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Effect of rhizobial inoculation and planting density on soybean (Glycine max [L.] Merr.) Growth and Yield in Goma, DRC

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ABSTRACT

The inoculation of soybean seeds *(Glycine max [L.] Merr.)* with *Bradyrhizobium japonicum* represents a sustainable strategy to improve productivity in nitrogen-poor tropical soils. This study evaluated the combined effect of rhizobial inoculation and different planting densities on vegetative growth and soybean yield in Goma, Democratic Republic of the Congo (DRC). A randomized complete factorial field trial with three replications was conducted with two inoculation treatments, the uninoculated control (T0) and seeds inoculated (T1) with the Biofix strain (*Bradyrhizobium japonicum* IRAT-F3) applied at a concentration of 1×10^9 CFU g^{-1}, combined with three planting densities, 250 000 (Spac1), 107 143 (Spac2), and 71 429 plants/ha (Spac3). Inoculation significantly increased plant height ($p < 0.0001$), canopy width ($p < 1.6 \times 10^{-10}$), number of leaves ($p < 1.24 \times 10^{-11}$), and branches ($p < 0.0001$) at 56 days after sowing. The T1Spac3 combination produced the highest yield (6.107 t/ha) compared to 5.088 t/ha for T0Spac3. These results indicate that rhizobial inoculation, when optimized with planting density, can sustainably enhances soybean production in Goma.

Keywords: *soybean, rhizobial inoculation, Bradyrhizobium japonicum, planting density*

1. INTRODUCTION

Soybean *(Glycine max [L.] Merr.)* is a legume of strategic importance for food and nutritional security due to the high protein content of its seeds (approximately 40%) [1], and for improving soil fertility through its ability to symbiotically fix atmospheric nitrogen [2]**.** Over the past decades, the increase in global soybean yields has been primarily attributed to genetic improvements, the judicious intensification of inputs, and the enhancement of agronomic practices, notably the optimization of seeding density and soil management [3,4]. These advances represent a key step toward low-input sustainable production systems, reducing the environmental impacts of agriculture without compromising yields [5]**.**

In Africa, low soil fertility remains one of the main factors limiting the productivity of food crops, including soybean, thereby compromising food security [6]**.** Paradoxically, the excessive or inappropriate use of mineral fertilizers contributes to soil degradation, water pollution, and increased greenhouse gas emissions, thereby exacerbating climate change [7]. In this context, integrated soil fertility management, combining appropriate inputs with good agronomic practices, emerges as a promising approach to sustainably improve crop yields [8]. Although legume yields in smallholder farming systems often remain below their potential, numerous studies have shown that they can be significantly improved through the use of improved varieties [9,10], rhizobial inoculants [11], phosphate fertilizers [12]**.**

The effectiveness of soybean inoculation depends on the rhizobial strains used, with several studies reporting good performance for strains of *Bradyrhizobium* and *Sinorhizobium*, particularly *Bradyrhizobium japonicum*, which is recognized for enhancing nodulation, nitrogen uptake, and soybean yield at low cost [13,14]**,** However, it has been shown that the survival of rhizobia in the soil is affected by extreme pH, desiccation, nutrient deficiencies, extreme temperatures, and toxicities [15]. Moreover, planting density represents a major agronomic factor influencing canopy architecture, the utilization of light, water, and nutrients, and consequently, the number of pods, seed weight, and final soybean yield [16]**.** High planting

density can reduce the light intensity reaching the lower canopy layers, thereby affecting crop productivity through light and nutrient use [17].

In North Kivu, particularly around Goma, agriculture is the main pillar of the local economy, and the demand for soybean is rising due to urbanization, livestock development, and agro-processing activities [18]. Soybean, widely consumed as milk, flour, and other derivatives, provides essential proteins and nutrients, contributing significantly to household food and nutritional security [19]. Despite its agronomic potential, smallholder farms dominate the region, and continuous cropping on fertile Andosols with minimal inputs limits yields, reinforcing local food insecurity [20**,** 21]. Consequently, soybean cultivation is declining, even though it remains a crucial food source in this war-affected area. We hypothesize that seed inoculation can significantly improve soybean yield, with its effectiveness depending on planting density**.** The present study aims to evaluate the combined effect of *Bradyrhizobium japonicum* inoculation and varying planting densities on growth and yield to enhance food security in Goma.

2. METHODOLOGY

2.1. Study Area

The experiment was conducted in 2024 at Buhimba, Lac Vert neighborhood in Goma (North Kivu, DRC), within the volcanic highlands of the Great Lakes region, located at 29°7′30″E, 1°34′30″S, at an altitude of 1,530 m. The area is characterized by a temperate tropical savanna climate, with an average annual temperature of 21.2 °C. The geographical location of the experimental site is shown in Fig. 1.

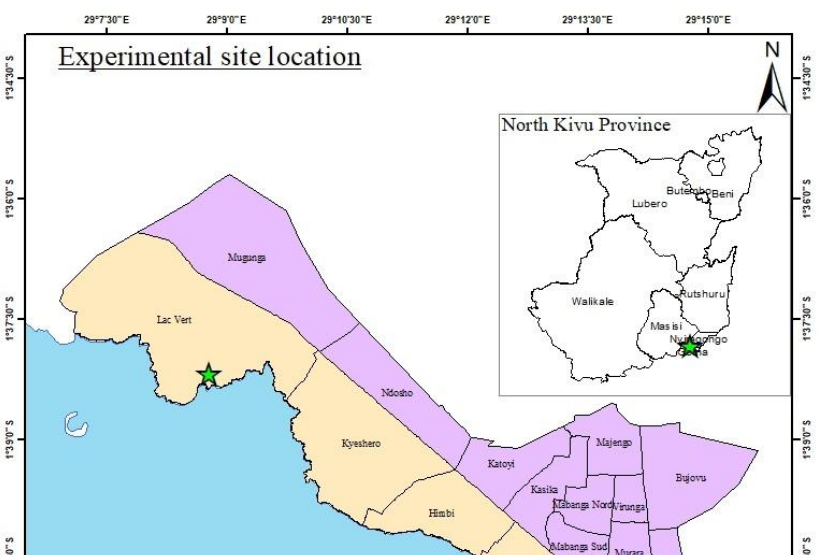

Figure 1. Location of the Buhimba experimental site Goma-DRC (Authors in ArcGIS 10.7).

2.2. Plant Material and inoculum

The plant material consisted of soybean *(Glycine max [L.] Merr.)* seeds of the Imperial variety, provided by the National Institute for Agronomic Study and Research (INERA/Mulungu). The inoculum used was a *B. japonicum* strain (IRAT-F3), specific for soybean nodulation, supplied by the Microbiology Laboratory of the International Institute of Tropical Agriculture (IITA/Kalambo).

2.3 Experimental Design and Treatments

A field experiment was conducted using a full factorial randomized complete block design (RCBD) with three replications. The treatments consisted of two inoculation levels: non-inoculated control (T0) and inoculated with *Biofix* (*B. japonicum* IRAT-F3) applied at a concentration of 1×10^{9} CFU g^{-1} (T1), combined with three planting densities: 250,000 (Spac1), 107,143 (Spac2), and 71,429 plants ha^{-1} (Spac3). Experimental units measured 1.4 m × 2 m, with six plots per replication.

2.4. Field Management

2.4.1. Land Preparation

The previous crop was cabbage (*Brassica oleracea* L.). The field was prepared by two medium plowings (15-20 cm) using a hoe, the first to loosen the soil and the second to finalize the seedbed one day before sowing. Experimental plots were demarcated with stakes the day before sowing.

2.4.2. Inoculation

Soybean seeds were manually inoculated following the recommendations of the inoculant strain. For 1 kg of seeds, 10 ml of adhesive solution (sugar water) was added and thoroughly mixed to ensure uniform coating. Subsequently, 50 g of inoculum was incorporated, and the seeds placed in a basin were gently shaken for one minute to avoid damaging the bacteria [22].

Immediately after inoculation, the seeds were spread on a clean surface and left to air-dry in the shade before being sown as soon as possible to ensure maximum inoculum viability [23].

2.4.3. Sowing

To prevent any contamination or mixing with the inoculated seeds, the non-inoculated seeds were sown first in the experimental units. Planting density was determined using the following formula :

$$Number\ of\ plants = \frac{Experimental\ Unit\ Area\ (m^2)}{Intra-row\ spacing\ x\ Inter-\ row\ spacing\ (m^2)}$$

Spacings of 20 × 20 cm, 40 × 20 cm, and 65 × 20 cm corresponded to 70, 30, and 20 plants per experimental unit, respectively. Two seeds were sown per hill to ensure proper plant establishment. Gaps caused by missing seedlings were filled 10 days after sowing to ensure uniform plant establishment. For observations, 10 central plants per plot were selected to avoid border effects. For the third planting density, where the number of central rows was very limited, plants were selected from the center of the two available rows to constitute the 10 observed plants, thereby ensuring representativeness despite the low number of rows.

2.5 Vegetative and Yield Parameters

Plant growth was assessed by measuring plant height, canopy width, number of leaves, and number of branches at 28 and 56 days after sowing. Yield parameters were determined at harvest on the observable plants. The 100-seed weight was measured using an electronic LCD balance (accuracy 1 g, capacity 10 kg), while the number of pods per plant, number of seeds per pod, and number of nodules per plant were obtained by manual counting. Grain yield was estimated by weighing the total production of each plot and then extrapolated to per hectare based on the harvested area, using the following formula:

$$Grain\ yield\ (t\ ha^{-1}) = \frac{Dry\ seed\ weight\ (kg) \times 10\ 000}{Plot\ area\ (m^2)}/1000$$

2.6. Statistical Analysis

Statistical analyses were performed using R software (version 4.5.2) through the R Studio interface. The effects of inoculation, planting density, and their interaction were evaluated using

136 analysis of variance (ANOVA) when the assumptions of residual normality and homogeneity
137 of variances were met. Multiple mean comparisons were performed using Tukey's Honest
138 Significant Difference (HSD) test at a 95% confidence interval. When the assumptions of
139 ANOVA were not met, a non-parametric Scheirer-Ray-Hare test was applied, followed by a
140 post hoc Dunn test with significance threshold adjustment.
141

3. RESULTS

3.1 Effects of inoculation and Planting density on vegetative growth

The vegetative growth parameters of soybean, including plant height, canopy width, number of branches, and number of leaves per plant at 28 days after sowing, are presented in Table 1.

Table 1. Vegetative growth parameters of soybean at 28 days after sowing

	T0			T1			H-test (α=0.05)					
Var	**Spacing1**	**Spacing2**	**Spacing3**	**Spacing1**	**Spacing2**	**Spacing3**	**Spacing**		**Treatments**		**Spacing x Treatments**	
PH28	26.6 [b] ±0,516	26±1.054	25.37±0.67	24.5 [a] ±0.527	24.36±0.32	24.12±0.104	3.695	p=0.157	34.92	p<0.000***	0.85	p=0.655
CW28	20.5±0.527	20,6 [b] ±0.51	20.2±0.63	21.5±0.52	22.6±0.51	23.2 [a] ±0.78	2.62	p=0.269	39.97	p<0.000***	4.57	p=0.101
NB28	2.3 [b] ±0.48	2.3 [b] ±0.48	2.2±0.42	3±0.66	3.1 [a] ±0.56	3.1±0.73	0.141	p=0.931	21.19	p<0.000***	0.19	p=0.911
LP28	8.4±1.26	8.1±1.44	10.8 [b] ±1.54	12±0.00	11.7±0.94	12.6±1.26	7.965	p=0.018*	34.31	p<0.000***	2.79	p=0.248

Signif. codes : 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Abbreviations: PH: *Plant height,* ***CW28****: Canopy width,* ***NB28****: Number of branches,* ***LP28****: Leaves per plant;* ***H-test****: Scheirer–Ray–Hare test,* ***T0:*** *non-inoculated (control),* ***T1****: inoculated,* ***Var****: Variables.*

At 28 days after sowing, the Scheirer–Ray–Hare test indicates that soybean vegetative growth is primarily influenced by inoculation, which has a highly significant effect on plant height (H = 34.92), canopy width (H = 39.97), number of branches (H = 21.19), and number of leaves per plant (H = 34.31), all with p-values < 0.0001. In contrast, planting density does not significantly affect plant height (H = 3.695, p = 0.157), canopy width (H = 2.62, p = 0.269), or number of branches (H = 0.141, p = 0.931), but it significantly influences the number of leaves per plant (H = 7.965, p = 0.018). However, no significant interaction between planting density and inoculation is observed.

Post hoc pairwise comparisons performed using Dunn's test with Bonferroni correction show that the treatments differ highly significantly with respect to plant height ($z = -5.909$; adjusted $p = 3.444 \times 10^{-9}$), canopy width ($z = 6.322$; adjusted $p = 2.576 \times 10^{-10}$), number of branches ($z = 4.603$; adjusted

$p = 4.159 \times 10^{-6}$), as well as number of leaves per plant ($z = 5.857$; adjusted $p = 4.709 \times 10^{-9}$). The same parameters are also assessed at 56 days after sowing, and the corresponding results are presented in Table 2.

Table 2. Vegetative growth parameters of soybean at 56 days after sowing

	T0			T1			Statistical tests (α=0.05)		
Var	**Spacing1**	**Spacing2**	**Spacing3**	**Spacing1**	**Spacing2**	**Spacing3**	**Spacing**	**Treatments**	**Spacing x Treatments**
PH56	55,7 [b] ±0,67	54,4±0,69	54,5±0,52	58,3±0,94	58,8±0,73	59,7 [a] ±0,67	H=0.906 p=0,635	H= 45,08 p<0.000***	H= 4.99 p=0.082
CW56	35,4±1,26	39,8 [b] ±1,13	39,1±1,44	41,1±1,52	40±1,41	41,8 [a] ±1,61	F= 13.8 $p=2.18^{-05}$***	F=61.98 $p=1.6^{-10}$***	F= 19.06 $p=5.44^{-07}$***
NB56	7±0,47	8,5 [b] ±0,85	8,3±0,67	9,7 [a] ±1,05	9,6±0,84	9,7±0,67	H= 4.96 p=0.01*	H=73.3 $p=1.24^{-11}$***	H=5.88 p=0.004**
LP56	24±1,05	32,8 [b] ±1,03	32±1,33	37±1,41	36,2±1,47	37,9 [a] ±1,37	H= 2.9 p=0.235	H= 43.8 p<0.000***	H=4.14 p=0.126
								Signif. codes : 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1	

Abbreviations: ***PH56****: Plant height,* ***CW56****: Canopy width,* ***NB56****: Number of branches,* ***LP56****: Leaves per plant;* ***H-test****: Scheirer–Ray–Hare test,* ***F-Test****: Anova,* ***T0****: non-inoculated (control),* ***T1****: inoculated,* ***Var****: Variables.*

At 56 days after sowing (DAS), analyses performed using the Scheirer-Ray-Hare test and two-way ANOVA reveal a marked and differentiated influence of the studied factors. Rhizobial inoculation exerts a highly significant effect on all growth parameters, as indicated by the respective statistics ($H = 45.08$, $p < 0.0001$), ($F = 61.98$, $p = 1.6 \times 10^{-10}$), ($H = 73.30$, $p = 1.24 \times 10^{-11}$), and ($F = 43.80$, $p < 0.0001$), thereby confirming its sustained impact on soybean vegetative vigor.

Planting density exerts a highly significant effect on canopy width ($F = 13.80$; $p < 2.18 \times 10^{-5}$) and on the number of branches ($H = 4.96$; $p = 0.01$). Highly significant density × inoculation interactions are also observed for canopy width ($F = 19.06$; $p < 5.44 \times 10^{-7}$) and the number of branches ($H = 5.88$; $p = 0.004$), indicating that the effectiveness of rhizobial inoculation depends on planting density. In contrast, plant height and the number of leaves per plant are not significantly affected by either planting density or the density × inoculation interaction, suggesting that these traits are primarily regulated by the inoculation treatment.

Post hoc pairwise comparisons performed using Dunn's test with Bonferroni correction reveal extremely significant differences between treatments for plant height (z = 6.714; adjusted $p = 1.891 \times 10^{-11}$), number of branches (z = 5.489; adjusted $p = 4.021 \times 10^{-8}$), and number of leaves per plant (z = 6.618; adjusted $p = 3.631 \times 10^{-11}$). In addition, Tukey's HSD post hoc test for pairwise mean comparisons indicates a highly significant difference between treatments for canopy width (mean difference = −2.866; adjusted $p < 0.001$).

3.2. Grain yield

3.2.1. Effects of inoculation and planting density on yield components

The results related to soybean production are presented in Table 3.

Table 3. Effects of inoculation and planting density on soybean yield components

	T0			T1			Statistical tests (α=0.05)		
Var	**Spacing1**	**Spacing2**	**Spacing3**	**Spacing1**	**Spacing2**	**Spacing3**	**Spacing**	**Treatments**	**Spacing x Treatments**
W100	19,40±1,21	20,47±0,87	21,11 [b] ±0,76	22,57±0,54	22,82±1,26	24,52[a] ±0,7	F=19.57 p=4.1^{-07} ***	F= 151.65 p = 2^{-16} ***	F=1.79 p=0.177
PP	9,73±1,196	10,5±0,849	12,37 [b] ±1,31	12,36±1,70	12,08±0,76	13,01[a] ±0,7	F=11.94 p=5.1^{-05}***	F=29.78 p=1.25^{-06} ***	F= 3.75 p= 0.03 *
SP	2,34±0,248	2,66 [b] ±0,55	2,63±0,342	2,69±0,394	3,19 [a] ±0,57	3,18±0,59	H=6.99 p=0.03*	H=15.64 p=0.00008***	H=0.297 p=0.86
NP	10,64±0,88	10,9±1,37	10,99 [b] ±1,36	12,49±1,42	12,78±1,66	13,75 [a] ±1,5	F=1.76 p= 0.181	F=36.79 p= 1.34^{-07} ***	F=0.7 p= 0.501
Y	1485,1±61,3	1543,3 [b] ±16,4	1424,7±148,6	1641,6±14,8	1645,2±71,4	1709,9 [a] ±52,5	H=0.75 p= 0.687	H=8.56 p=0.000***	H=3.3 p= 0.189
								Signif. codes : 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1	

Abbreviations: W100*:100-seed weight (g),* ***PP****: Pods per plant,* ***SP****: Seeds per pod,* ***NP****: Nodule per Plant,* ***Y****: Yield (g),* ***H-test****: Scheirer–Ray–Hare test,* ***F-Test****: Anova,* ***T0****: non-inoculated (control),* ***T1****: inoculated,* ***Var****: Variables****, Mean diff****: Mean difference*

Analysis of the effects of inoculation and planting density on soybean yield components shows a dominant influence of inoculation. 100-seed weight (F = 151.65, $p = 2 \times 10^{-16}$), number of pods per plant (F = 29.78, $p = 1.25 \times 10^{-6}$), number of seeds per pod (H = 15.64, $p = 8 \times 10^{-5}$), number of nodules per plant (F = 36.79, $p = 1.34 \times 10^{-7}$), and grain yield (H = 8.56, $p < 0.001$) are all highly significantly increased by inoculation.

Regarding planting density, it has a highly significant effect on 100-seed weight ($F = 19.57$, $p = 4.1 \times 10^{-7}$), number of pods per plant ($F = 11.94$, $p = 5.1 \times 10^{-5}$), and number of seeds per pod ($H = 6.99$, $p = 0.03$), whereas no significant effect is observed on the number of nodules per plant ($F = 1.76$, $p = 0.181$) or on grain yield ($H = 0.75$, $p = 0.687$). The density × inoculation interaction is significant only for the number of pods per plant.

Tukey's HSD post hoc test for pairwise mean comparisons indicates a highly significant difference between treatments for 100-seed weight (mean difference = 2.975, adjusted $p < 0.001$), number of pods per plant (mean difference = 1.619, adjusted $p = 1.2 \times 10^{-6}$), and number of nodules per plant (mean difference = 2.166, adjusted $p = 1 \times 10^{-7}$). Additionally, pairwise post hoc comparisons performed using Dunn's test with Bonferroni correction show a highly significant difference between treatments for number of seeds per pod ($z = -3.955$, adjusted $p = 7.661 \times 10^{-5}$) and grain yield ($z = -6.209$, adjusted $p = 5.32 \times 10^{-10}$).

3.2.2. Visualization of yield components by treatment

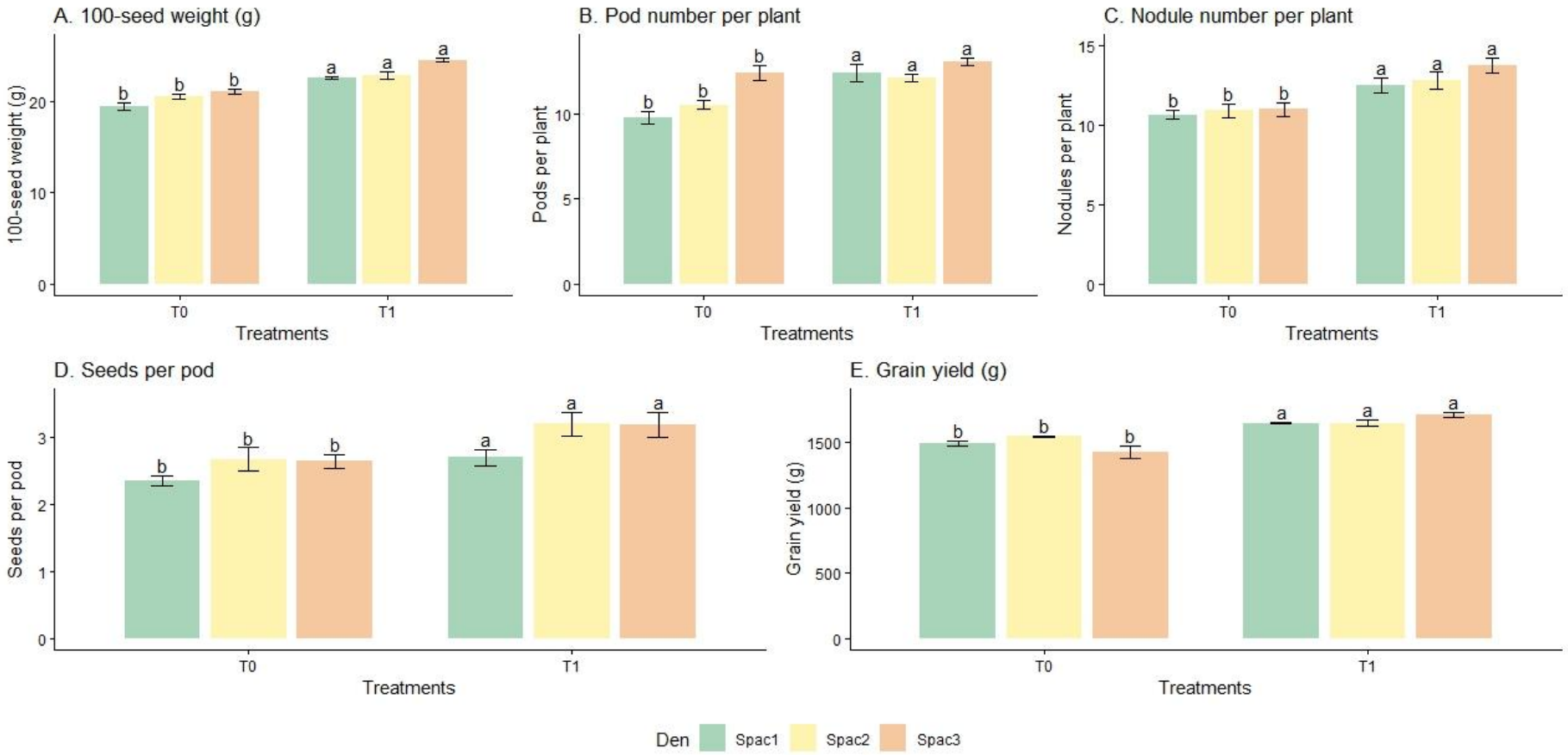

Figure 2. Effects of inoculation on soybean yield components

Figure 2 illustrates the effect of inoculation on yield variables, including 100-seed weight, number of pods per plant, number of nodules per plant, number of seeds per pod, and grain yield. Overall, inoculation leads to a significant improvement in all assessed variables. Under non-inoculated conditions, differences between planting densities are more pronounced for certain yield components, notably the number of pods per plant, number of nodules per plant, and grain yield.

3.2.2. Linear relationship between grain yield and its components

The relationships between grain yield and its components under inoculated and non-inoculated conditions are presented in Fig. 3.

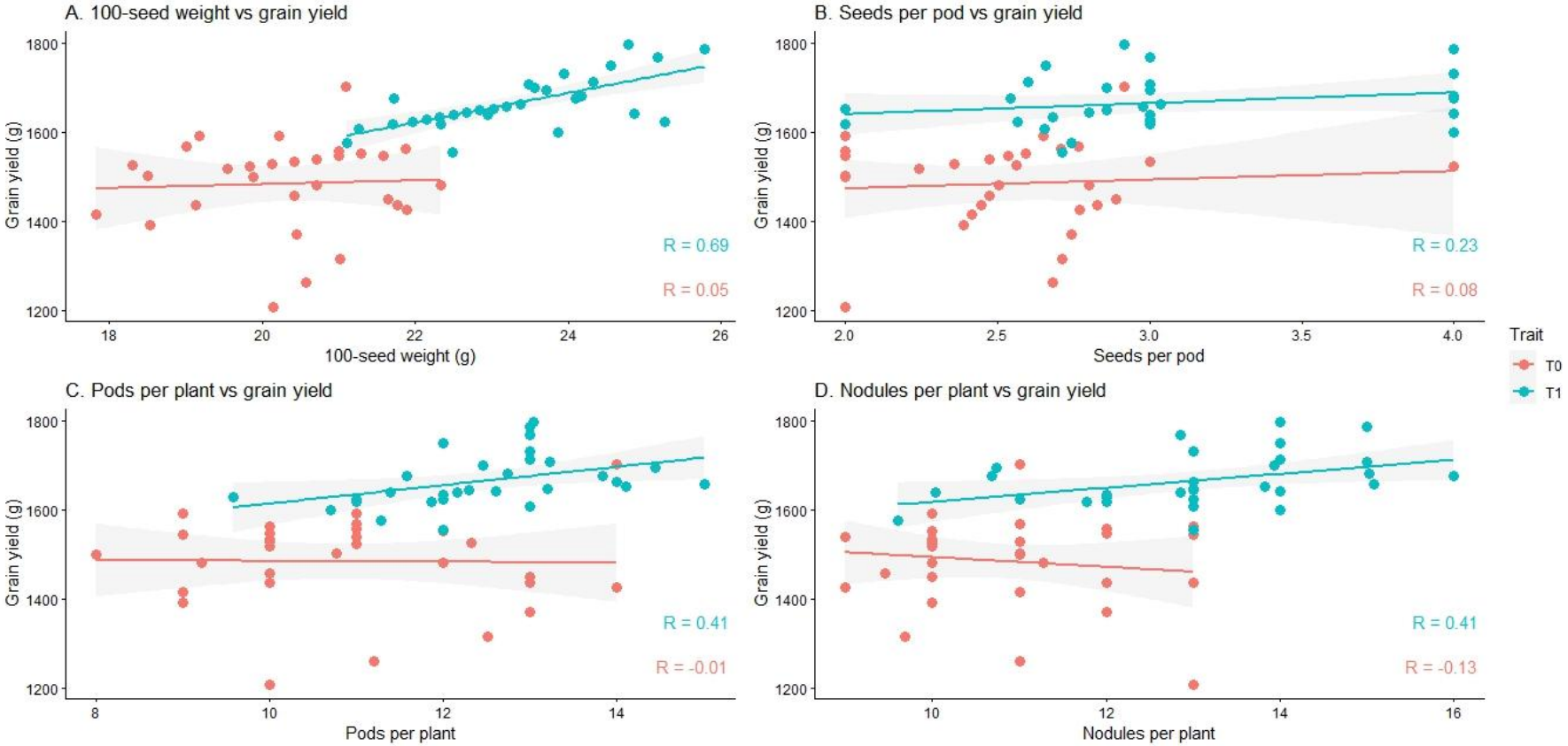

Figure 3. Relationships between grain yield and its components by treatment

The figure 3 illustrates the linear relationships between grain yield (GY) and its components by inoculation treatment. In inoculated plants (T1), GY is positively correlated with hundred-seed weight (R = 0.69), number of pods per plant (R = 0.41), and number of nodules per plant (R = 0.41), with

247 a weaker correlation for number of seeds per pod (R = 0.23). In the non-inoculated control (T0), correlations are weak or negligible (R = −0.13 to
248 0.08). For comparable component levels, yields are consistently higher under inoculation.

3.2.3. PCA of yield components and factor grouping (treatment and planting density)

The PCA biplot showing the relationships among treatments and agronomic traits is presented in Fig. 4.

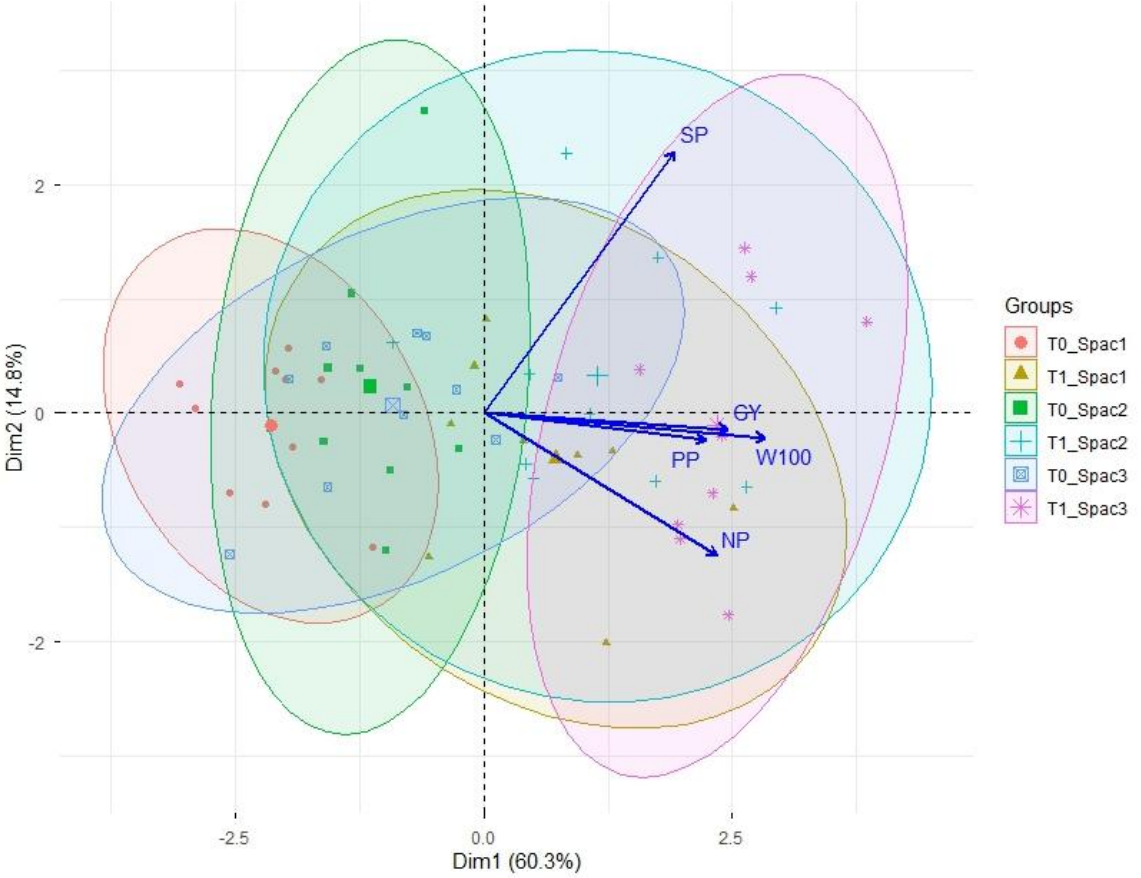

Figure 4. PCA Biplot of treatments based on agronomic traits

As shown in Fig. 4, the biplot of the principal component analysis (PCA) shows that the first principal axis (Dim1), explaining 60.3% of the total variance, is primarily associated with grain yield, 100-seed weight, and number of pods per plant. This axis thus represents a gradient of agronomic performance, highlighting the major contribution of yield components to treatment differentiation. The second axis (Dim2), which explains 14.8% of the variance. Inoculated treatments, particularly at the highest planting densities, are mostly positioned on the positive side of Dim1, reflecting better agronomic performance.

4. DISCUSSION

The results show that inoculation of soybean seeds with *B. japonicum* promotes vegetative growth in Goma, particularly plant height, canopy width, and the number of leaves and branches at 28 and 56 days after sowing. This response reflects an integrated physiological adaptation, associated with improved nitrogen nutrition through biological nitrogen fixation, which stimulates cell division and expansion, resulting in more vigorous plants [24].

Issa & Hayatu [25] also showed that, compared with the control, inoculated treatments had a significant effect on plant height. Similar results were reported by Sajid [26]. The lack of a significant effect at 28 days is likely related to the early developmental stage, where the symbiosis is not yet fully functional and the plants still rely primarily on seed reserves for their initial growth. In contrast, the significant interactions observed at 56 days for canopy width ($p = 5.44 \times 10^{-7}$***) and number of branches (p = 0.004**) reflect the onset of more complex physiological mechanisms associated with nitrogen fixation, resource competition, and the progressive expression of inoculation benefits over time. Thus, the observed interaction represents the combined effect of increased nitrogen availability through inoculation and competition induced by planting density, influencing canopy expansion and branching only as resources become limiting.

These observations are consistent with those of Zhou et al. [27], who showed that rhizobial inoculation increased photosynthetic rates and stomatal conductance, enabling a more efficient use of light energy and promoting growth. Nitrogen supplied through the symbiosis also contributes to hormonal regulation by stimulating gibberellin biosynthesis and destabilizing DELLA proteins, which are growth repressors, thereby promoting cell elongation and vegetative biomass accumulation [28]. However, maximum aboveground biomass is reached only when the soybean variety and the Rhizobium strain are compatible, ensuring an effective symbiosis and optimal nitrogen supply [29, 30]. These results therefore highlight the importance of selecting strains adapted to the cultivated varieties, particularly for late-maturing

lines, which have a high nitrogen demand [31]. Overall, rhizobial inoculation appears as a key strategy to improve soybean growth and productivity in nitrate-poor soils.

Planting density, with 250,000 plants/ha for Spac1, 107,143 plants/ha for Spac2, and 71,429 plants/ha for Spac3, shows significant differences only for the number of leaves (7.965, p = 0.018) at 28 days after sowing. However, at 56 days after sowing, it has a highly significant influence on canopy width (F = 13.80; $p < 2.18 \times 10^{-5}$) and number of branches (H = 4.96; p = 0.01). Zhang et al. [32] found that a high planting density led to a significant increase in leaf area index and the amount of intercepted photosynthetically active radiation. However, it caused a reduction in the photosynthetic capacity of the plants, a decrease in stomatal conductance, and a decline in leaf chlorophyll content. However, De Bruin & Pedersen [33] and Boquet [34], emphasized that a moderate planting density favors optimal plant architecture development and more efficient use of environmental resources, including radiation, water, and nutrients. According to Friedman [19], at lower densities, improved access to resources enhances branching, pod formation, and seed weight at the individual plant level, leading to higher per-plant productivity.

Yield components, namely 100-seed weight (F = 151.65, $p = 2 \times 10^{-16}$), number of pods per plant (F = 29.78, $p = 1.25 \times 10^{-6}$), number of seeds per pod (H = 15.64, p = 0.00008), number of nodules per plant (F = 36.79, $p = 1.34 \times 10^{-7}$), and grain yield (H = 8.56, $p < 0.001$), were significantly improved by inoculation, while the latter two variables were not significantly affected by planting density. After extrapolation, the inoculated treatment (T1Spac3) performed best in terms of grain yield, reaching 6.107 t/ha compared with 5.088 t/ha for the control (T0Spac3), followed by the inoculated (T1Spac2) with 5.876 t/ha compared with 5.512 t/ha for the control (T0Spac2), and finally the inoculated (T1Spac1) with 5.862 t/ha compared with 5.304 t/ha for the control (T0Spac1). These results are similar to those of Jarecki [35], who reported that seed inoculation significantly improved nodulation, leaf chlorophyll index,

number of pods per plant, seed weight, and yield. It also increased seed protein content, whereas non-inoculated seeds showed the highest fat content.

They are also consistent with the findings of Argaw [36], who reported that soybean seed inoculation with *Bradyrhizobium* significantly affected yield. Similarly, Okereke et al. [30] reported that inoculation led to a significant increase in grain yield, confirming the observations of Thiès *et al*. [37] according to whom, initial soybean yield responses are observed in field soils where indigenous populations of *B. japonicum* are low or absent. Hu *et al*. [38] stated that nodulated soybean plants showed a remarkable increase in yield, ranging from 30.94% to 119.48%, compared with non-nodulated plants under similar conditions.

Inoculation resulted in a higher number of nodules, with 13 nodules for T1Spac3 compared with only 9 for the control (T0Spac3), and 12 versus 10 for T1Spac2 and T0Spac2, and T1Spac1 and T0Spac1, respectively. Some authors have shown that distinct bacterial communities can influence nodule size, shape, and number, thereby affecting nitrogen uptake and its utilization efficiency in soybean [39]. Moreover, Kamicker & Brill [40] found that a higher percentage of nodules were occupied by the inoculant when it was applied at high rates to both seeds and soil, compared with treatments where the inoculant was applied only to the seeds or at low rates to seeds and soil, highlighting the importance of optimizing inoculant formulation and application methodology. Martins *et al.* [41]reported that inoculant application resulted in a higher number of nodules and greater dry biomass, particularly during the wet cropping season, and added that combining seed inoculation with an additional inoculation at the V3 stage (third node) led to an 82.3% increase in nodule number and a 30.2% increase in nitrogen derived from the atmosphere compared with seed inoculation alone. In an experiment conducted in Congo, Mandimba & Mondibaye [42] reported that inoculation of soybean plants increased the number of nodules per plant by 355 to 380% and the dry mass of nodules per plant by 336 to 382% for the rhizobium strains TAL 377 and TAL 379, respectively. It is also worth noting that the symbiosis between rhizobia and legumes is controlled by both the bacteria and the host plant,

and that nodule formation varies according to the host and depends on environmental conditions as well as the rhizobial species involved. In soybean, significant differences in nodulation have been observed between *Sinorhizobium* and *Bradyrhizobium* depending on soil pH. These differences in pH tolerance help explain their geographic distribution [43, 44]**.**

5. CONCLUSION

This study evaluated the effect of *B. japonicum* inoculation and planting density on soybean (*Glycine max* (L.) Merrill) production in Goma (DRC). Inoculation significantly enhanced vegetative growth and yield components, confirming its role in improving soybean productivity and contributing to food security.

Planting density had a limited effect at 28 days after sowing, affecting only the number of leaves, but strongly influenced canopy width and number of branches at 56 days. It also impacted yield components, including 100-seed weight, number of pods per plant, and number of seeds per pod. After extrapolation, the inoculated (T1Spac3) treatment achieved the highest grain yield 6.107 t/ha, compared with 5.088 t/ha for the control (T0Spac3), followed by (T1Spac2) with 5.876 t/ha vs. 5.512 t/ha and (T1Spac1) with 5.862 t/ha vs. 5.304 t/ha.

This study presents some methodological limitations. Conducting the experiment over a single Season does not account for interannual variability in climatic and agronomic conditions, while the absence of detailed soil characterization restricts the accurate assessment of soil fertility and the interpretation of crop responses. Further studies across multiple seasons, locations, and rhizobial strains are needed to confirm these findings and support more robust recommendations for soybean cultivation in the region.

6. LIST OF ABBREVIATIONS

AI : artificial intelligence

ANOVA: Analysis of Variance

DRC: Democratic Republic of the Congo

EANSI : East African Nutritional Sciences Institute

FAO: Food and Agriculture Organization

GY: Grain Yield

INERA : Institut National

PCA : Principal Component Analysis

pH : Potentiel en Hydrogène

RCBD: Randomized Complete Block Design

THSD: Tukey's Honest Significant Difference

UCS : Université catholique La Sapientia

WFP: World Food Programme

7. DECLARATIONS

All authors declare that they have no conflicts of interest that could have influenced the findings presented in this study.

7.1. ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All authors reviewed this work to ensure compliance with ethical principles, particularly regarding plagiarism, duplication of work, and any inappropriate scientific conduct. No artificial intelligence (AI) tools were used in the preparation of this manuscript.

7.2. CONSENT FOR PUBLICATION

Not applicable.

7.3. AVAILABILITY OF DATA AND MATERIALS

The datasets generated or analysed during the current study are available from the corresponding author upon reasonable request.

7.4. COMPETING INTERESTS

The authors declare that they have no competing interests.

7.5. FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

7.6. AUTHOR'S CONTRIBUTION

All authors contributed to the study conception and defined the methodology. Katavali Katamba Aristote carried out the data collection. Basimine Lulemire Bienfait, Ntakobajira Karani Jean-Paul, and Niyorugira Sebigunda Jackson performed the data analysis. All authors contributed to the manuscript writing and critical revision, and approved the final version.

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