ($p < 0.05$) across Uyole, Kilosa, and Mkuyuni soils (Figure 1). In Uyole, all treatments except 30 kg P ha^{-1} + 60 kg N ha^{-1} produced similar low scores (2.0–2.3), while the combined N + P treatment scored highest (3.0). In Kilosa, the control and 30 kg P ha^{-1} alone had the lowest scores (1.3), inoculation treatments scored moderately higher (2.0), and N + P again scored highest (3.0). Similarly, in Mkuyuni, the control scored lowest (1.0), followed by 30kg P ha^{-1} (1.6), inoculation alone or with P (2.0), and N + P highest (3.0). Across all locations, the combined 30 kg P ha^{-1} + 60 kg N ha^{-1} treatment consistently produced the highest scores, while controls had the lowest. Inoculation, alone or with P, moderately increased scores relative to controls.

Shoot dry matter (g plant^{-1}) across locations

Shoot dry matter (g plant^{-1}) differed significantly among treatments ($p<0.05$) across Uyole, Kilosa, and Mkuyuni soils (Figure 2). In Uyole, 30 kg P ha^{-1} + 60 kg N ha^{-1} produced the highest value (2.2 g plant^{-1}), exceeding the control and 30 kg P ha^{-1} alone (1.5 g plant^{-1}); inoculation alone or with P yielded the lowest (0.9 g plant^{-1}). A similar pattern occurred in Kilosa, where N + P was highest (2.2 g plant^{-1}), control, P alone, and inoculant alone were intermediate (1.4–1.5 g plant^{-1}), and P + inoculant was moderately higher (1.9 g plant^{-1}). In Mkuyuni, values were generally lower, with inoculant alone performing best (1.6 g plant^{-1}), followed by P + inoculant (1.4 g), while control, P alone, and N + P were similar and lowest (1.1–1.3 g). Moreover, P + N consistently maximized shoot dry matter in Uyole and Kilosa, but the inoculant alone excelled in Mkuyuni.

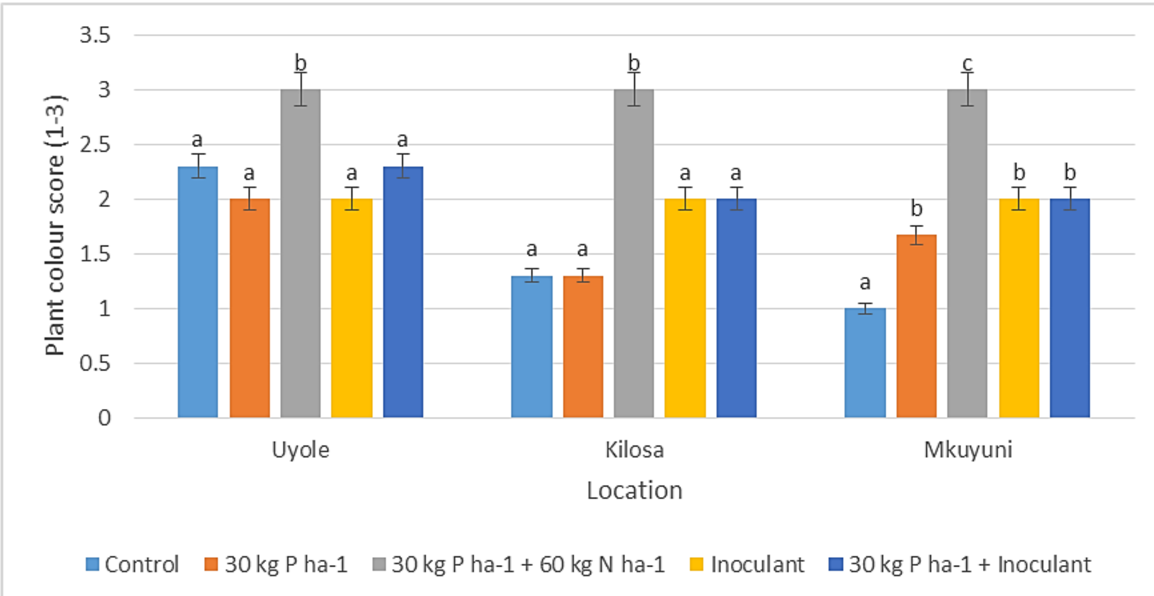

Figure 1. Effects of rhizobial inoculation and fertilizer P and N on soybean plant colour score in the Uyole, Kilosa and Mkuyuni soils

Effects of rhizobial inoculation and fertilizer P and N on soybean pod number plant^{-1} in the Uyole, Kilosa and Mkuyuni soils

Figure 3 illustrates the effects of rhizobial inoculation and varying rates of phosphorus (P) and nitrogen (N) fertilizers on soybean pod production across three locations: Uyole, Kilosa, and Mkuyuni. The results revealed that soybean performance differed significantly among the various treatment applications. In both Uyole and Kilosa, the application of 30 kg P ha^{-1} + 60 kg N ha^{-1} produced the highest number of pods per plant, which significantly outperformed the control and other individual treatments. Although pod numbers in Mkuyuni were generally lower than at the other sites, the 30 kg P ha^{-1} + 60 kg N ha^{-1} combination remained the most effective, showing a statistically significant improvement over the control. Across all three locations, this combined application consistently resulted in the highest average number of pods per plant. Conversely, individual applications of phosphorus or simple rhizobial inoculation yielded lower, less consistent results that often performed near the level of the control group. These findings suggest that soybean pod production in these soil types is significantly more responsive to a balanced supply of P and N than to inoculation or phosphorus fertilization alone.

Effects of Rhizobial Inoculation and Varying Phosphorus (P) and Nitrogen (N) Rates on Soybean Yield

Effects of rhizobial inoculation and varying rates of phosphorus (P) and nitrogen (N) fertilizers on soybean yield (g plant^{-1}) are presented in Figure 4. The results revealed that soybean yield differed significantly among treatments at all sites. In both Uyole and Kilosa, the combined application of 30 kg P ha^{-1} + 60 kg N ha^{-1} produced the highest yields (g plant^{-1}), significantly outperforming the control and other individual treatments. Yields in Mkuyuni were generally lower than at the other sites. However, the 30 kg P ha^{-1} + 60 kg N ha^{-1} combination remained the most effective, showing statistically significant improvements over the control and 30 kg P ha-^{1}. Across all three locations, this treatment consistently resulted in the highest average soybean yield (g plant^{-1}). In contrast, individual applications of phosphorus or rhizobial inoculation alone yielded lower results, with less consistent results that often performed similarly to the control. These findings suggest that soybean pod production in these soil types responds more strongly to balanced P and N fertilization than to inoculation or phosphorus alone.

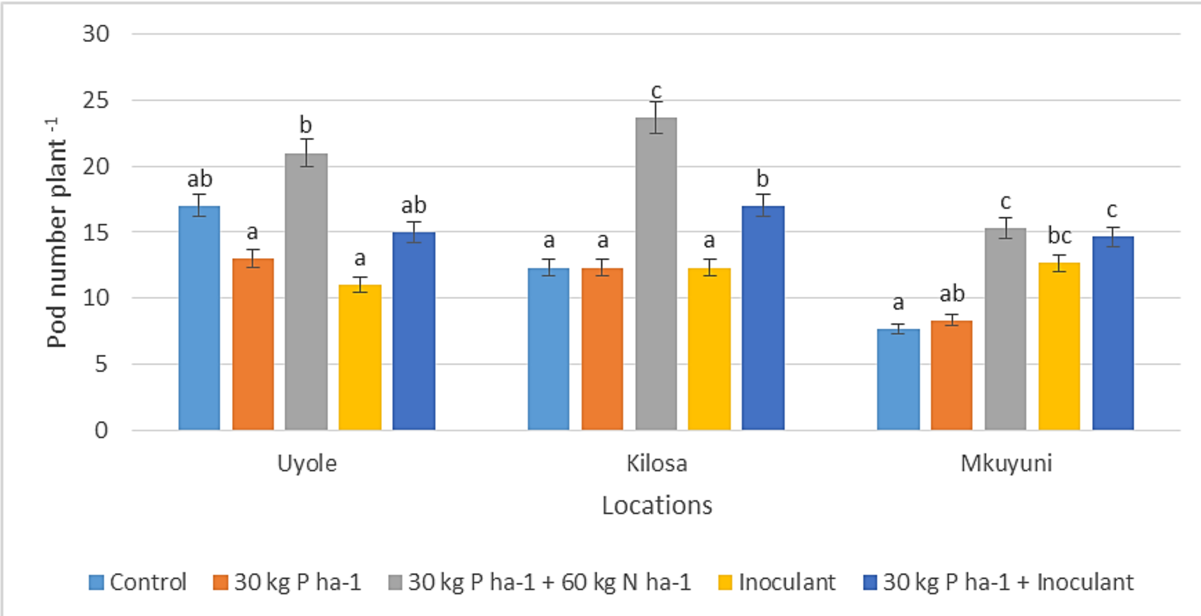

Figure 2. Effects of rhizobial inoculation and fertilizer P and N on soybean pod number plant $^{-1}$ in the Uyole, Kilosa and Mkuyuni soils

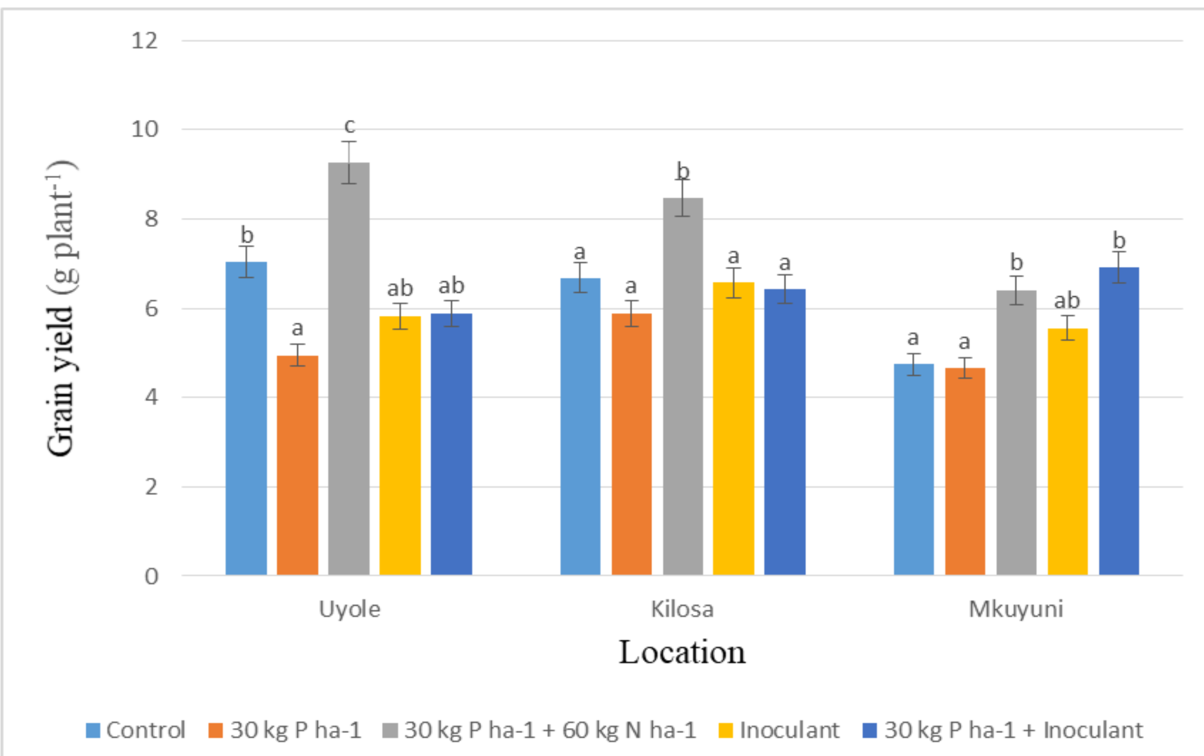

Figure 3. Effects of rhizobial inoculation and fertilizer P and N on soybean plant growth and yields in the Mkuyuni soil.

DISCUSSION

Nodule numbers and weights

For each soil, the effect of each treatment on nodulation was assessed by quantifying two key nodulation parameters, namely nodule number and dry nodule weight per plant. Results show that for all soils, nodule numbers per plant were significantly (*P≤0.05*) higher in inoculant-only and inoculant plus mineral P fertilizer applied treatments than in mineral P or mineral P plus N fertilizer-applied treatments. Similarly, dry nodule weight per plant values were significantly (P≤0.05) greater in the inoculant-only treatment, followed by the fertilizer P plus inoculant-applied treatment. As expected, in all three soils, nodulation was poorest in the treatments that received a combination of recommended rates of mineral fertilizer P plus N without any inoculant (Tables 3, 4, and 5). The significant (P≤0.05) effects of Legumefix inoculant and P on nodulation of soybean (Tables 3, 4, and 5) indicate a positive response of soybean to inoculation and P treatments. The very few nodules in the reference treatment where P and N were both applied may be due to N's suppressive effect on nodulation (Liu et al., 2010).

Results show that the Legumefix inoculant rhizobia were less competitive than the native rhizobia (control treatment) when the inoculant was added together with P in all the soils. The reasons for this are not clear, as it contradicts other research, such as that by Ibrahim and Ibrahim (2019), Akello et al. (2022), and Szpunar-Krok et al. (2023), which reported an increase in nodulation and yield response when P was used. The relatively higher nodule numbers observed on inoculated treatments across all three soils are consistent with observations by Salih et al. (2015), who reported an increase in nodule numbers per plant when soybean was inoculated with *Bradyrhizobium* inoculants.

Shoot total Nitrogen (TN)

N2 fixation, on the other hand, was assessed by monitoring shoot total nitrogen (TN) accumulation. Overall, compared to the control, the treatment that received mineral fertilizer P plus N at the recommended rates had the highest shoot total N, though not significantly different ($P < 0.05$), in the Mkuyuni and Uyole soils (Tables 3 and 5). Generally, no significant ($P< 0.05$) differences were observed between inoculant only, inoculant plus fertilizer P, and fertilizer P only treatments on total shoot N accumulation (Tables 3, 4, and 5). The similarity in total N values in plant biomass was probably due to the presence of resident native rhizobia in these soils, which are more effective and competitive than introduced strains. Literature suggests that when legumes are applied with mineral nitrogen, less N accumulates in shoots than when inoculated with rhizobia (Getachew & Dagnaw, 2020; Księżak & Bojarszczuk, 2022; Win et al., 2023). A study by Bais et al. (2023) and Santos et al. (2022) presented results similar to those observed in this study, where total N accumulation did not differ significantly between the applied treatment combinations. However, when N is applied in abundance, its effects on dry matter (DM) and TN accumulation can be observed. However, reports on N accumulation by legumes in the field following mineral N application have been variable, ranging from no response to substantial (Księżak & Bojarszczuk, 2022).

Plant color

In all three soils, the plant colors varied according to treatments applied as presented in Tables 6, 7 and 8 for Uyole, Kilosa and Mkuyuni soils, respectively. There were significant (*P≤0.05*) differences in color for soybean plants in all the three soils, whereas most plants in the control and P-only treatments were yellowish in color, a symptom of N deficiency, while a deep green was observed in plants applied with N (and P). For the inoculated-only treatment and P + inoculant, the plants were green in color but not as deep as in the P + N treatment, where the color of plants was deep green. Nitrogen deficiency can be identified as a yellowing or chlorosis of the lower leaves in the canopy, as N is important for formation of chlorophyll (Sakuraba, 2022; Kiba & Kojima (2026). Chlorosis of plants in the control and other uninoculated plants is due to low N supply in soils (Table 1), which is also reflected by low percent Total N of plant material (Tables 3, 4 and 5).

Shoot dry matter

Shoot dry matter (DM) results for Uyole, Kilosa, and Mkuyuni soils are presented in Tables 6, 7, and 8, respectively. There were significant (*P≤0.05*) differences in shoot DM values among treatments of the Uyole and Mkuyuni soils, but not the Kilosa soil. Results further showed that DM was significantly (P≤0.05) higher in the N + P treatment in the Uyole and Mkuyuni soils, due to the applied N fertilizer. The lack of significant

($P \leq 0.05$) differences in soybean shoot DM for Kilosa soil (Table 7) may be due to the effectiveness of rhizobia in the soil, which fixed nitrogen and thus provided shoot DM that was statistically similar between the inoculated and fertilizer treatments. The observations from Uyole and Kilosa soils are consistent with those of Patra et al. (2012) and Bekere and Hailemariam (2012), who reported no statistical differences in soybean shoot biomass between inoculated and control treatments. These observations are consistent with reports by Salih et al. (2015) that, in general, the interaction between chemical and bio-fertilizers significantly increased the relative growth rate of soybean, which was associated with higher shoot DM. In contrast, Rechiatu et al. (2015) also showed that Legumefix increased soybean shoot biomass as compared to uninoculated treatments.

Pod numbers

Similar to observations made on shoot DM in the present study, significant ($P \leq 0.05$) effects of P + N fertilizers on soybean pod number per plant for Uyole, Kilosa and Mkuyuni soils (Tables 6, 7 and 8) were probably due to the effect of the N and P fertilizers on growth of soybean plants. The lowest number of pods in the inoculated-only treatment may imply a lesser competitive ability of the inoculant rhizobia against native rhizobial strains in colonizing and infecting soybean roots. There have been mixed results from similar studies on the comparative effects of inoculants and chemical fertilizers on plant growth and yield. For example, Janagard et al. (2013) reported no statistical differences in pod numbers and yield between the inoculated, non-inoculated, and chemical fertilizers treatments. In a different study, Jaiswal and Dakora (2019) and Kanomanyanga et al (2022) reported good performance of the control treatment compared to the inoculated treatment, with no significant difference in pod numbers between the treatments, which already had high numbers of soybean rhizobia in the soil. On the other hand, there have been reports of increased pod formation when soybean plants are supplied with P and inoculated (Salih et al*.,* 2015). Similarly, in two independent studies, both Dominic et al. (2014) reported increases in pod number, biomass production, and soybean yield due to rhizobia inoculation in an experiment conducted under farmers' conditions.

Grain yields

The grain yields for the Uyole, Kilosa, and Mkuyuni soils are presented in Tables 6, 7, and 8, respectively. In all three soils, significant ($P \leq 0.05$) differences were observed, with higher yields recorded in Uyole and Kilosa soils under the P + N treatment. This could be attributed to the fertilizers, especially nitrogen (N). The Mkuyuni soil had a significantly ($P \leq 0.05$) higher grain yield under P + Legumefix inoculant, followed by the P + N treatment. Similar to Uyole and Kilosa soil observations, studies by Cordeiro & Echer, 2019 reported that N application significantly ($P \leq 0.05$) decreased nodulation. However, they increased vegetative growth and yield in leguminous crops, although this did not necessarily increase the indigenous Bradyrhizobium population density in soil. The observation from the Mkuyuni soil is supported by Merriam et al. (2023) and Chibeba et al. (2023), who reported that the yield and nutritional quality of legumes are greatly influenced by the combined application of phosphorus fertilizers and rhizobial biofertilizers, which enhance biological nitrogen fixation and overall plant performance.

CONCLUSIONS

According to the soil analysis, all three experimental soils were fertile to moderately fertile and therefore suitable for growing soybeans and most other crops. The soils used in this study contained few indigenous rhizobia, which are effective in fixing nitrogen with soybeans. When inoculated in an N-free nutrient solution, the native rhizobial isolates from Uyole and Kilosa soils did not produce a significant ($P \leq 0.05$) difference in nodule numbers for soybeans when compared to the Legumefix inoculant. Regardless of differences in soybean response to applied treatments, soybean-growing soils must be analyzed for general fertility status to assess their suitability for the crop and determine the types and amounts of inputs to improve overall crop growth and yield. Also, experiments to test for the presence of native rhizobia in these and other soils should be conducted before applying the inoculants, because there may be infective and effective rhizobia within the soil capable of nodulating and fixing N with soybeans. This study should serve as a baseline for other researchers to explore additional areas in the country producing soybeans and to assess their suitability and the inputs needed to improve the production of this crop.

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AUTHORS' CONTRIBUTIONS

All the authors contributed equally to this research work.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Not applicable.

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Not Applicable.

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AVAILABILITY OF DATA AND MATERIALS

All datasets analyzed and described during the present study are available from the corresponding author upon reasonable request

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