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Optimizing soybean (*Glycine max* L. Merr.) performance through rhizobial inoculation and planting density in Kétou, Benin

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ABSTRACT

Declining soil fertility, particularly nitrogen deficiency, remains a major constraint to soybean productivity in Benin. This study evaluated the effects of rhizobial inoculation and planting density on the growth and grain yield of soybean (*Glycine max* (L.) Merr.) variety TGX1910-14F under field conditions in Kétou, central Benin. A factorial field experiment comprising two inoculation levels (inoculated and non-inoculated) and three planting densities corresponding to row spacings of 50, 60, and 70 cm (20 cm within rows) was conducted in six farmers' fields. The six treatment combinations were replicated six times, giving a total of 36 experimental units. Plant height, leaf number, leaf length, and leaf width were measured at four growth stages (S1–S4), while grain yield was determined at harvest. Data were analyzed using two-way analysis of variance (ANOVA) followed by Tukey's HSD test at the 5% significance level. Rhizobial inoculation significantly increased leaf number (p= 0.0125) and plant height (p= 0.0055), whereas planting density had no significant effect on these traits. Inoculated plants also exhibited greater leaf dimensions than non-inoculated plants throughout the growth period. Grain yield was significantly affected by inoculation ($p < 0.001$), planting density ($p < 0.001$), and their interaction (p= 0.0014). The highest grain yield (1,855.53 kg ha^{-1}) was obtained from the combination of inoculation and 50 cm × 20 cm spacing (T1), while the lowest yield (677.77 kg ha^{-1}) was recorded in the non-inoculated treatment at 70 cm × 20 cm spacing (T6). The results demonstrate that rhizobial inoculation substantially improves soybean growth and productivity and that its benefits are maximized at a planting density of 50 cm × 20 cm. This management combination is recommended for sustainable soybean production in the Kétou agro-ecological zone of Benin.

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INTRODUCTION

Soybean (*Glycine max* (L.) Merr.) is one of the most important grain legumes cultivated worldwide because of its high protein and oil contents, multiple food and industrial uses, and capacity to improve soil fertility through biological nitrogen fixation. In sub-Saharan Africa, soybean production has expanded considerably during the last two decades owing to increasing demand for human consumption, livestock feed, and agro-industrial processing. In Benin, soybean has become a strategic crop for both food security and income generation, particularly in the central and northern regions of the country (Kpenavoun *et al.*, 2018).

Despite the expansion of cultivated area, soybean productivity in Benin remains relatively low compared with its genetic potential. Soil fertility degradation, especially nitrogen deficiency, is recognized as one of the major factors limiting crop productivity in many soybean-growing areas (Chabi *et al.*, 2019). Recent studies have further identified nitrogen and zinc deficiencies as key nutritional constraints affecting soybean performance in central and northern Benin (Chabi *et al.*, 2023). Consequently, the development of sustainable and affordable soil-fertility management strategies is essential for improving soybean production under smallholder farming conditions.

Rhizobial inoculation has emerged as an effective technology for enhancing soybean productivity through biological nitrogen fixation. The symbiotic association between soybean roots and compatible *Bradyrhizobium* strains improves nitrogen availability, promotes vegetative growth, and increases grain yield while reducing dependence on mineral nitrogen fertilizers. Several studies conducted in Africa have demonstrated significant improvements in soybean growth and productivity following inoculation with effective rhizobial strains (Ulzen *et al.*, 2020; Deka *et al.*, 2024). In Benin, Houngnandan *et al.* (2020) reported substantial increases in soybean growth and grain yield following rhizobial inoculation, highlighting the potential of this technology for sustainable crop intensification.

In addition to biological nitrogen fixation, planting density is a key agronomic factor influencing crop performance. Appropriate plant population affects canopy development, light interception, nutrient uptake, and resource-use efficiency, which ultimately determine grain yield. Previous studies have shown that soybean yield can be significantly affected by row spacing and plant density, although the optimum spacing often varies according to environmental conditions, cultivar characteristics, and management practices (Baboy *et al.*, 2015; Kena *et al.*, 2022; Li *et al.*, 2025). Therefore, identifying suitable planting densities is necessary to maximize the benefits of improved agronomic technologies.

The municipality of Kétou, located in central Benin, is an important soybean-producing area characterized by ferruginous soils with declining fertility. Although rhizobial inoculation has shown promising results in several regions of Benin, limited information is available regarding its interaction with planting density under the agro-ecological conditions of Kétou. Understanding how these factors influence soybean growth and yield is essential for developing practical recommendations for local farmers.

The present study was therefore conducted to evaluate the effects of rhizobial inoculation and planting density on the growth and grain yield of soybean variety TGX1910-14F under field conditions in Kétou, Benin. Specifically, the study aimed to determine the extent to which inoculation and planting density influence vegetative growth, leaf development, and grain yield, and to identify the most productive management combination for soybean production in the study area.

MATERIALS AND METHODS

Study area

This study was conducted in the municipality of Kétou, located in the northern part of the Plateau Department, Republic of Benin (7°15′–7°30′ N; 2°20′–2°40′ E; Fig. 1). Kétou is situated approximately 140 km from Cotonou and 108 km from Porto-Novo, the political capital of Benin, and covers an area of about 2,183 km^2. The municipality is bordered by Savè to the north, Pobè to the south, Ouinhi and Zangnanado to the west, and the Federal Republic of Nigeria to the east. The climate is tropical with a bimodal rainfall regime characterized by two rainy seasons and two dry seasons. Mean annual rainfall is approximately 1,003.4 mm, while the average annual temperature is about 27°C, with maximum temperatures reaching 34.5°C (INSAE, 2016). The dominant vegetation consists of wooded savanna established on ferruginous and ferralitic soils.

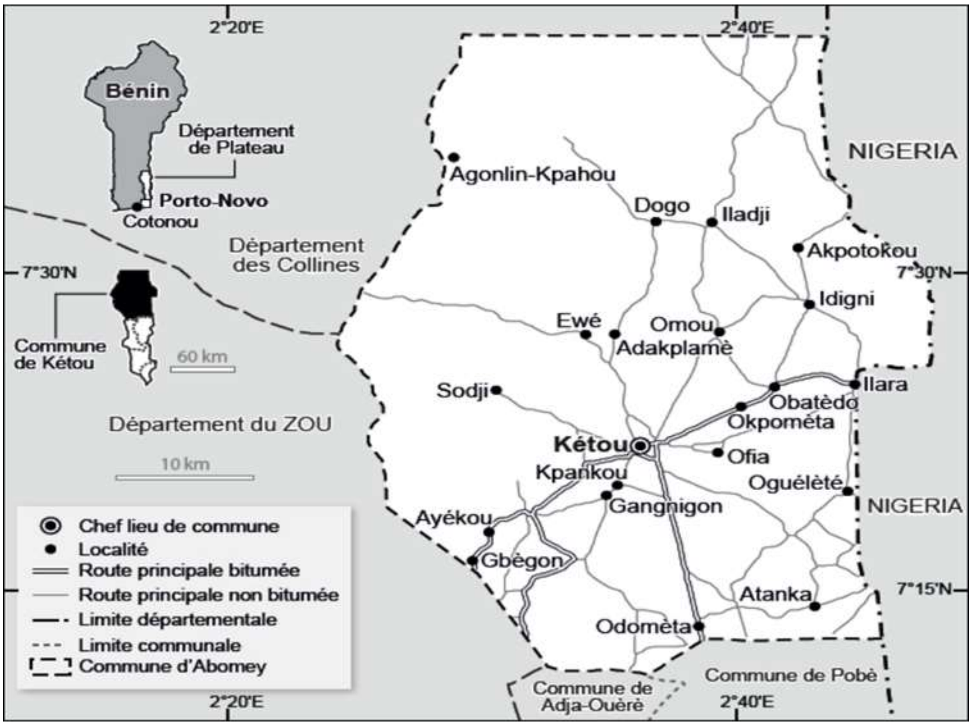

Fig. 1. Map of the municipality of Kétou (Plateau Department, Benin)

Experimental design and crop management

The experiment was established in six farmers' fields during the soybean growing season. A factorial treatment arrangement comprising two factors was used: rhizobial inoculation and planting density. The inoculation factor consisted of two levels:

1. Inoculated with *Bradyrhizobium japonicum*
2. Non-inoculated control

The planting-density factor consisted of three inter-row spacings:

1. 50 cm
2. 60 cm
3. 70 cm

For all treatments, intra-row spacing was maintained at 20 cm with three plants per hill after establishment.

The factorial combination of these factors generated six treatments:

T1: 50 cm × 20 cm + inoculation (300,000 plants ha^{-1})

T2: 60 cm × 20 cm + inoculation (250,000 plants ha^{-1})

T3: 70 cm × 20 cm + inoculation (214,286 plants ha^{-1})

T4: 50 cm × 20 cm without inoculation (300,000 plants ha^{-1})

T5: 60 cm × 20 cm without inoculation (250,000 plants ha^{-1})

T6: 70 cm × 20 cm without inoculation (214,286 plants ha^{-1})

Each treatment was replicated six times, with each farmer's field serving as one replicate, resulting in a total of 36 experimental units. Individual plots measured 3 m

× 3 m ($9\ m^2$) and were separated by 1-m buffer zones to minimize interference among treatments.

Certified seeds of soybean variety TGX1910-14F were obtained from the communal unit of the Territorial Agricultural Development Agency No. 6 (ATDA-6), Kétou. For inoculated treatments, seeds were coated immediately before sowing using a commercial *Bradyrhizobium japonicum* inoculum. The inoculum suspension was prepared by dissolving 25 sugar cubes in clean water and thoroughly mixing the solution with one 100-g sachet of inoculum before coating 15 kg of seed. Seeds assigned to the control treatments were sown without inoculation.

Manual weeding was carried out at 15, 30, and 45 days after sowing to maintain weed-free experimental plots throughout the cropping season.

Data collection

Growth observations were conducted at four successive sampling periods designated S1, S2, S3, and S4, corresponding to major vegetative growth stages of soybean development.

At each sampling period, ten plants were randomly selected from the central rows of each plot and tagged for measurement. The following variables were recorded:

Plant height

Plant height (cm) was measured from the collar to the terminal bud using a graduated ruler.

Number of leaves

The number of fully expanded leaves per plant was counted manually.

Leaf length

Leaf length (cm) was measured on the central leaflet of the most recently fully expanded trifoliate leaf.

Leaf width

Leaf width (cm) was measured at the widest portion of the central leaflet of the same trifoliate leaf.

For each variable, the average value of the ten sampled plants was calculated and used as the plot mean for subsequent analyses and graphical presentation.

Grain yield

At physiological maturity, soybean plants from the net plot area were harvested, threshed, cleaned, and air-dried. Grain weight was recorded and converted to kilograms per hectare ($kg\ ha^{-1}$).

Statistical analysis

Data were analyzed using R statistical software (version 4.0.5). Plot means were subjected to two-way analysis of variance (ANOVA) to evaluate the effects of inoculation, planting density, and their interaction on leaf number, plant height, and grain yield.
The statistical model included:

$$Y_{ijk}=\mu + I_i + D_j + (ID)_{ij} + \varepsilon_{ijk}$$

where:
(Y_{ijk})= observed value of the response variable,
(μ) = overall mean,
(I_i) = effect of inoculation,
(D_j)= effect of planting density,
($(ID)_{ij}$)= interaction between inoculation and planting density,
(ε_{ijk})= experimental error.

The ANOVA partitioned the total variation into inoculation (df= 1), planting density (df= 2), inoculation × density interaction (df= 2), and residual error (df= 30), giving a total of 35 degrees of freedom. When significant differences were detected, treatment means were separated using Tukey's Honest Significant Difference (HSD) test (Tukey, 1949) at the 5% probability level. Growth responses measured at S1–S4 were illustrated graphically to show temporal trends among treatments.

RESULTS

Effect of inoculation and planting density on leaf number

Leaf number increased progressively from S1 to S4 across all treatments (Fig. 2). Plants grown under inoculation generally produced more leaves than non-inoculated plants, particularly at the closer planting densities. At S4, treatment T1 (50 cm × 20 cm + inoculation) recorded the highest mean number of leaves (approximately 33 leaves plant^{-1}), whereas T5 (60 cm × 20 cm without inoculation) produced the lowest values (approximately 26 leaves plant^{-1}).

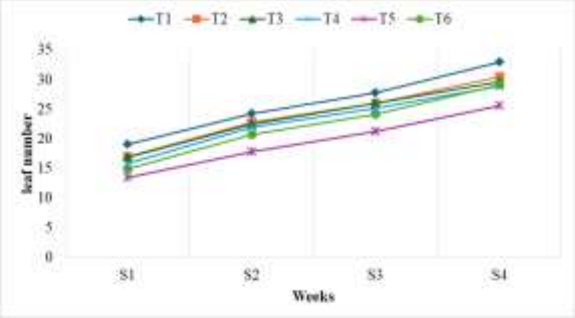

Fig. 2. Evolution of the number of leaves per plant across treatments over time (weeks after emergence)

Analysis of variance revealed that inoculation significantly affected leaf number (F = 7.06, *p* = 0.0125), while the effects of planting density (F = 1.82, *p* = 0.1795) and the inoculation × density interaction (F = 0.57, *p* = 0.5723) were not significant (Table 1). These results indicate that rhizobial inoculation was the principal factor influencing leaf production in soybean under the conditions of the study.

Table 1. ANOVA for leaf number

Source of variation	df	SS	MS	F-value	*p*-value
Inoculation (I)	1	1677	1677.00	7.06	0.0125 *
Density (D)	2	864	432.00	1.82	0.1795 ns
I×D	2	270	135.00	0.57	0.5723 ns
Error	30	7122	237.40	—	—
Total	35	9933	—	—	—

ns= not significant; *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$.

Effect of inoculation and planting density on plant height

Plant height increased steadily throughout the observation period for all treatments (Fig. 3). Treatment T1 consistently produced the tallest plants, reaching approximately 30.35 cm at S4, whereas T5 recorded the lowest plant height values. Inoculated treatments generally exhibited greater plant height than their corresponding non-inoculated controls.

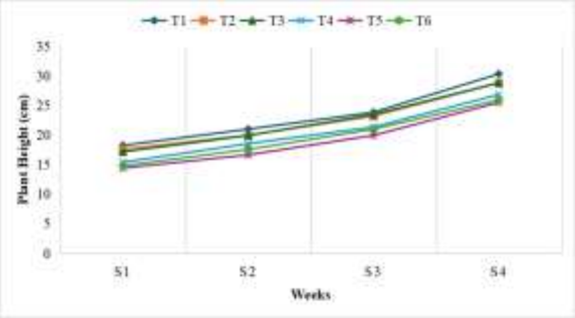

Fig. 3. Evolution of soybean plant height (cm) across treatments over time (weeks after emergence).

The ANOVA showed a significant effect of inoculation on plant height (F = 8.97, *p* = 0.0055), whereas planting density (F = 0.54, *p* = 0.5885) and the inoculation × density interaction (F = 0.05, *p* = 0.9501) were not significant (Table 2). These findings suggest that plant height was primarily influenced by rhizobial inoculation rather than by planting density.

Table 2. ANOVA for plant height

Source of variation	df	SS	MS	F-value	*p*-value
Inoculation (I)	1	1487	1487.00	8.97	0.0055 **
Density (D)	2	179	89.50	0.54	0.5885 ns
I×D	2	17	8.50	0.05	0.9501 ns
Error	30	4976	165.87	—	—
Total	35	6659	—	—	—

ns= not significant; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

Effect of inoculation and planting density on leaf dimensions

Leaf length and leaf width increased progressively from S1 to S4 in all treatments (Figs 4 and 5). For leaf length, the highest values were observed under T3 (70 cm × 20 cm + inoculation), which reached approximately 10.5 cm at S4. Treatments receiving inoculation generally exhibited longer leaves than non-inoculated treatments.

Similarly, leaf width was greatest in T1 (50 cm × 20 cm + inoculation), reaching approximately 6.9 cm at S4. The narrowest leaves were recorded in T5 (60 cm × 20 cm without inoculation). Across all observation periods, inoculated treatments maintained larger leaf dimensions than the corresponding non-inoculated controls, indicating improved vegetative development following rhizobial inoculation.

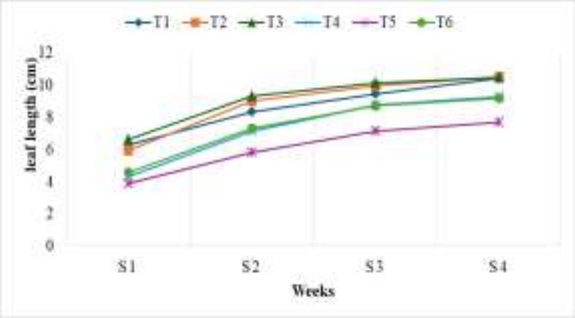

Fig. 4. Evolution of leaf length (cm) across treatments over time (weeks after emergence)

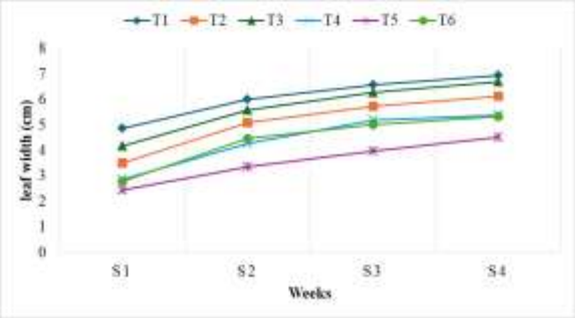

Fig. 5. Evolution of leaf width (cm) across treatments over time (weeks after emergence)

Grain yield response to inoculation and planting density

Grain yield varied significantly among treatments (Table 3). The highest yield was obtained from T1 (50 cm × 20 cm + inoculation), which produced 1,855.53 kg ha^{-1}. This yield exceeded that of the corresponding non-inoculated treatment (T4), which produced 1,266.66 kg ha^{-1}. Among the inoculated treatments, yield declined as row spacing increased, with T2 and T3 producing 1,022.22 and 877.77 kg ha^{-1}, respectively. The lowest yield was recorded in T6 (70 cm × 20 cm without inoculation), with 677.77 kg ha^{-1}.

The ANOVA indicated highly significant effects of inoculation (F = 140.20, $p < 0.001$), planting density (F = 10.34, $p < 0.001$), and the inoculation × density interaction (F = 8.22, $p = 0.0014$) on grain yield (Table 4). The significant interaction demonstrates that the response to inoculation depended on planting density. The combination of rhizobial inoculation and the highest planting density (50 cm × 20 cm) generated the greatest yield advantage, confirming T1 as the most productive treatment under the agro-ecological conditions of Kétou.

Table 3. Grain yield (kg ha^{-1}) by treatment

Treatment	Spacing (row × hill)	Inoculation	Mean yield (kg ha^{-1})
T1	50 cm × 20 cm	Yes	1,855.53 a
T2	60 cm × 20 cm	Yes	1,022.22 c
T3	70 cm × 20 cm	Yes	877.77 d
T4	50 cm × 20 cm	No	1,266.66 b
T5	60 cm × 20 cm	No	911.11 c
T6	70 cm × 20 cm	No	677.77 e

Means followed by the same letter are not significantly different according to Tukey's HSD test ($p < 0.05$).

Table 4. ANOVA for grain yield

Source of variation	df	SS	MS	F-value	*p*-value
Inoculation (I)	1	0.9000	0.9000	140.20	<0.001***
Density (D)	2	0.1327	0.0664	10.34	0.0004***
I×D	2	0.1055	0.0527	8.22	0.0014**
Error	30	0.1926	0.00642	—	—
Total	35	1.3308	—	—	—

ns = not significant; *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$.

DISCUSSION

Effect of rhizobial inoculation on soybean growth

The significant increases in leaf number and plant height observed in inoculated treatments demonstrate the positive contribution of rhizobial inoculation to soybean vegetative development. Inoculated plants consistently produced more leaves and attained greater height than non-inoculated plants throughout the growth period, indicating improved nutrient acquisition and plant vigor. These findings are consistent with those reported by Houngnandan *et al.* (2020), who observed significant increases in soybean growth and biomass following inoculation with effective Bradyrhizobium strains in Benin. Similarly, Deka *et al.* (2024) reported that inoculation with *Bradyrhizobium japonicum* significantly improved plant height, nodulation, biomass accumulation, and grain yield of soybean in southern Ethiopia.

The beneficial effects of inoculation can be attributed primarily to enhanced biological nitrogen fixation. Soybean has a high nitrogen requirement, and in the ferruginous soils of central Benin, nitrogen deficiency remains one of the principal constraints to productivity (Chabi *et al.*, 2023). By establishing an effective symbiosis with soybean roots, rhizobia increase the availability of biologically fixed nitrogen, thereby promoting vegetative growth, leaf formation, and photosynthetic capacity. The superior performance of inoculated plants observed in the present study therefore confirms the importance of inoculation as a low-cost and environmentally sustainable fertility-management strategy for soybean production.

Effect of planting density on soybean growth

Although treatment means showed some variation among planting densities, the effects of density on leaf number and plant height were not statistically significant. This suggests that within the range of spacings evaluated (50–70 cm between rows), soybean plants were able to compensate for differences in plant population through adjustments in vegetative growth. Similar compensatory responses have been reported in soybean, where individual plants growing at lower population densities often develop larger canopies and greater branching, partially offsetting reductions in plant number per unit area.

The absence of significant density effects on vegetative growth contrasts with findings reported by Kena *et al.* (2022) and Li *et al.* (2025), who observed substantial influences of plant spacing on soybean growth and productivity. However, the response of soybean to planting density is highly dependent on genotype, environmental conditions, soil fertility status, and management practices. The relatively narrow range of spacings evaluated in the present study may have reduced the magnitude of growth differences among treatments, resulting in comparable vegetative performance across planting densities.

Leaf development and canopy formation

Leaf length and leaf width increased progressively throughout the observation period, reflecting normal canopy development as plants advanced through their vegetative stages. Inoculated treatments generally produced larger leaves than non-inoculated treatments, indicating improved nutritional status and greater photosynthetic potential. Larger leaf area enhances light interception and carbon assimilation, which ultimately contributes to biomass production and grain filling.

The superior leaf dimensions observed in inoculated treatments are consistent with the physiological effects of improved nitrogen nutrition. Nitrogen plays a fundamental role in chlorophyll synthesis, protein formation, and leaf expansion. Consequently, enhanced nitrogen fixation resulting from inoculation likely promoted greater leaf development and improved canopy structure. Similar responses have been documented in soybean and other grain legumes following inoculation with effective rhizobial strains (Ulzen *et al.*, 2020; Deka *et al.*, 2024).

Grain yield response to inoculation and planting density

Grain yield was strongly influenced by both inoculation and planting density. The highest yield was obtained under T1 (50 cm × 20 cm with inoculation), while the lowest yield was recorded under T6 (70 cm × 20 cm without inoculation). Furthermore, the significant inoculation × density interaction indicates that the effectiveness of inoculation depended on plant population density.

The substantial yield advantage of inoculated treatments confirms previous findings from Benin and other African countries. Houngnandan *et al.* (2020) demonstrated that rhizobial inoculation significantly increased soybean grain yield under field conditions in Benin, while Ulzen *et al.* (2020) reported enhanced soybean productivity in northern Ghana when inoculation was integrated with improved crop management practices. Likewise, Tovihoudji *et al.* (2025) observed significant yield increases in soybean following inoculation combined with phosphorus management in northern Benin.

The superior performance of T1 suggests that the 50 cm × 20 cm spacing provided a more favorable balance between plant population and resource utilization. At this density, the crop canopy likely intercepted more solar radiation and achieved more efficient use of available land area while maintaining sufficient access to soil moisture and nutrients. When combined with effective rhizobial inoculation, these conditions promoted greater biomass accumulation and grain production. Similar observations were reported by Baboy *et al.* (2015), who identified 50 cm × 20 cm as an optimal spacing for soybean production in the Democratic Republic of Congo.

The significant interaction between inoculation and density further suggests that agronomic management practices can influence the expression of biological nitrogen fixation benefits. In the present study, inoculation generated the greatest yield advantage under the highest plant population density, indicating that improved nitrogen supply was particularly important when crop demand for nutrients was greatest. This finding highlights the importance of integrating biological and agronomic approaches to maximize soybean productivity.

Implications for soybean production in Benin

The results demonstrate that rhizobial inoculation represents an effective strategy for improving soybean productivity in the Kétou agro-ecological zone. While planting density alone had limited influence on vegetative growth traits, its interaction with inoculation significantly affected grain yield. The combination of inoculation and a spacing of 50 cm × 20 cm produced the highest yield and therefore appears to be the most suitable management option under the environmental conditions of the study area.

Given the increasing need for sustainable intensification of soybean production in Benin, the adoption of rhizobial inoculation together with appropriate planting density could contribute substantially to improving yields while reducing dependence on mineral nitrogen fertilizers. Future studies should evaluate the combined effects of inoculation, phosphorus fertilization, and organic amendments in order to further optimize soybean productivity under smallholder farming conditions.

CONCLUSION

This study evaluated the effects of rhizobial inoculation and planting density on the growth and grain yield of soybean (*Glycine max* (L.) Merr.) variety TGX1910-14F under field conditions in Kétou, Benin. Rhizobial inoculation significantly improved vegetative growth, as evidenced by increases in leaf number, plant height, and leaf dimensions throughout the cropping cycle. In contrast, planting density alone had limited effects on vegetative growth traits within the range of spacings tested.

Grain yield was significantly influenced by inoculation, planting density, and their interaction. The combination of rhizobial inoculation and a spacing of 50 cm × 20 cm (T1) produced the highest grain yield (1,855.53 kg ha^{-1}), whereas the lowest yield was recorded in the non-inoculated treatment at 70 cm × 20 cm (T6). These findings demonstrate that the benefits of inoculation are maximized when combined with an appropriate plant population density.

The results highlight the importance of integrating biological nitrogen fixation technologies with suitable crop management practices to enhance soybean productivity in the ferruginous soils of central Benin. Under the agro-ecological conditions of Kétou, the use of *Bradyrhizobium japonicum* inoculation together with a planting density corresponding to 50 cm × 20 cm spacing is recommended for improving soybean yield and promoting sustainable production.

Further research should investigate the combined effects of rhizobial inoculation, phosphorus fertilization, and organic soil amendments across multiple seasons and locations to develop integrated nutrient-management strategies capable of further increasing soybean productivity in Benin.

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