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Effect of biopriming with *Bacillus subtilis* on nodulation and growth of peanuts (*Arachis hypogaea*) in the agro-pedoclimatic conditions of Mali

**Bagayoko Issiaka*¹, Tiendrebeogo Assiata², Bereté Brahim¹, Koné Yaya¹, Konaté Fatoumata¹,
Togo Agnès³**

Institute of Rural Economy, BP 258, Bamako, Mali¹

Nazi Boni University, 01 BP 1091, Bobo Dioulasso 01, Burkina Faso²

National Water Laboratory, BP 66, Bamako, Mali³

* Correspondance auteur : ibagayokoo@gmail.com

ABSTRACT : Seed biopriming using plant growth-promoting rhizobacteria (PGPR) is a promising alternative for improving legume productivity while reducing the use of chemical inputs. The present study aims to evaluate the effect of inoculation with the local *Bacillus subtilis* isolate (BS2b) on the growth, nodulation, and yield of two peanut varieties (Fleur11 and ICGV-IS 13825 also known as Keniana tige) in Mali's agro-pedoclimatic . A split-plot design with four replicates was set up in Sotuba (Bamako) to compare four treatments who are absolute control (Bs0A0), *B. subtilis* alone (Bs1A0), *B. subtilis* combined with a chemical seed treatment (Bs1A1), and a chemical treatment alone (Bs0A1). The results revealed a highly significant effect of *B. subtilis* on plant height at 25, 40, and 55 days after sowing, root length, grain weight, and nodule number and weight. The Bs1A0 treatment consistently yielded the highest values for all parameters, with notable yield gains compared to the control. However, a significant antagonistic interaction between *B. subtilis* and the chemical treatment was also observed, indicating that combining them reduces performance compared using the bioinoculant alone. No significant effect of variety or interaction with treatments was detected, suggesting a stable response of both genotypes. These results demonstrate the potential of biopriming with *B. subtilis* as an agroecological lever to improve peanut productivity in Mali as an agroecological lever, while highlighting the importance of avoiding its combination with certain chemical seed treatments.

KEYWORDS : Biopriming, *Bacillus subtilis*, chemical seed treatments, nodulation, peanut, Mali.

I. INTRODUCTION

The peanut (*Arachis hypogaea* L.) is an annual, self-pollinating oilseed legume belonging to the Fabaceae family. It is a globally important food and cash crop (Kamalasundari et al. 2025). It is mainly grown in tropical and subtropical regions due to its high adaptability and ranks third among oilseeds after soybeans and rapeseed (Krishnan et al. 2025). It has exceptional economic and nutritional value is exceptional, as the seed is rich in vegetable protein, unsaturated fatty acids, fibre, and essential micronutrients such as vitamin E and magnesium (Syed et al. 2020). This composition makes it a pillar of food and nutritional security for millions of people.

In Mali, for example, peanuts occupy a central socio-economic and historical place, particularly in the former production regions of Kayes, Koulikoro, and Sikasso. They remain a cornerstone of family farming systems, providing both subsistence and income generation (Clavel et al. 2005). Production systems, which are mainly manual and practiced as monoculture or in association with cereals, are concentrated in the southern and western regions of the country, where annual rainfall of 500-800 mm allows for their cultivation (Yattara, M. 2024). However, the sustainability and productivity of this crop are severely compromised by a set of interdependent constraints. Production, which fluctuates around several hundred thousand tons per year, including 497,000 tons in 2024 (USDA Mali peanut

production 2024), and is marked by chronic instability. This volatility is mainly attributable to climatic hazards, the impact of which is exacerbated by climate change (e.g. droughts and irregular rainfall). These abiotic stresses interact with significant biotic pressures, including fungal diseases such as cercosporiosis (caused by *Cercospora arachidicola* and *Cercosporidium personatum*) and root rot. These diseases can cause substantial yield losses, often exceeding 30% (Waliyar et al. 2007; Waliyar, F. 2000; Subrahmanyam, P. 2011; Adam et al. 2016). Added to this are structural socio-economic challenges, including limited access to quality inputs, low mechanization, and market instability.

Faced with these multiple challenges, adopting resilient and sustainable agricultural practices is imperative for securing production. Dependence on chemical inputs, in addition to its costs and environmental impacts, can disrupt beneficial soil microbial communities, including symbiotic nitrogen-fixing bacteria such as *Rhizobium* and *Bradyrhizobium*, which play a crucial role in the nitrogen nutrition of legumes (Nihorimbere et al. 2025; Kour, D., & Yadav, A. N. 2025). In this context, seed biopriming, a pretreatment technique involving the controlled hydration of seeds in the presence of beneficial biological agents, is emerging as a promising strategy for biocontrol and biostimulation.

Among the most studied microorganisms, plant growth-promoting rhizobacteria (PGPR), particularly *B. subtilis*, offer remarkable potential (Nihorimbere et al. 2024). This bacterium combats pathogens through three synergistic mechanisms, namely the production of antifungal metabolites (iturins and fengycins), making it a biofungicide, competition for ecological niches, and the induction of systemic resistance in the plant (Pérez-García et al. 2011). As a biostimulant, *B. subtilis* improves seed vigor, promoting early germination and increased root development, allowing the plant to explore the soil more efficiently for water and nutrients (Hashem et al. 2019). Furthermore, it can potentially facilitate symbiosis with rhizobia by creating a favorable rhizospheric environment, thereby improving nodulation and biological nitrogen fixation. While the beneficial effects of *B. subtilis* have been documented for various crops against a range of soil-borne pathogens (Zhang et al. 2021), its application to peanuts in Mali's agro-pedoclimatic remains insufficiently explored. The effectiveness of this approach must be evaluated in the context of local constraints, such as the prevalence of fungal diseases, the frequency of water stress, and the quality of often degraded soils.

This study therefore aims to evaluate the effect of biopriming peanut seeds with a strain of *B. subtilis* on the agronomic performance of the peanuts in a Malian context. It is hypothesised that this treatment will (i) improve early growth parameters, (ii) stimulate nodulation and plant vigor, and (iii) ultimately increase productivity and resilience to water stress. The results will contribute to developing an environmentally friendly and economically accessible production strategy to support the sustainability of the Malian peanut sector.

II. MATERIALS AND METHODS

2.1 Study site

The trial was set up at the Regional Agricultural Research Center (CARR) in Sotuba, located near Bamako, Mali (12.65277° N, -7.92614° W). Covering an area of 267 hectares, this center is bordered by the Niger River and experiences a Sudano-Sahelian climate characterized by a dry season from November to May and a rainy season from June to October with annual rainfall ranging from 1,000 to 1,200 mm. The soils in the area are mainly ferruginous with a silty-clay or sandy-clay texture (Coulibaly, M. 2023).

2.2 Biological material

The study focused on two peanut varieties, Fleur 11 and ICGV-IS 13825 or Keniana tige. These genotypes were selected for their drought tolerance and high yield potential, with a short growing cycle of 90 to 95 days. The bacterial inoculum used consisted of the local strain BS2b of *B. subtilis* isolated in Burkina Faso and was kindly provided by the plant clinic at Nazi BONI University in Bobo-Dioulasso.

2.3 Preparation of inoculum and seed maceration

Bio-priming was performed according to the method developed by Singh et al. (2023). The strain was grown in nutrient broth and then incubated at 28°C with agitation at 120 rpm for 48 hours. The cells were harvested by centrifugation, and the final bacterial suspension was adjusted to a density of 3.75×10^8 CFU/mL with sterile distilled water. The peanut seeds were first disinfected by immersing them for one minute in a 1 % sodium hypochlorite solution, followed

by three washes with sterile distilled water for 15 minutes each. For inoculation, the sterile seeds were immersed for two hours at 28° C in the bacterial suspension containing 2 % carboxymethylcellulose (CMC) had been added as an adhesive agent. Finally, the coated seeds were air-dried in a laminar flow hood for two hours before use.

2.4 Experimental device

A split-plot design with four replicates was adopted to study the two main factors separately and in interaction. The experimental design adopted was a split-plot with four replicates, in order to study the two main factors separately and in interaction. The factor assigned to the main plots was the peanut variety, with two levels: FLEUR 11 and ICGV-IS 13825. The secondary factor, applied to the subplots, was the treatment with four distinct modalities, namely the absolute control or T1 (Bs0A0: untreated seed and no inoculation with *B. subtilis*), bacterial inoculation alone or T2 (Bs1A0 seed inoculated with the *Bacillus subtilis* strain, without fungicide treatment), combined treatment or T3 (Bs1A1 seed treated with a protective fungicide, then inoculated with *B. subtilis*), and fungicide treatment alone or T4 (Bs0A1 seed treated with a fungicide, without subsequent bacterial inoculation). This factorial design enabled the specific effects of each variety and the effect of the different seed treatments to be analysed, as well as their potential interactions. The seed treatment applied during the experiment was “Caïman Rouge”, consisting of permethrin and thiram at a dosage of one 25 g package per 10 kg of seeds.

2.5 Measured parameters

Several agronomic and physiological parameters were measured to evaluate the growth and development of peanut plants. Plant height was recorded at three key phenological stages, namely 25, 40, and 55 days after sowing (denoted PHS25, PHS40, and PHS55, respectively). At harvest, the length of the main root (LRH) and the relative humidity of the plants (RHP) were also determined in order to assess their water status at harvest. Regarding nitrogen-fixing symbiosis, the total number of nodules (NNoD) and their weight (WNoD) were counted. Finally, grain yield was estimated based on grain weight (GW) per treatment.

2.6 Statistical analyses

The data were subjected to a two-factor analysis of variance (ANOVA) using a split-plot design. Comparisons of means between treatments were performed using the Least Significant Difference (LSD) test at a 5% probability threshold. All statistical analyses were performed using R software (version 3.6.1).

III. RESULTS

3.1 Vegetative development parameters

3.1.1 Plant height

- **PHS25** : A two-factor analysis of variance (variety and treatment) was performed on peanut plant heights 25 days after sowing. The results showed a significant effect of treatment ($F = 14.19$; $p < 0.001$), but no significant effect of variety ($F = 0.008$) or the interaction between variety and treatment ($F = 1.005$). The treatment means, for all varieties combined, are as follows: Bs0A0 = 10.5 cm, Bs1A0 = 17.7 cm, Bs1A1 = 15.1 cm, and Bs0A1 = 14.4 cm. The corresponding histogram (Fig. 1.A) shows that treatment Bs1A0 produces the tallest plants, followed by Bs1A1 and Bs0A1, while Bs0A0 produces the shortest. A breakdown of the treatment factor into its components (Bs and A) reveals a significant interaction between Bs and A: the effect of Bs is positive in the absence of A, but negative in its presence. Thus, the Bs1A0 combination is the most favorable for early growth. These results highlight the importance of selecting an appropriate treatment to optimize plant height at 25 days after sowing.

- **PHS40** : Forty days after sowing, analysis of variance reveals that only the treatment effect is highly significant on height ($p < 0.001$). The effects of variety and its interaction with treatment are not significant, indicating that both varieties respond similarly to the treatments. breakdown of the treatment effect shows that factor Bs has a highly significant effect ($p < 0.001$), whereas factor A alone is not significant. However, the Bs and A interaction is significant ($p < 0.01$), meaning that the effect of Bs depends on the level of A. In the absence of factor A (A0), the application of factor Bs (Bs1A0) increases height by 10.0 cm compared to the control (Bs0A0). In the presence of A (A1), the increase due to Bs is only 2.9 cm (Bs1A1 vs. Bs0A1). The application of A alone (Bs0A1) increases height by 4.1 cm compared to the control, however in combination with Bs, it slightly reduces height by 3.0 cm compared to Bs alone. This antagonistic interaction suggests that the positive effect of Bs on growth is reduced in the presence of A. The most favorable treatment is Bs1A0 (34.85 cm), followed by Bs1A1 (31.81 cm) and Bs0A1 (28.95 cm), with the control

Bs0A0 giving the lowest height (24.86 cm) (Fig. 1.B). The Bs factor significantly improves plant height, but this effect is reduced by the addition of A. The variety does not influence the response. These results suggest that the technical choice should be to use Bs alone to maximize growth at 40 days.

- **PHS55** : At 55 days after sowing, the analysis of variance highlights a highly significant effect of factor Bs ($F = 21.08$; $p < 0.001$) and a significant Bs vs. A interaction ($F = 12.18$; $p < 0.01$). However, the effects of factor A, variety, and its interactions with the treatments were not significant. Treatment Bs1A0 produced the highest height (43.9 cm), followed by Bs1A1 (38.3 cm), while treatment Bs0A0 produced the lowest height (31.6 cm) (Fig. 1.C). The significant interaction can be explained by a contrasting effects of A depending on the level of Bs. In the absence of Bs (Bs0), the application of A increased height (from 31.6 to 36.6 cm). In the presence of Bs (Bs1), the application of A reduced height (from 43.9 to 38.3 cm). The variety did not significantly influence height, although Fleur11 had a slightly higher average (38.7 cm) than Keniana tiga (36.5 cm). For height at 55 days, the Bs factor is decisive, and should be avoided in combination with A should be avoided as it does not promote growth. The optimal treatment is Bs1A0, regardless of variety.

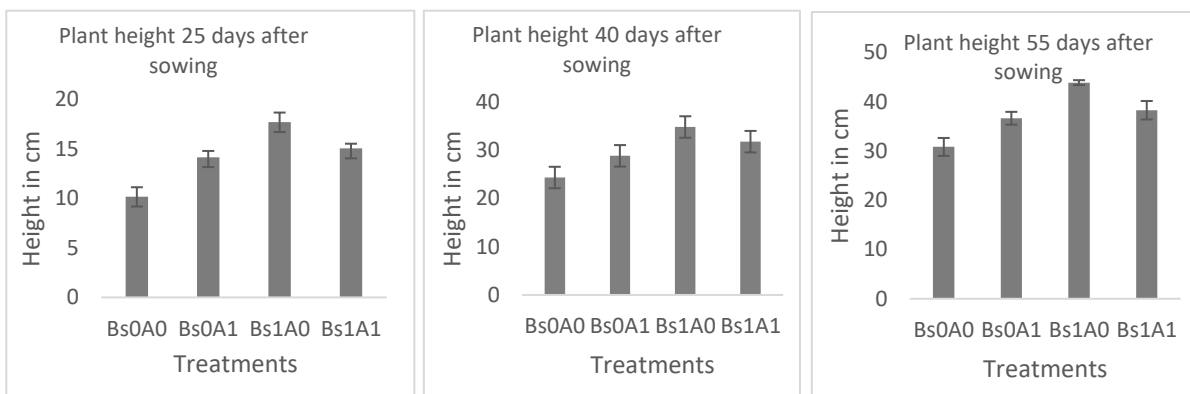


Figure 1 : Effect of bacterial inoculation with strain BSb2 on the growth of two peanut varieties. A: Plant height 25 days after sowing; B: Plant height 40 days after sowing; C: Plant height 55 days after sowing.

3.1.2 Main root length, relative humidity, and grain weight

- **Length of the main root after harvesting (LRH)** : The analysis of variance revealed a highly significant effect of the factors *Bacillus subtilis* (Bs) ($F = 14.78$; $p < 0.01$) and seed treatment (A) ($F = 9.94$; $p < 0.01$) on root length. However, no significant interactions were found between Bs and A, variety and factors, or triple interactions. The effect of variety did not reach the significance threshold ($F = 1.30$; $p > 0.05$). The Bs1A0 treatment (mean = 16.69 cm) produced significantly longer roots than the control treatment (13.77 cm). The A0 treatment (no seed treatment, mean = 16.43 cm) produced longer roots than the A1 seed treatment (14.03 cm). Figure 2 shows the distribution of root length for each level of the significant factors. The box corresponding to the Bs1A0 treatment is clearly shifted towards higher values than those of the other treatments, and the Bs1A0 combination stands out with the longest median length (approximately 18.2 cm) and low variability. These results demonstrate that combining application of *B. subtilis* with no seed treatment (Bs1A0) maximizes root growth, regardless of variety. The absence of significant differences between the two varieties indicates that this response is stable for the plant material tested.

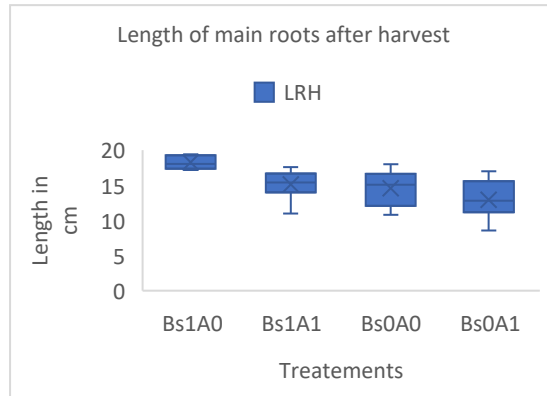


Figure 2 : Effect of bacterial infection by strain BSb2 on the length of the main root of plants of two peanut varieties.

Relative Humidity of Plants (RHP) : ANOVA on the relative humidity data revealed a significant effect of the Bs1A0 treatment compared to the other treatments. These differences are not due to chance. The results suggest that the Bs1A0 combination promotes better moisture retention at harvest, whereas the Bs0A1, Bs0A0, and Bs1A are unfavorable. The Bs1A0 treatment had the highest median and moderate dispersion, indicating higher relative humidity. In contrast, the Bs0A1 treatment has the lowest median and significant variability, with particularly low values (minimum around 39%). The Bs0A0 and Bs1A1 treatments exhibit intermediate levels, but Bs1A1 exhibits a broader range (Fig. 3).

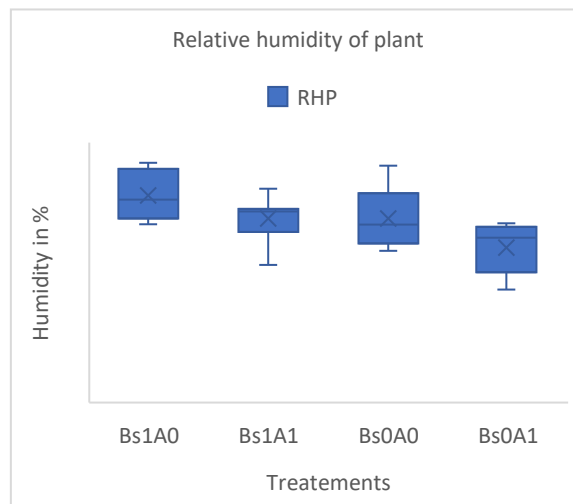


Figure 3 : Effect of bacterial infection by strain BSb2 on the relative humidity of plants of two peanut varieties at harvest.

- Grain weight (GW) : The effect of factor Bs1A0 is highly significant ($p < 0.01$) compared to the other treatments. However, the effects of variety and its interactions with treatments are not significant. Figure 4 shows the distribution of grain weight for the four treatment combinations (Bs0A0, Bs0A1, Bs1A0, and Bs1A1), when all blocks and varieties are combined, given the absence of interaction with the variety.

Bs1A0 stands out with the highest median value (approximately 73.4 g) and an average of 86.3 g. However, its dispersion is high (interquartile range of 39.2 g), reflecting significant variability between plots. Treatment Bs1A1 has a median of 52.3 g and an average of 57.8 g, also with high dispersion (IQR = 38.8 g). Treatment Bs0A1 has a median of 50.0 g and an average of 54.3 g, with lower dispersion (IQR = 19.1 g). Treatment Bs0A0 (control) showed the lowest

median (44.0 g) and a mean (46.5 g), and moderate dispersion (IQR = 27.6 g). The Bs1A0 combination appears to be the most promising for maximizing yield, although its variability must be taken into account for any agronomic recommendations.

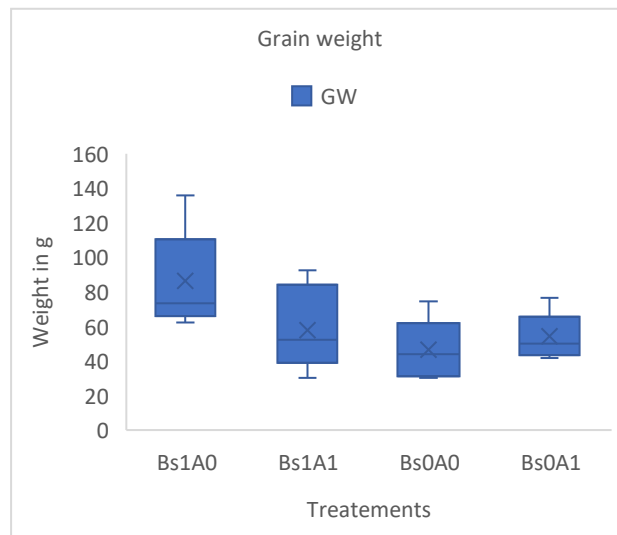


Figure 4 : Effect of bacterial treatment with strain BSb2 on the weight of grains from different treatments after harvest

2.7 Nodulation

- **Number of nodules (NNoD)** : The difference between the four treatments is highly significant ($F = 7.26$; $p < 0.01$). Therefore, the number of nodules therefore varies depending on the treatment applied. No significant difference was detected between Fleur11 and Keniana tige ($F = 0.07$). These two varieties react similarly, and the absence of a significant interaction ($F = 0.23$) suggests that the effect of the treatments is consistent between the two varieties. Figure 5 shows the distribution of the number of nodules for each of the four treatments, all varieties combined. Treatment Bs0A0 shows an average of 127.3 nodules, moderate spread, and a few extreme values. Bs1A0 shows an average of 211.4 nodules, high values, and high variability (notably a very high value of 347.2). Treatment Bs1A1, with an average of 193.1 nodules, has a fairly symmetrical distribution, and treatment Bs0A1, with an average of 146.2 nodules, has a distribution comparable to Bs0A0. The treatments containing *B. subtilis* (Bs1A0 and Bs1A1) show significantly higher medians than the treatments without *B. subtilis* (Bs0A0 and Bs0A1). The averages suggest that Bs1A0 is the treatment most favorable to nodulation, followed by Bs1A1, while Bs0A0 and Bs0A1 produce the lowest numbers of nodules.

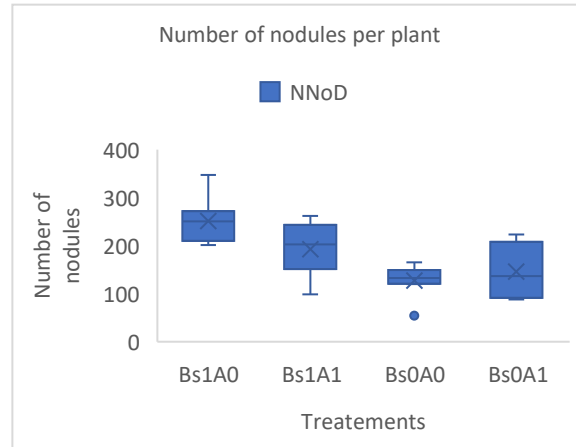


Figure 5 : Effect of bacterial inoculation with strain BSb2 on the number of nodules in plants of two peanut varieties at harvest.

Weight of nodules (WNoD): Treatments with *Bacillus subtilis* (Bs1A0 and Bs1A1) produced significantly higher nodule weight than treatments without *B. subtilis* (Bs0A0 and Bs0A1). Among the treatments containing Bs, the absence of A (A0) produced better result than the presence of A1. The Bs0A1 treatment resulted in the lowest nodule weight. These results suggest that adding *B. subtilis* promotes nodulation, while the presence of seed treatment A reduces it, regardless of variety. Figure 6 shows the whisker plots of nodule weight for each treatment (raw data, all varieties combined). The hierarchy of treatments is clearly visible, namely Bs1A0 > Bs1A1 > Bs0A0 > Bs0A1. There is relatively low dispersion within each group, confirming the robustness of the observed differences.

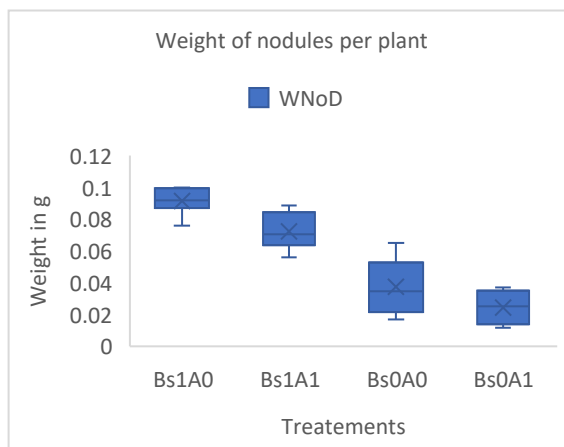


Figure 6 : Effect of inoculation with strain BSb2 on nodule weight in plants of two peanut varieties

III. DISCUSSION

The results of this study underscore the pivotal role of *Bacillus subtilis* in promoting the growth and development of peanuts (*Arachis hypogaea* L.), while emphasizing the importance of interactions among production factors. Bioinoculants such as *B. subtilis* or *P. fluorescens* have been shown to produce indole-3-acetic acid (IAA), siderophores, and hydrogen cyanide (HCN). They also have the ability to solubilize phosphorus (P), thereby contributing to improved plant growth (Nithyapriya et al. 2021; Bayat et al. 2017). The main objective of this study was to evaluate the effects of *B. subtilis* treatment and conventional seed treatment (red caiman), both alone and in combination, on two peanut varieties. Our results show that, regardless of variety, the application of *B. subtilis* without

seed treatment (Bs1A0) is the most effective strategy for optimizing aerial and root growth, nodulation, cell turgidity, and yield.

Analysis of plant height data at different stages (25, 40, and 55 days) reveals a consistent dynamic interaction between the Bs and A factors. Starting at 25 days after sowing (DAS), the positive effect of *B. subtilis* depends on the level of factor A. The significant interaction ($p < 0.05$) observed can be explained by a substitution effect in the absence of seed treatment (A0), where the addition of Bs (Bs1A0) strongly stimulates growth, while in the presence of seed treatment (A1), however the benefit provided by Bs is minimal (0.69 cm). This pattern is confirmed and accentuated at 40 and 55 days after planting (DAP).

At 40 JAS, the highly significant interaction between Bs and A ($p < 0.01$) illustrates antagonism. The application of Bs alone (Bs1A0) results in a gain of 10 cm compared to the absolute control (Bs0A0). However, when Bs is applied in combination with A (Bs1A1), the gain decreases to 2.9 cm compared to treatment A alone (Bs0A1). Furthermore, the Bs1A1 combination is less effective than Bs1A0, resulting in a loss of 3 cm. This trend is even more pronounced at 55 JAS, where the application of A in the presence of Bs reduces height (from 43.9 to 38.3 cm), whereas it increases it in the absence of Bs. These results suggest that seed treatment A, consisting of a fungicide and a chemical insecticide, could have a negative effect on the efficacy of *B. subtilis*. This is consistent with previous studies reporting the sensitivity of certain strains of PGPR (Plant Growth-Promoting Rhizobacteria) to phytosanitary molecules, altering their ability to colonize the rhizosphere or produce phytohormones (Ahmad & Khan, 2012).

The beneficial effects of *B. subtilis* are evident in root growth. The Bs1A0 treatment produces roots that are significantly longer (16.69 cm) than those of Bs. The lack of a significant Bs \times A interaction for this parameter suggests that the negative effects of A on height do not result in a significant reduction in root length in the presence of Bs. However, the trend is similar, with root length under Bs1A1 (unspecified but lower) following the logic of the results. A more developed root system, such as that observed under Bs1A0, potentially improves soil exploration and access to water and nutrients. This could explain, at least in part, the significantly higher relative humidity measured at harvest for this same treatment. Better root exploration allows for more efficient water uptake at the end of the cycle, thus delaying the dehydration of the organs.

The most significant impact of *B. subtilis* concerns nodulation. Treatments including Bs (Bs1A0 and Bs1A1) significantly increase the number and weight of nodules compared to treatments without Bs. These results are consistent with the known properties of certain PGPR bacteria, which can stimulate the nodulation process by Rhizobium bacteria naturally present in the soil through mechanisms such as the production of flavonoid-like compounds or the modification of root physiology (Zhang et al. 2017; Cheng et al. 2025; Alemneh et al. 2020; Smith et al. 2015; Swarnalakshmi et al. 2020; Abrham et al. 2025; Adeel Ahmad et al. 2022). Again, antagonism with seed treatment A is evident, although Bs1A0 and Bs1A1 are both superior to the controls, the presence of A significantly reduces nodulation compared to the use of Bs alone. The Bs0A1 treatment even shows the lowest nodulation, suggesting a direct depressive effect of the chemical on symbiotic bacteria or on nodule initiation. This reduction in nodulation in the presence of treatment A could be a key mechanism explaining the lower plant growth in the combined treatments, through a limitation of symbiotic nitrogen supply.

All of these effects converge on grain yield, the ultimate parameter of interest to farmers. The Bs1A0 treatment stands out with the highest average grain weight (86.3 g), confirming its potential to maximize production. However, the high variability observed for this treatment (IQR = 39.2 g) and for Bs1A1 (IQR = 38.8 g) is notable. This dispersion could reflect an increased sensitivity of *B. subtilis*-treated plants to micro-variations in environmental conditions (soil heterogeneity, microclimate), or a more complex interaction with the indigenous soil microflora that was not controlled. Conversely, treatments without Bs, although less productive, appear to be more stable (lower IQR for Bs0A1). This trade-off between high yield potential and stability is a crucial point to consider for agronomic recommendations.

A major and consistent finding across all analyses was the absence of any significant effect of variety (Fleur11 vs. Keniana tiga) and its interactions with treatments. The two varieties tested responded similarly to the different treatments for all measured parameters. These results suggest that the mechanisms of action of *B. subtilis* and the

antagonistic interaction with seed treatment are sufficiently general to be expressed on this genetic material, and that the recommendations arising from this study can be applied to both varieties without distinction

This study demonstrates the strong potential of *B. subtilis* as a bioinoculant for improving peanut growth and yield. However, it cautions against the systematic combination of this type of PGPR with certain chemical seed treatments, which can inhibit its beneficial effects, as shown in several studies (Illa et al. 2020; Eswaran et al. 2023). The Bs1A0 combination appears to be the most promising for maximizing production. Further research is needed to (i) evaluate the colonization dynamics of *B. subtilis* in the rhizosphere in the presence and absence of seed treatment, and (ii) test this winning combination under different soil and climate conditions to assess the stability of its performance and variability.

IV. CONCLUSION

This study demonstrates the major role of *Bacillus subtilis* as a biofertilizer in enhancing the agronomic performance of peanuts. The effect of this factor is highly significant at all stages of development (height at 25, 40, and 55 days after sowing), confirming its potential to stimulate aerial growth, root development, turgidity, nodulation, and grain yield. A key finding is the significant antagonistic interaction between *B. subtilis* (Bs) and chemical seed treatment (A). Although seed treatment alone can improve growth in the absence of Bs, its joint application with the bacterium systematically reduces performance compared to the use of Bs alone. This unfavorable trend is observed for all measured parameters: plant height, root length, grain weight, and nodulation. Seed treatment alone never showed a significant effect without *B. subtilis*, except in negative interaction with it. Additionally, the absence of a significant effect of the varieties (Fleur11 and Keniana tiga) and their interactions with the treatments show almost identical responses, indicating the robustness and stability of the observed effects, regardless of the genetic background. The Bs1A0 combination (inoculation with *B. subtilis* without seed treatment) clearly stands out as the optimal treatment. It consistently produces the highest values for plant height at all stages, root length, relative humidity at harvest, grain weight, and the number and weight of nodules, with marked gains compared to the control. These results open up interesting prospects for sustainable and biostimulant management of peanut cultivation, pending larger-scale validation.

Additional materials : Summary table of statistical analyses representing averages and effects.

Table 1 below shows that the two peanut varieties have statistically equivalent performances. However, the treatments applied have a very significant influence on all yields. Treatment T2 (Bs1A0) is the most effective, while T1 (Bs0A0) is the least effective. The absence of interaction indicates that these effects are valid for both varieties.

Table 1 : Summary data on averages and effects

Factors	HPS 25	HPS 40	HPS 55	LRH	GW	RHP	NNoD	WNoD	
Varieties (V)									
Fleur 11	14,45	31,2	38,8	16	66	63	181	0,0571	
Keniana tiga	14,38	29	36,5	15	56	64	178	0,0558	
Probabilité	NS	NS	NS	NS	NS	NS	NS	NS	
Traitements (T)									
T1 Bs0A0	10,5	b 24,9	b 31,6	b 15	ab 47	b 67	ab 128	b 0,0383	ab
T2 Bs1A0	17,72	a 34,9	a 43,9	a 18	a 86	a 70	a 251	a 0,0915	a
T3 Bs1A1	15,06	ab 31,8	ab 38,3	ab 15	ab 58	ab 64	ab 193	ab 0,0724	ab
T4 Bs0A1	14,37	ab 29	ab 36,6	ab 13	b 54	ab 54	b 144	ab 0,0253	b
Probability	0,0231	0,0002	0,001	0,0008	0,002	0,0096	0,0003	0,001	
Interactions									
V x T	NS	NS	NS	NS	NS	NS	NS	NS	

a : Significant difference from the highest value ; **b** : Significant difference with the lowest value ; **ab** : Intermediate difference ; **NS** : Statistically insignificant.

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