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Potential of Bioinoculants Based on Plant Growth-Promoting Rhizobacteria (PGPR) Isolated in the Municipality of N'Zérékoré (Guinea) for the *in Vitro* Germination of Maize and Rice

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Abstract

Against a backdrop of steadily declining soil fertility, falling crop productivity, dependence on imported chemical inputs, and mounting pressures on food security, Guinea's agricultural sector must rethink its production models by adopting endogenous, sustainable, and agroecological solutions. Against this backdrop, the objective of this study was to evaluate the potential of bioinoculants based on indigenous rhizobacteria isolated from the rhizosphere soils of the urban municipality of N'Zérékoré on the *in vitro* germination of maize and rice. The experimental approach involved collecting soil samples from the rhizosphere of maize and rice plants. Next, rhizobacteria strains were isolated, identified, and characterized using Gram staining, catalase, and tests for the production of ammonia, indole acetic acid, hydrogen cyanide, etc. The identified strains were then evaluated for their agronomic performance through an *in vitro* germination test. To do this, maize and rice seeds, which had been previously disinfected, were treated by soaking in bioinoculants based on the isolated rhizobacteria at bacterial concentrations of approximately 10^8 CFU/mL and then transferred to Petri dishes for *in vitro* germination at 30˚C for seven days. The results show that all strains produced catalase, indoleacetic acid (IAA), and ammonia. The Ri2, M5, Ri1, and M4 strains significantly improved

several growth parameters, including root length, seedling length, and vigor index in maize and rice, as well as seedling and root weight in maize. Although no statistically significant differences were observed in germination rates, these strains showed the best values compared to the control. These results confirm the ability of local rhizobacteria to play a central role in improving the germination of rice and maize seeds. This study also opens up concrete prospects for establishing local bioinoculant production chains that generate added value and are adapted to the soil and climate conditions of Forest Guinea.

Keywords

Improvement, Biostimulants, PGPR, Growth, Sustainable Agriculture

1. Introduction

The rhizosphere, the zone of soil surrounding and influenced by plant roots—is home to a complex microbial community [1]. These beneficial microorganisms form symbiotic relationships there that enhance the overall health and resilience of crops. Among them, plant growth-promoting rhizobacteria (PGPRs) are attracting growing interest due to their ability to stimulate crop development and mitigate various environmental stresses. This interest has intensified particularly for PGPR associated with cereals, whose positive effects on growth and yield have been demonstrated under various ecological conditions [2]-[7]. In this global context of PGPR promotion, their potential appears particularly relevant for low-productivity agricultural systems such as those in the Republic of Guinea. Indeed, agriculture is a crucial pillar of Guinea's economy, contributing approximately 28 to 30% of GDP and providing employment for more than half of the working population [8].

In Guinea, rice is the primary food crop and plays a major role in the population's diet and food security. Cultivated in all regions of the country and accounting for approximately 67% of the area planted with cereals, its production is particularly concentrated in the major rice-growing regions of N'Zérékoré, in Forest Guinea, and Boké [9] [10]. As for maize, it is one of Guinea's main food crops, particularly in the Faranah region, where it is an essential source of food and income for rural households [11]. Despite favorable agroecological potential, rainfed rice yields in Guinea remain low, ranging from 0.8 to 1.5 t/ha, well below the current global average of around 5 t/ha [12]. Similarly, a significant decline in maize yields has recently been reported in the Faranah region [11]. This low productivity is attributed in particular to the gradual depletion of soils, inappropriate farming practices, and the increasing irregularity of rainfall patterns [11] [13] [14]. In Forest Guinea, particularly in the N'Zérékoré region a major agricultural production hub with an estimated output of 520,824 metric tons of rice and 60,390 metric tons of maize [9], the intense leaching of ferrallitic soils is driving farmers toward increased dependence on chemical inputs.

The excessive use of fertilizers and pesticides [15] not only imposes heavy eco-

nomic burdens but also exacerbates soil acidification and water pollution [16]. According to [17], this chemical degradation disrupts native microbial communities and gradually alters the soil's physicochemical properties. In light of these agro-environmental constraints, the use of sustainable biological alternatives appears essential for restoring soil fertility and sustainably improving agricultural productivity. From the perspective of sustainable agriculture, interest in beneficial rhizobacteria associated with cereals, in particular, has recently increased [18] [19]. Several PGPR bacteria capable of enhancing plant development and increasing plant tolerance to various abiotic and biotic stresses have been selected from the rhizosphere of cereal crops worldwide [20]-[22]. However, despite the potential benefits of using plant growth-promoting rhizobacteria (PGPR) to improve crop productivity and enhance crop protection [23]-[25], these strategies remain largely underutilized in efforts to improve cereal production in Africa.

However, in Guinea, little research has been devoted to identifying and utilizing indigenous PGPRs to improve cereal growth and yield. Thus, the objective of this study is to evaluate the potential of growth-promoting rhizobacteria isolated from the rhizosphere in N'Zérékoré on the *in vitro* germination of maize and rice, with a view to their use as bioinoculants adapted to local agroecological conditions.

2. Materials and Methods

2.1. Isolation and Identification of Rhizobacteria from the Rhizosphere Soils of Rice and Maize

- **Collection of Soil Samples**

Rhizosphere soil samples were collected in the urban municipality of N'Zérékoré, Guinea, in April 2025. Four neighborhoods in the urban agricultural production zone were strategically selected based on the presence of target crops, notably maize (*Zea mays* L.) and rice (*Oryza sativa* L.).

The selected sites were: Horeya 1 (7.76592833˚ N; 8.80643833˚ W), Nakoyakpala (7.72554987˚ N; 8.83684901˚ W), Mohomou (7.73585699˚ N; 8.83269440˚ W), and Commercial-Ossud 1 (7.73846167˚ N; 8.82232833˚ W). The sampling sites are shown in **Figure 1**.

In the neighborhoods of Nakoyakpala, Mohomou, and Commercial-Ossud 1, only plots planted with maize were identified. In each of these three (3) areas, five (5) sampling points were randomly selected along the two (2) diagonals of each surveyed maize or rice field. At each point, two (2) maize plants were dug up. Their roots were cut into pieces, taking care to preserve the soil adhering to them. Three (3) composite samples of 300 g each of maize rhizosphere soil from this mixture were collected, for a total of nine (9) samples.

In the Horeya 1 neighborhood, which is characterized by rice cultivation, three (3) composite samples of rice rhizosphere soil were also collected using the same method, for a total of twelve (12) samples.

In total, twelve (12) rhizosphere soil samples were packaged in sterile bags, carefully labeled, and then transported to the Cell Biology Laboratory at the University

of N'Zérékoré in coolers containing ice packs to preserve their microbiological integrity. Upon arrival at the laboratory, the samples were stored at 4˚C and analyzed within a maximum of 72 hours for microbiological testing.

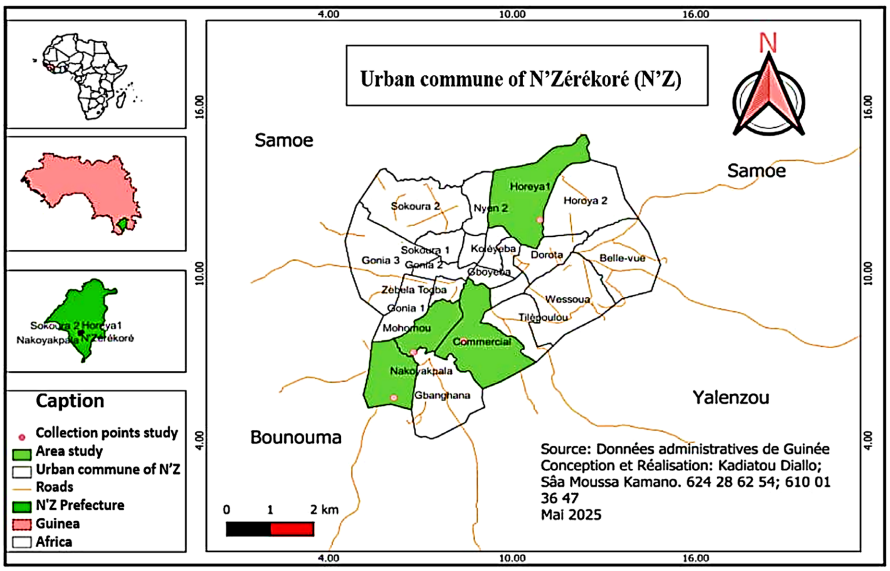


Figure 1. Map showing the sample collection areas in the urban municipality of N'Zérékoré (Guinea).

- **Isolation of Rhizobacteria Strains**

As part of the microbiological analysis of the soils, the culture media required for bacterial growth were prepared. 10 g of each sample was weighed using an electronic balance and transferred to an Erlenmeyer flask containing 90 ml of Tryptone Salt liquid nutrient medium. The resulting mixture was vigorously shaken for approximately 2 minutes to prepare the stock solution. Next, a series of dilutions was performed by transferring 1 mL of the stock solution into 9 mL of Tryptone-Salt diluent. This process was repeated in series to achieve dilutions ranging from 10^{-1}; 10^{-2} through 10^{-5}. At each step, the mixture was shaken vigorously to ensure the suspension was completely homogeneous. For inoculation, 1 mL of the final dilution was transferred to a sterile Petri dish. Approximately 25 mL of solid nutrient medium (Nutrient Agar) kept at a temperature of 45˚C was added, and the mixture was homogenized by gently rotating the dish. The dishes were then left at room temperature to allow the medium to solidify. After complete solidification, a second layer (approximately 5 mL) of the same agar was added, and the Petri dish was incubated at 30˚C for 48 hours. The rhizobacteria strains were selected for subsequent morphological and microbiological analyses [26] [27].

- **Identification of Isolated Rhizobacteria Strains**

The identification of the isolated rhizobacteria began with macroscopic observations (colony morphology, pigmentation, etc.) and microscopic observations (Gram stain, cell shape). This initial identification was followed by several other enzymatic tests, such as the production of oxidase, catalase, and indole.

- **Gram Stain, Catalase, and Oxidase Tests**

The Gram stain is used to determine the type of cell wall in bacteria. The Gram stain is a laboratory technique used to differentiate bacteria based on the composition of their cell walls. It involves preparing a bacterial smear, successively applying gentian violet, Lugol's solution, decolorizing alcohol, and fuchsin, and then observing the sample under a microscope. Bacteria appear purple if they are Gram-positive and pink if they are Gram-negative. This distinction is essential for the identification and classification of bacteria.

The catalase test was performed by placing a colony of the rhizobacteria under study on a clean, dry slide, followed by a drop of hydrogen peroxide (H_2O_2), also known as oxygenated water. The immediate formation of bubbles indicates positive catalase activity [28].

The oxidase activity of the strains was assessed using a test strip kit impregnated with NNN'N'-tetramethyl-p-phenylenediamine dihydrochloride. A single colony from each isolated rhizobacterial strain was picked up using a sterile plastic loop and then applied to the surface of the test strip. The appearance of a purple or blue-violet color indicated a positive reaction.

2.2. Investigation of Certain Plant Growth-Promoting Properties of Isolated Rhizobacteria Strains

- **Hydrogen Cyanide (HCN) Production**

Hydrogen cyanide (HCN) production by the isolated bacterial strains was assessed using the method described by [29]. The isolates were streaked onto nutrient agar supplemented with glycine (4.4 g/L), which had been previously poured into Petri dishes. Each dish was then covered with Whatman No. 1 filter paper soaked in an alkaline solution consisting of 2% sodium carbonate and 0.5% picric acid. The Petri dishes were hermetically sealed with waxed paper and then incubated at 36 ± 2˚C for four (04) days. HCN production was assessed by the appearance of discoloration on the filter paper.

- **Ammonia Production (NH_3)**

The ability of the isolated bacterial strains to produce ammonia was evaluated using the method described by [30]. Fresh bacterial colonies were inoculated into 10 mL of peptone water and then incubated at 36˚C ± 2˚C for 48 to 72 hours. At the end of the incubation period, 0.5 mL of Nessler's reagent was added to each culture to detect ammonia production, which was indicated by the appearance of a characteristic color.

- **Production of Indole-3-Acetic Acid (IAA)**

The production of indole-3-acetic acid (IAA) was assessed using the method described by [31]. The bacterial isolates were cultured in Müller-Hinton (MH) liquid nutrient medium. After sterilization, the medium was supplemented with filter-sterilized L-tryptophan to achieve a final concentration of 0.1%. The enriched medium was dispensed into sterile tubes at a rate of 3 mL per tube. Each tube was inoculated with a bacterial isolate, with two replicates per strain. The

cultures were incubated at 30˚C for 48 hours. After incubation, 1 mL of culture was withdrawn and centrifuged at 10,000 rpm for 10 minutes according to the method of Patten and [32]. The resulting supernatant was then mixed with an equal volume of Salkowski's reagent, prepared by combining 1 mL of ferric chloride ($FeCl_3$) at 0.5 mol/L with 49 mL of 35% perchloric acid ($HClO_4$). The appearance of a pink color after 10 to 15 minutes was considered indicative of AIA production.

2.3. Evaluation of the Effects of Bioinoculants Based on Isolated Strains on the *in Vitro* Germination of Maize and Rice

- **Refreshing the Selected Strains**

After the isolation stage, the bacterial strains underwent a purification and multiplication process. To this end, the colonies initially isolated on nutrient agar were subcultured into new Petri dishes containing MH Agar (Müller-Hinton Agar) to obtain pure, fresh cultures. The transfer was performed using a platinum loop sterilized over the flame of an alcohol lamp, under strict aseptic conditions to prevent any cross-contamination by external bacteria. Only clearly distinct and homogeneous colonies were selected for this process.

- **Preparation of bioinoculants**

First, the previously isolated bacterial strains were picked up using a sterilized platinum loop and then transferred to liquid MH (Müller-Hinton) medium in sterile tubes. The inoculated tubes were then incubated at 30˚C for 24 hours in an incubator to allow the strains to reactivate and multiply. After 24 hours, another culture was prepared from the preculture and incubated at 30˚C for 24 hours. Finally, the concentration of the bioinoculants was adjusted by measuring the optical density (OD) using a spectrophotometer at 620 nm, aiming for a target value corresponding to 10^8 CFU/mL (OD = 0.45) [33]. Suspensions with an optical density exceeding this value were diluted by adding sterile liquid MH until a uniform OD of 0.45 was achieved in each Erlenmeyer flask, thereby ensuring standardization of the inocula prior to application.

➢ **Inoculation and Germination of Maize and Rice Seeds**

Maize and rice seeds were disinfected using 0.024% sodium hypochlorite and rinsed five (05) times with sterile distilled water [34]. Next, the maize and rice seeds were immersed in the bioinoculants for 30 minutes, depending on the specific strain [35]. It should be noted that for the rice seeds, after disinfection, they were first soaked for 24 hours in sterile distilled water before being transferred to the bioinoculants. The control seeds were immersed in the nutrient medium without bacteria.

Subsequently, twenty (20) maize or rice seeds, which had been previously inoculated, were arranged at equal intervals using sterile forceps in a Petri dish lined with sterile blotting paper that had been pre-soaked in 10 mL of sterile distilled water (SDW). The seeds were then covered with a second layer of blotting paper also impregnated with 10 mL of EDS. The Petri dishes were closed, sealed tightly with a rubber band, and then incubated at 30˚C for seven (7) days, in accordance with the method described by [36].

- **Evaluation of Germination Parameters**

At the end of the seven (07) days of germination, the following parameters were evaluated:

Germination rate per tray: This was determined by counting the number of germinated seeds relative to the number of seeds sown. The germination percentage was calculated by dividing the number of germinated seeds by the number of seeds sown [37]. The length of the root and seedling of each germinated seed: this was measured using a cotton string and a graduated ruler. The vigor index was calculated by summing the average lengths of the roots and seedlings from a single tray and multiplying that sum by the seed germination percentage [38]. The weights of the germinated seed, seedling, and roots were determined by weighing them on a precision scale accurate to 0.01 g.

2.4. Statistical Analysis of the Data

Statistical analyses were performed using the R software (RStudio version 2026.01.1 + 403). Several libraries were used for data processing and analysis. The tidyverse package was used for data manipulation and organization, while agricolae was used for multiple comparison tests. The car package was used to perform statistical tests related to the assumptions of analysis of variance (ANOVA), including the test for homogeneity of variances. In addition, the FactoMineR and factoextra packages were used for multivariate analyses and the creation of graphs. The data were subjected to a one-way analysis of variance (ANOVA) to evaluate the effect of treatments on the various measured parameters. The SNK post-hoc test was performed to compare means at a 5% significance level. In addition, a Principal Component Analysis (PCA) was conducted to explore the relationships among the various measured variables and to characterize the structure of the treatments. The PCA was performed on the variables after centering and data reduction to ensure comparability of the variables.

3. Results and Discussion

3.1. Isolation and Identification of Rhizobacteria Strains

➢ **Isolation of Potential Rhizobacteria**

Microbiological analysis of rhizosphere soils revealed greater bacterial diversity in the rhizosphere of maize and rice. Based on morphological criteria (shape, color, and colony appearance), six (06) potential rhizobacteria strains isolated from maize designated M1 through M6 as well as two (02) strains from rice—designated Ri1 and Ri2 were selected for further testing. In total, eight (08) strains were selected, distributed according to the target crops and sampling areas within the municipality (Table 1).

➢ **Identification of Rhizobacteria Strains (Gram Stain, Catalase, and Oxidase)**

Table 2 presents the preliminary morphological and biochemical characteristics of the eight (08) rhizobacteria strains isolated from the rhizosphere soils of maize (M1 through M6) and rice (Ri1 and Ri2).

Table 1. Distribution of rhizobacterial strains isolated from the rhizosphere soils of maize and rice and their origin.

Soil sample	Soil sample code	Neighborhoods
Maize rhizosphere soils	M_1	Mohomou
	M_2	
	M_3	Commercial (osud1)
	M_4	
	M_5	Nakoyakpala
	M_6	
Rice rhizosphere soils	Ri_1	Horeya 1
	Ri_2	

Table 2. Gram stain and catalase production.

Isolated rhizobacteria strains	Gram+/−	Shape	Catalase production	Oxidase production
M1	+	Rod-shaped	+	+
M2	+	Rod-shaped	+	+
M3	+	Rod-shaped	+	+
M4	+	Rod-shaped	+	+
M5	+	Rod-shaped	+	+
M6	−	Rod-shaped	+	+
Ri1	+	Rod-shaped	+	+
Ri2	−	Rod-shaped	+	+

Gram+/−: Bacteria Gram+, Bacteria Gram−; +: positive; −: negative.

The Gram stain test revealed a predominance of Gram-positive bacteria, with six (06) of the eight strains (M1 through M5 and Ri1) being Gram-positive, while two (02) strains (M6 and Ri2) were Gram-negative. This distribution suggests structural diversity among the isolates, although Gram-positive bacteria are in the majority.

From a morphological standpoint, all strains exhibit a rod-shaped form, indicating morphological homogeneity among the selected isolates.

Regarding enzymatic tests, all strains tested positive for catalase and oxidase activity, indicating their ability to produce catalase and cytochrome oxidase, respectively. A positive catalase test result indicates an ability to break down hydrogen peroxide into water and oxygen, which serves as a protective mechanism against oxidative stress. Similarly, the production of oxidase suggests the presence of a functional respiratory chain involving cytochrome c oxidase, a characteristic of aerobic or facultative aerobe-anaerobic bacteria.

3.2. Characteristics of the Plant Growth-Promoting Properties of Isolated Rhizobacteria Strains

Table 3 presents a qualitative assessment of certain plant growth-promoting properties of the isolated rhizobacteria strains, including the production of hydrogen cyanide (HCN), ammonia (NH_3), and indoleacetic acid (IAA). These metabolites are recognized for their important role in stimulating plant growth and protecting plants against certain pathogens.

Table 3. Qualitative assessment of selected growth-promoting properties of isolated rhizobacterial strains.

Isolated rhizobacteria strains	Hydrogen cyanide (HCN) production	Ammonia (NH3) production	Indole acetic acid (IAA) production
M1	+	+	+
M2	++	++	++
M3	++	++	++
M4	+++	+++	+++
M5	+++	+++	+++
M6	++	++	++
Ri1	+++	+++	+++
Ri2	+++	+++	+++

(+) positive; (−) negative; (++) moderate; (+++): high

The results show that all strains exhibit at least one positive activity for the three parameters studied, indicating their potential as plant growth-promoting rhizobacteria (PGPR). However, the intensity of production varies among strains.

Strains M4, M5, Ri1, and Ri2 are characterized by high production (+++) of HCN, NH_3, and IAA, suggesting a high potential for stimulating plant growth. Strains M2, M3, and M6 exhibit moderate production (++) of the three compounds, reflecting moderate growth-promoting activity. Strain M1, on the other hand, shows low production (+), which could explain its less pronounced stimulating effect compared to the other strains.

3.3. Effects of Rhizobacteria-Based Bioinoculants on the Germination Parameters of Maize Seeds

- **Morphology of Germinated Maize and Rice Seeds**

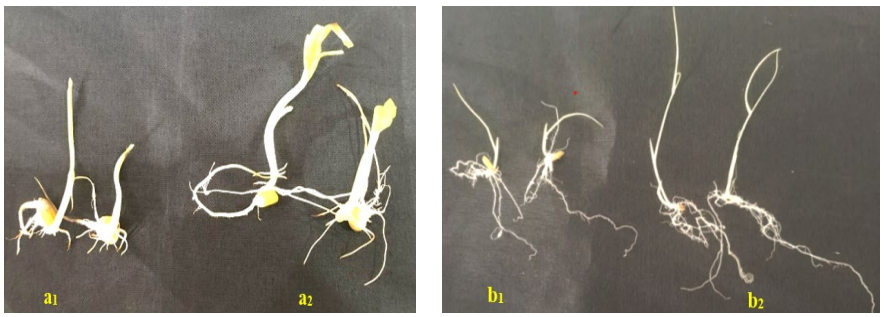
Figure 2 shows the morphology of germinated maize and rice seeds. The germination setup developed during this study allowed us to observe, after seven (7) days of germination, good growth of seedlings and roots from seeds treated with bioinoculants compared to control seeds (without bioinoculants).

- **Effects of Rhizobacteria-Based Bioinoculants on Germination Rate, Seedling Length, and Root Length of Germinated Maize Seeds**

Table 4 presents the effects of rhizobacteria-based bioinoculants on the germi-

nation rate, seedling length, and root length of maize seeds, compared to the non-inoculated control. The same observations were made regarding the seed vigor index (Figure 3).

Overall, inoculating the seeds with bacterial strains led to an improvement in the parameters studied compared to the control. The germination rate of the control was 75%, while the inoculated treatments ranged from 80% to 85%. Strains M4, M5, Ri1, and Ri2 exhibited the highest rates (85%), suggesting a positive effect of the bioinoculants on seed germination capacity. The statistical results ($p = 0.553$) show that there is no significant difference between the treatments ($p \geq 0.05$) (Table 4).



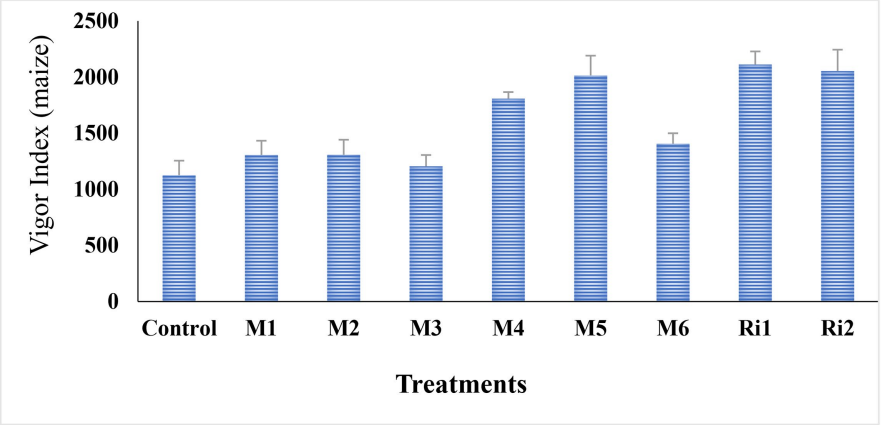
(a) (b)

Figure 2. Picture showing the morphology of germinated seeds: (a) Maize; (b) Rice. (a_1) Maize seed without bioinoculant (control); (a_2) Maize seed with bioinoculant; (b_2): Rice seed without bioinoculant (control); (b_2): Rice seed with bioinoculant.

Table 4. Effects of Rhizobacteria-based bioinoculants on the germination rate, seedling length, and root length of germinated maize seeds.

Treatments	Germination rate (%)		Shoot length (cm)		Root length (cm)	
	Mean	Standard error	Mean	Standard error	Mean	Standard error
Control	75^{a}	7.070	6.261^{c}	0.400	8.705^{b}	0.320
M1	80^{a}	0.000	7.572^{bc}	0.393	8.752^{b}	1.600
M2	80^{a}	0.000	7.583^{bc}	0.780	8.766^{b}	1.700
M3	80^{a}	0.000	6.316^{c}	0.940	8.738^{b}	0.610
M4	85^{a}	7.070	8.127^{ab}	0.200	13.181^{a}	0.730
M5	85^{a}	7.070	10.323^{a}	0.430	13.355^{a}	1.000
M6	80^{a}	0.000	8.291^{ab}	0.650	9.283^{b}	0.780
Ri1	85^{a}	0.000	9.690^{ab}	0.830	15.159^{a}	1.210
Ri2	85^{a}	7.070	9.417^{ab}	0.090	14.709^{a}	0.650
P-value	0.553		0.0007		0.0001	
Significance	ns		***		***	

***: Very highly significant ($p \leq 0.001$); **: highly significant ($0.001 < p \leq 0.01$); ns: not significant ($p \geq 0.05$). Controls: Seeds without rhizobacterial bioinoculants. M1; M2; M3; M4; M5; M6; Ri1; Ri2: Rhizobacterial strains used for bioinoculants.



Control: Seeds without rhizobacterial bioinoculants. M1; M2; M3; M4; M5; M6; Ri1; Ri2: Rhizobacterial strains used for bioinoculants.

Figure 3. Effects of rhizobacteria-based bioinoculants on the vigor index of germinated maize seeds.

Regarding seedling length, all seeds treated with bioinoculants showed greater seedling elongation compared to the control seeds. Seeds treated with strains M5 (10.323 cm); Ri1 (9.690 cm); and Ri2 (9.417 cm) recorded the longest seedling lengths, with increases of 64.87%, 54.76%, and 50.41%, respectively, compared to the control (6.261 cm). The statistical results ($p = 0.0007$) show a highly significant difference among the treatments ($p \leq 0.001$) (Table 4).

Root length also showed a marked improvement under the influence of the treatments. The highest performance in root elongation was obtained with the Ri1 strain (15.159 cm), followed by Ri2 (14.709 cm), M5 (13.355 cm), and M4 (13.181 cm), with respective increases of 74.14%, 68.97%, 53.42%, and 51.41%, respectively, compared to the control seeds. The statistical results ($p = 0.0001$) show a highly significant difference among the treatments ($p \leq 0.001$) (Table 4).

Figure 3 shows the effects of rhizobacteria on the average vigor index of maize seeds. The vigor index is an agronomic parameter used to assess the physiological quality of seeds, particularly their ability to germinate rapidly and produce strong seedlings in a given environment. This indicator reflects not only germination capacity but also the initial development of seedlings and roots in the presence of the various bacterial strains tested. Figure 3 clearly shows that seeds treated with rhizobacteria exhibited greater vigor compared to the control (1124.94). The highest values were obtained with seeds treated with Ri1 (2110.9), followed by Ri2 (2053.3), M5 (2014.60), and M4 (1807.89), resulting in respective increases of 87.64%; 82.52%, 79.085%, and 60.71%, respectively, in the vigor of the inoculated seeds compared to the control (1124.94). The statistical results ($p = 0.0005$) show a highly significant difference between the treatments ($p \leq 0.001$).

Among all the parameters evaluated (Table 4 and Figure 3), strains M5, Ri1, Ri2, and M4 stood out for their most pronounced effects on maize germination and early growth, highlighting their potential as promising bioinoculants for im-

proving agronomic performance.

- **Effects of Rhizobacteria-Based Bioinoculants on the Weight of Germinated Maize Seeds, Seedlings, and Roots**

Table 5 shows the effect of different treatments based on rhizobacteria strains on the weight of germinated seeds, seedlings, and roots, compared to the non-inoculated control. In general, all inoculated treatments showed improved growth parameters compared to the control, indicating that the bioinoculants promote germination and early seedling development.

Table 5. Effects of rhizobacteria-based bioinoculants on the weight of maize seeds, seedlings, and roots.

Treatments	Germinated seed weight (g)		Shoot weight (g)		Root weight (g)	
	Mean	Standard	Mean	Standard	Mean	Standard
Control	0.897^{b}	0.024	0.313^{b}	0.012	0.206^{b}	0.012
M1	1.036^{ab}	0.043	0.369^{ab}	0.029	0.212^{b}	0.012
M2	1.079^{ab}	0.035	0.409^{ab}	0.032	0.207^{b}	0.012
M3	1.039^{ab}	0.160	0.322^{b}	0.072	0.209^{b}	0.011
M4	1.189^{ab}	0.025	0.477^{ab}	0.053	0.317^{a}	0.014
M5	1.398^{a}	0.106	0.574^{a}	0.023	0.336^{a}	0.011
M6	1.120^{ab}	0.048	0.463^{ab}	0.009	0.233^{b}	0.026
Ri1	1.282^{a}	0.079	0.569^{a}	0.041	0.341^{a}	0.020
Ri2	1.215^{ab}	0.062	0.559^{a}	0.093	0.323^{a}	0.014
P-value	0.0137		0.0073		0.00005	
Significance	*		**		***	

***: Very highly significant ($p \leq 0.001$); **: highly significant ($0.001 < p \leq 0.01$); *: significant ($0.01 < p \leq 0.05$). Controls: Seeds without rhizobacterial bioinoculants. M1; M2; M3; M4; M5; M6; Ri1; Ri2: Rhizobacteria strains used for bioinoculants.

Regarding the weight of germinated seeds, all strains resulted in an increase compared to the control (0.897 g). The most pronounced effect was observed in seeds inoculated with strain M5 (1.398 g), followed by Ri1 (1.282 g), Ri2 (1.215 g), and M4 (1.189 g), with respective increases of 55.85%, 42.92%, 35.45%, and 32.55%, respectively, compared to the control (0.897 g). The statistical results ($p = 0.0137$) show a significant difference among the treatments ($p \leq 0.05$) (Table 5).

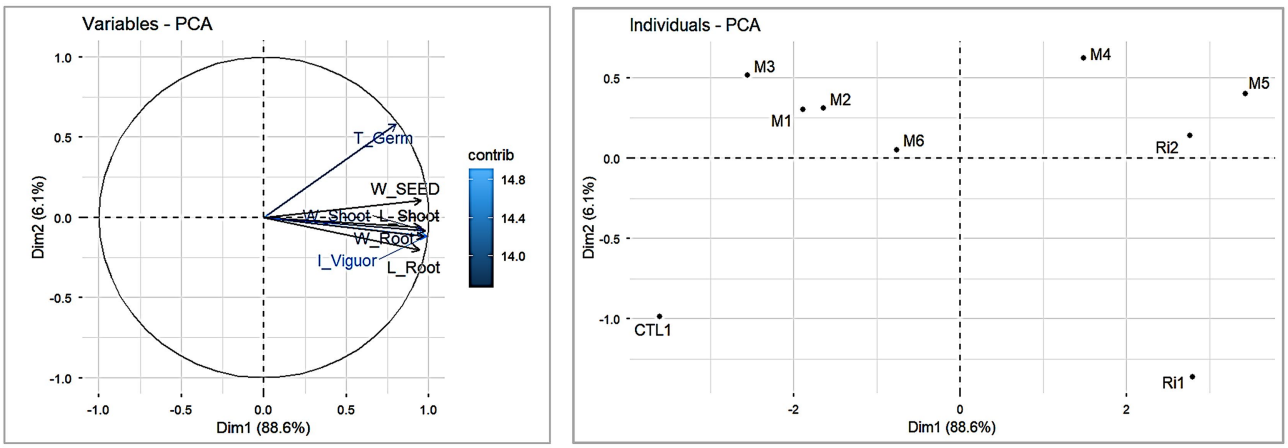
The same trend was observed for seedling weight. The highest values were recorded for maize seeds treated with strains M5 (0.574 g), Ri1 (0.569 g), Ri2 (0.559 g), and M4 (0.477 g), with respective increases of 83.38%; 81.78%, 78.59%, and 52.39% compared to the control (0.313 g). - The statistical results ($p = 0.0073$) show a highly significant difference between the treatments ($p \leq 0.01$) (Table 5).

Regarding root weight, the treatments also promoted root development compared to the control. Seeds treated with bioinoculants based on strains Ri1 (0.341

g); M5 (0.336 g), Ri2 (0.323 g), and M4 (0.317 g) yielded the best root weight performance, with respective increases of 65.53%, 63.11%, 56.79%, and 53.88% compared to the control (0.206 g). The statistical results ($p = 0.00005$) show a highly significant difference among the treatments ($p \leq 0.001$) (Table 5).

- **Principal Component Analysis (PCA) of germination of maize seeds inoculated with PGPR-Based Bioinoculants**

The principal component analysis (PCA) performed on the germination parameters of corn inoculated with PGPR-based bioinoculants shows that the first two axes account for 94.70% of the total variance, with 88.57% for axis 1 (Dim.1) and 6.13% for axis 2 (Dim.2) (Figure 4). Axis 1 (Dim.1) is strongly and positively correlated with all the parameters studied, particularly with the vigor index ($r = 0.987$), shoot weight ($r = 0.974$), root weight ($r = 0.969$), seed weight ($r = 0.952$), shoot length ($r = 0.950$), and root length ($r = 0.942$). The germination rate also shows a strong correlation with Axis 1 (Dim.1) ($r = 0.801$) and is the variable most strongly associated with Axis 2 (Dim.2) ($r = 0.581$). Thus, Dim.1 primarily represents a gradient of seedling vigor and overall growth, while Axis 2 (Dim.2) more closely reflects variations in germination rate.



I.Viguor (Viguor index); L.Root (Length root); W.Root (Root weight); L.Shoot (Length shoot); W.Shoot (Shoot weight); W.seed (Germinated seed weight). (M1; M2; M3; M4; M5; M6; Ri1; Ri2): Rhizobacterial strains used for bioinoculants.

Figure 4. Principal component analysis (PCA) of germination of maize seeds inoculated with PGPR-based bioinoculants.

A joint analysis of the PCA structure and the average performance of the treatments identifies four particularly high-performing treatments among the eight PGPRs evaluated: M5, Ri1, Ri2, and M4. M5 is characterized by the best performance in terms of above-ground growth and biomass, with the highest values for shoot length, seed weight, and shoot weight. Ri1 shows a strong association with root development and vigor parameters, notably with the highest values for root length, root weight, and vigor index. Ri2 also exhibits high performance in root length, vigor index, and above-ground growth parameters. M4, although slightly inferior to M5 and the Ri1 and Ri2 treatments for several variables, shows a significant overall improvement in growth and vigor compared to the other treat-

ments. The control treatment CTL1 is characterized by a strong negative contribution to Dim.1.

Overall, the position of these treatments in the PCA profile and their average performance indicate that M5, Ri1, Ri2, and M4 are the four highest-performing PGPR treatments for maize germination and early growth. This performance is primarily explained by their association with the positive pole of Dim.1, which encompasses the main growth and vigor parameters.

3.4. Effects of Rhizobacteria-Based Bioinoculants on Rice Seed Germination Parameters

- **Effects of Rhizobacteria-Based Bioinoculants on Rice Germination Rate, Seedling Length, and Root Length**

Table 6 shows the effect of rhizobacteria-based bioinoculants on rice seed germination rate, seedling length, and root length.

Table 6. Effects of rhizobacteria-based bioinoculants on germination rate, seedling length, and root length in rice.

Treatments	Germination rate (%)		Shoot length (cm)		Root Length (cm)	
	Mean	Standard error	Mean	Standard error	Mean	Standard error
Control	90[a]	0.000	4.210[b]	0.570	8.352[b]	0.490
M1	90[a]	14.140	5.043[a]	0.020	10.6435[a]	0.020
M2	90[a]	0.000	5.056[a]	0.270	10.656[a]	0.150
M3	90[a]	14.140	5.076[a]	0.090	10.298[a]	0.020
M4	90[a]	0.000	5.107[a]	0.060	11.054[a]	0.020
M5	90[a]	0.000	5.538[a]	0.240	11.160[a]	0.400
M6	95[a]	7.070	4.945[a]	0.090	10.240[a]	0.240
Ri1	95[a]	7.070	5.538[a]	0.210	11.089[a]	0.280
Ri2	100[a]	0.000	5.795[a]	0.010	11.270[a]	0.270
P-value	0.846		0.0038		0.00008	
Significance	ns		**		***	

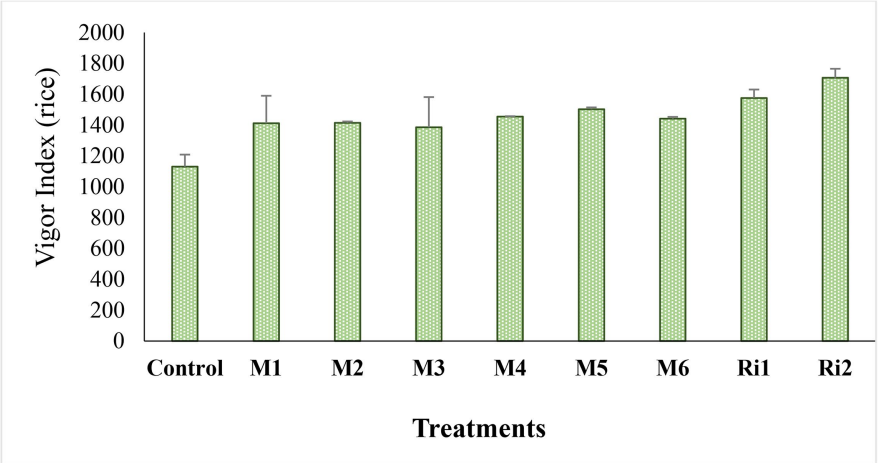
***: Very highly significant ($p \leq 0.001$); **: highly significant ($0.001 < p \leq 0.01$); ns: not significant ($p \geq 0.05$). Control: Seeds without rhizobacterial bioinoculants. M1; M2; M3; M4; M5; M6; Ri1; Ri2: Rhizobacterial strains used for bioinoculants.

The rice seed germination rate was generally high, at 90% for the control. Inoculation with the bioinoculants resulted in a slight increase, reaching 95% for strains M6 and Ri1, and 100% for Ri2, suggesting a positive effect on seed germination capacity. Statistical results show that there is no significant difference among the treatments ($p \geq 0.05$) (Table 6).

Regarding seedling length, all strains resulted in an increase compared to the control (4.210 cm). Treatments with strains Ri2 (5.795 cm), M5 (5.538 cm), Ri1 (5.538 cm), and M4 (5.107 cm) resulted in the longest seedling lengths, corresponding to increases of 37.63%, 31.54%, 30.98%, and 21.31%, respectively, compared to the control. The statistical results ($p = 0.0038$) show a highly significant difference among the treatments ($p \leq 0.01$) (Table 6).

With regard to root growth, root development is also promoted by inoculation. All rice seeds inoculated with rhizobacteria showed a notable improvement in root length compared to the control. In particular, treatments with Ri2 (11.27 cm), followed by M5 (11.1605 cm); Ri1 (11.089 cm), and M4 (11.054 cm) yielded the best root performance, with increases of 34.94%, 33.62%, and 32.77%, respectively, and 32.35% compared to the control (8.352 cm). The statistical results ($p = 0.00008$) show a highly significant difference among the treatments ($p \leq 0.001$) (Table 6).

Figure 5, meanwhile, illustrates the impact of rhizobacteria on the vigor index of rice seeds. Seeds inoculated with the bacterial strains exhibited higher vigor than the control (1130.63). The highest values were recorded for treatment Ri2 (1706.5), followed by Ri1 (1575.51), M5 (1502.86), and M4 (1454.51), corresponding to increases of 50.93%, 39.34%, 32.92%, and 28.64%, respectively, compared to the control. The statistical results ($p = 0.0452$) show a significant difference among the treatments ($p \leq 0.05$).



(Control): Seeds without rhizobacterial bioinoculants. (M1; M2; M3; M4; M5; M6; Ri1; Ri2): Rhizobacterial strains used for bioinoculants.

Figure 5. Effects of rhizobacteria-based bioinoculants on the vigor index of germinated rice seeds.

- **Effects of Rhizobacteria-Based Bioinoculants on the Weight of Germinated Rice Seeds, Seedlings, and Roots**

Table 7 shows an increase in the weight of germinated rice seeds, seedlings, and roots following treatment with rhizobacteria-based bioinoculants, compared to the control.

Table 7. Effects of rhizobacteria-based bioinoculants on the weight of seeds, seedlings, and roots of germinated rice seeds.

Treatments	Germinated seed weight (g)		Shoot weight (g)		Root weight (g)	
	Mean	Standard	Mean	Ecartype	Mean	Standard
Control	0.115[b]	0.010	0.0186[a]	0.003	0.0184[a]	0.003
M1	0.137[ab]	0.000	0.0205[a]	0.0001	0.0221[a]	0.001
M2	0.138[ab]	0.010	0.0217[a]	0.0001	0.0223[a]	0.001
M3	0.139[ab]	0.000	0.0219[a]	0.001	0.0221[a]	0.001
M4	0.142[ab]	0.000	0.0222[a]	0.002	0.0229[a]	0.002
M5	0.154[ab]	0.020	0.0232[a]	0.001	0.0241[a]	0.001
M6	0.137[ab]	0.010	0.0207[a]	0.001	0.0205[a]	0.002
Ri1	0.149[ab]	0.010	0.0230[a]	0.001	0.0236[a]	0.001
Ri2	0.163[a]	0.020	0.0245[a]	0.0017	0.0255[a]	0.002
P-value	0.0447		0.1781		0.1220	
Significance	*		ns		ns	

*: significant ($p \leq 0.05$); ns: not significant ($p \geq 0.05$). Controls: Rice husks without rhizobacterial bioinoculants. Rhizobacterial strains used: M1; M2; M3; M4; M5; M6; Ri1; Ri2.

Regarding the weight of germinated rice seeds, the highest values were recorded for treatments Ri2 (0.163 g), M5 (0.153 g), Ri1 (0.149 g), and M4 (0.142 g), corresponding to increases of 41.74%, 33.91%, 29.56%, and 23.47%, respectively, compared to the control (0.115 g). The statistical results ($p = 0.0447$) show a significant difference among the treatments ($p \leq 0.05$) (Table 7).

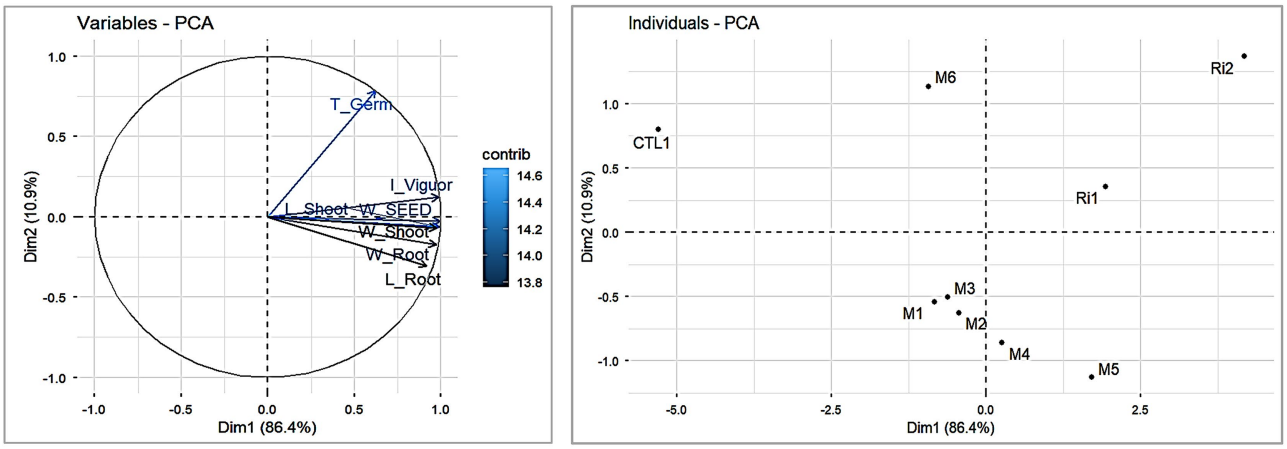
Regarding seedling weight, all seeds treated with bioinoculants had a higher weight than the controls (0.0186 g), as shown in Table 6. The most effective treatments were obtained with strains Ri2 (0.0245 g); M5 (0.0232 g); Ri1 (0.0230 g); and M4 (0.0222 g), resulting in increases of 31.72%, 24.73%, 23.65%, and 19.35%, respectively, compared to the control. The statistical results ($p = 0.1781$) show that there is no significant difference between the treatments ($p \geq 0.05$) (Table 7).

With regard to root weight, all treatments exceeded the control (0.0184 g). The highest values were observed in seeds treated with Ri2 (0.0255 g); M5 (0.0241 g) and Ri1 (0.0236 g), and M4 (0.2229 g), representing increases of 38.58%, 30.97%, 28.26%, and 11.11%, respectively, compared to the control. The statistical results ($p = 0.1220$) show that there is no significant difference among the treatments ($p \geq 0.05$) (Table 7).

- **Principal Component Analysis (PCA) of germination of rice seeds inoculated with PGPR-Based Bioinoculants**

Principal component analysis (PCA) revealed the multivariate structure of rice responses to PGPR-based bioinoculants. The first two principal components of the PCA explain 97.36% of the total variance, with 86.44% accounted for by principal

component 1 (Dim.1) and 10.93% by principal component 2 (Dim.2) (Figure 6).



I.Viguor (Viguor index); L.Root (Length root); W.Root (Root weight); L.Shoot (Length shoot); W.Shoot (Shoot weight); W.Seed (Germinated seed weight). (M1; M2; M3; M4; M5; M6; Ri1; Ri2): Rhizobacterial strains used for bioinoculants.

Figure 6. Principal component analysis (PCA) of germination of rice seeds inoculated with PGPR-based bioinoculants.

Axis 1 (Dim.1) is strongly and positively associated with most of the analyzed variables, notably shoot length (L_Shoot; 0.991), seed weight (W_SEED; 0.992), the vigor index (I_Vigor; 0.985), shoot weight (W_Shoot; 0.972), root weight (W_Root; 0.971), and root length (L_Root; 0.918). The germination rate (T_Germ) is also positively associated with this axis (0.619). Axis 1 (Dim.1) can thus be interpreted as an overall axis of rice growth performance and biomass production. Treatments with high positive scores on this axis would therefore be characterized by a concurrent improvement in above-ground and root growth parameters, vigor, and biomass accumulation. The second axis (Dim.2), which explains 10.93% of the variance, is primarily associated with the germination rate (T_Germ; 0.785).

A cross-analysis of the PCA results and the average performance recorded for the various treatments reveals four particularly effective treatments among the eight PGPRs evaluated: Ri2, M5, Ri1, and M4.

Overall, the PCA structure highlights a strong association between rice growth and biomass parameters. The strong positive coordinates observed simultaneously for shoot and root length, the vigor index, and the weights of seeds, shoots, and roots indicate that these variables respond consistently to the effects of PGPR bioinoculants. These results suggest that the effect of PGPRs is not limited to a single parameter but results in an overall improvement in the vegetative performance of rice, particularly through root development, above-ground growth, seedling vigor, and biomass accumulation.

4. Discussion

Low soil fertility remains one of the main factors limiting agricultural growth and yields in developing countries. However, the adoption of sustainable agricultural

practices particularly the use of plant growth-promoting rhizobacteria (PGPR) as bioinoculants is a promising strategy for improving soil fertility and crop productivity.

The study conducted in the urban municipality of N'Zérékoré (Guinea) made it possible to isolate, based on morphological criteria, six (06) potential strains of rhizobacteria from the maize rhizosphere, designated M1 through M6, as well as two (02) strains from the rice rhizosphere, designated Ri1 and Ri2. Although [39] identified four species of PGPR, including Bacillus flexus and Klebsiella variicola, in banana systems in North Kivu, Democratic Republic of the Congo, our results reveal a marked predominance of Gram-positive bacilli (75%). This characteristic is often associated with the genus Bacillus, known for its ability to form spores, which confer high resistance in leached ferrallitic soils. This taxonomic difference could be explained by the specificity of the ecological niches associated with the rice and maize crops studied, in contrast to the complex crop mixtures (cassava, taro, sorghum, and beans) analyzed in North Kivu. Furthermore, our results are consistent with those reported by [40], who highlight the abundance of Gram-positive bacteria, particularly those of the genus Bacillus, in the rhizosphere. All strains exhibited positive catalase and oxidase activity, confirming their ability to survive oxidative stress and their aerobic metabolism, which is essential for root colonization (**Table 2**).

The production of indole-3-acetic acid (IAA) by bacterial isolates is an important factor in enhancing plant growth. This phytohormone is produced by approximately 80% of the bacteria isolated from the rhizosphere [41] [42]. In the present study, all bacterial strains tested demonstrated the ability to produce IAA (**Table 3**). These results are consistent with those reported by [43] who also observed that all bacteria isolated from rhizosphere soils in China produced this phytohormone. AIA contributes to root elongation, the regulation of cell division, and root branching, thereby promoting the uptake of water and minerals as well as root colonization by PGPR bacteria. This could explain plants' adaptation to extreme soils [44] [45].

In addition to producing phytohormones such as AIA, certain rhizobacteria also promote plant growth by participating in the cycle of essential nutrients, particularly nitrogen. Ammonia production is thus an important characteristic of PGPRs. It has been reported that ammonia-producing bacteria contribute to the nitrogen cycle by converting organic nitrogen or amino compounds into ammonia. This process can also promote the mineralization of organic matter and, consequently, the availability of nutrients in the rhizosphere [46]. Our study revealed that all strains were capable of producing ammonia (**Table 3**). Similarly, numerous PGPR strains have already been identified as ammonia producers [47] [48]. Ammonia-producing PGPR bacteria have a significant impact on soil nitrogen dynamics, particularly in cereal crops such as rice and maize [46]. Thus, inoculating such bacteria may be a beneficial strategy for improving the availability of plant-available nitrogen, particularly when mineral nitrogen inputs are reduced

or replaced by organic sources.

PGPR strains capable of producing hydrogen cyanide (HCN) secrete hydrogen cyanide synthases, which contribute to the degradation of the cell walls of pathogenic microorganisms [49]. Although hydrocyanic acid is a general metabolic inhibitor with toxic properties [50], it is considered an important trait in the selection of PGPRs, particularly those sought for use in the biocontrol of plant pathogens [20] [51]. All strains (100%) tested in our study produced this volatile compound. These results exceed the 75% and 20% rates reported, respectively, by [52] for Bacillus spp. and by [53] for 76 isolates collected from the rhizosphere in arid and semiarid regions of Algeria. By inhibiting the growth of certain pathogenic fungi or bacteria present in the rhizosphere, these PGPRs can indirectly promote plant germination and growth, thereby reducing pathogen pressure.

Regarding maize germination, the results showed that inoculating seeds with the bacterial strains selected in our study significantly improved the germination parameters of maize and rice. Among these, strains Ri1 and Ri2 (isolated at Horeya 1); M5 (isolated at Nakoyakpala) and M4 (isolated from Commercial (osud1)) proved to be the most effective. In maize, these strains strongly stimulated seedling elongation, reaching 10.323 cm (M5), 9.690 cm (Ri1), and 9.417 cm (Ri2), compared to 6.261 cm for the control, as well as root growth, with maximum values of 15.159 cm (Ri1), 14.709 cm (Ri2), and 13.355 cm (M5). This improvement is also reflected in a high vigor index, reaching 2110.9 (Ri1), 2053.3 (Ri2), and 2,014.60 (M5). In rice, although the control's germination rate was already high (90%), inoculation increased it to 95% (M6 and Ri1) and 100% (Ri2). The strains Ri2, M5, Ri1, and M4 also promoted seedling growth (up to 5.795 cm compared to 4.210 cm for the control) and root growth (up to 11.27 cm), with vigor indices reaching 1706.5, 1575.51, and 1502.86, respectively. These results are consistent with those reported by [54] in Türkiye. The authors of this study found that inoculation with several PGPR bacteria, including *Pseudomonas mohnii* SS7 (5) and *Bacillus siamensis* SS4 (5) isolated from the rhizosphere of *M. domesticade* and *A. pseudoplatanus*, respectively contributed to a significant increase in germination characteristics, root and aboveground biomass, growth, and the vigor of young rice seedlings. Similarly, several studies have reported the effectiveness of *Bacillus siamensis* inoculation on rice growth parameters under conditions of nitrogen deficiency and salinity stress [55] [56]. Significant improvements in the vigor index, root length, seedling weight, and germination rate of maize were observed following inoculation with PGPR bacteria isolated from the maize rhizosphere in Benin and Cameroon [57]-[60]. The same observations were made in the work of [61]. Indeed, these authors demonstrated in their studies that all the bacterial strains tested were capable of producing IAA, siderophore, HCN, and ammonia, and significantly improved the germination rate and vigor index of wheat.

The effectiveness of the strains studied may be linked to their ability to produce several metabolites that promote plant growth, including indole-3-acetic acid (IAA), ammonia, and hydrogen cyanide (HCN). Several authors have shown that

the positive effects of PGPRs on plant germination and growth are closely associated with their ability to produce or modulate plant phytohormones such as IAA [62]-[64]. Furthermore, certain ammonium-producing rhizosphere bacteria also contribute to improving plant nitrogen nutrition by participating in the transformation or mineralization of soil nitrogen compounds, thereby making nitrogen more available to plants [53].

Overall, the results highlight the agronomic potential of bacterial strains Ri1, Ri2, M5, and M4 as bioinoculants capable of improving the germination and early growth of maize and rice. Thanks to their multiple plant-growth-promoting traits notably AIA production and ammonium production—these strains could promote better plant nutrition and faster crop establishment, thereby contributing to improved agricultural productivity while reducing dependence on chemical fertilizers. These results therefore suggest that Ri1, Ri2, and M5 are promising candidates for the development of biofertilizers for maize and rice cropping systems. However, although the performance observed under controlled conditions is encouraging, further greenhouse and field trials are needed to confirm their effectiveness under real-world agricultural conditions, where environmental factors, interactions with the soil microbiome, and soil conditions can influence their agronomic performance.

5. Conclusion

This study evaluated the potential of rhizobacteria isolated from the rhizosphere soils of maize and rice as bioinoculants capable of improving the germination and early growth of these crops. A total of eight (8) strains were identified and selected from among the isolated strains to evaluate the various parameters under test. Qualitative analysis of growth-promoting properties revealed that several strains, notably M4, M5, Ri1, and Ri2, exhibit a high capacity to produce indoleacetic acid (IAA), ammonia (NH_3), and hydrogen cyanide (HCN). These metabolites are known for their role in stimulating root development, mobilizing nutrients, and suppressing certain pathogenic microorganisms. *In vitro* germination assays showed that inoculated seeds exhibited improved germination parameters compared to the non-inoculated controls. Strains M5, Ri1, Ri2, and M4 stood out in particular for their high performance on both maize and rice. These results demonstrate that certain rhizobacteria isolated in N'Zérékoré have strong potential as bioinoculants to improve maize and rice germination. Their use could contribute to more sustainable agriculture by reducing dependence on chemical inputs. However, greenhouse and field trials will be necessary to validate the effectiveness of these strains. In addition, further molecular biology studies would help identify the scientific name of each species and characterize the genes.

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Author Contributions

NAA participated in the concept, design and implementation of the study. NAA, SMK, M-M S, MPZ and MK were responsible for setting up the trial and collecting data. NAA, MMK, JFK, and OA participated in the literature review and data analysis. NAA, MMK, OA, and CMK contributed to the preparation of the manuscript. NAA, CMK, and OA contributed to the revision of the manuscript. NAA and LB-M contributed to the supervision and proofreading of the manuscript. All authors read and approved the final version of the manuscript.

Conflicts of Interest

Authors have declared that no competing interests exist.

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