Response of Indigenous Rhizobia to the Inoculation of Soybean \([Glycine \textit{max} (L.) \textit{Merrill}]\) Varieties Cultivated under Controlled Conditions in Côte d’Ivoire

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Abstract

Soybean \([Glycine \textit{max} (L.) \textit{Merrill}]\) is an important crop known to improve population nutritional status and increase soil fertility and its productivity through biological nitrogen fixation. In Côte d’Ivoire, the introduced \textit{Bradyrhizobium japonicum} used as inoculum had slight compatibility to several soybean varieties compromising their vulgarization. Therefore, the present study was conducted to examine the infectiveness and the effectiveness of indigenous rhizobia isolates on three soybean varieties (Canarana, Doko and Piramana) cultivated in Côte d’Ivoire. The experiment was conducted with potted plant filled with sterilized sand and was statistically laid in Completely Randomized Design (CRD) with sixteen (16) natives rhizobia, one (01) reference strain and uninoculated control (with or without nitrogen) with three replications. The results showed that inoculation significantly improved nodule number, nodule dry weight, plant height and total dry matter of soybean over the negative control treatment. Among the inoculated treatments, five indigenous rhizobia RSC119, RSC324, RSC502, RSC504 and RSC508 significantly (\(P < 0.05\)) increased the nodulation and plant growth parameters than \textit{B. japonicum} (IRAT FA3) with Doko and Piramana. RSC502 produced highest nodule number (64) on Piramama, nodule dry weight was most promoted with RSC504 (321 mg plant\(^{-1}\)) compared to IRAT FA3 strain (95 mg plant\(^{-1}\)) on Doko. The higher effectiveness was recorded with RSC119, RSC504 and RSC502 with 206.73%; 201.79% and 200.45% respectively compared to TN
(100%). The correlation analysis indicated significant association of nodule number and total biomass indicating the importance of symbiotic nitrogen fixation. Based on their infectiveness and their effectiveness, the isolates RSC119, RSC324, RSC502, RSC504 and RSC508 could be used as elite local rhizobia and tested in field conditions to establish their potential contribution on soybean productivity.

Keywords
Selection, Indigenous Rhizobia, Inoculation, Soybean, Côte d’Ivoire

1. Introduction

Soybean [Glycine max (L.) Merrill] is one of the most important grain legumes cultivated in the world for its richness in protein (40%) and oil (20%). It is the most available crop that provides a cheap and high quality source of protein comparable to meat, poultry and eggs. It is used for production of cooking oil, human food, stock feed and industrial products [1] [2]. This legume was introduced in Côte d’Ivoire for crops diversification and its contribution to improve population nutritional status. Soybean also increases soil fertility and productivity through biological nitrogen fixation (BNF) [3].

The BNF plays an essential role in crop establishment and fulfills most of plants need for nitrogen. The amount of nitrogen fixed by soybean in symbiosis with Bradyrhizobium japonicum is estimated yearly to 450 kg·ha⁻¹ [4]. It considerably reduces the need of synthetic nitrogen fertilizers supply of crops which continuous application could be harmful to soil fertility and accelerate environmental pollution [5] [6] [7]. Thereby, maintaining this natural significant nitrogen input should be better for economical and ecological sustainable soybean yields. However, the ability of this crop to fix the desired amount of nitrogen depends on many factors such as the effectiveness of the rhizobia strain and plant varieties [8] [9].

In Côte d’Ivoire, initiative researches have been undertook to vulgarize soybean crop in several areas of the country. Hence, researchers have evaluated in these studies the effective compatibility of soybean varieties to the introduced B. japonicum strain IRAT FA3 used as inoculum [10] [11] [12] [13]. Results of these studies have shown that B. japonicum IRAT FA3 was compatible with some soybean cultivars than others, compromising therefore their vulgarization in the country. However, recent investigations reported by N’Gbesso et al. [14] and Amani et al. [15], have revealed that indigenous rhizobia isolated in Cote d’Ivoire soil was able to improve other soybean cultivars growth and productivity. Thus, the present investigation was carried out to 1) evaluate the infectiveness and the effectiveness of indigenous rhizobia on three soybeans [Glycine max (L.) Merrill] varieties under controlled conditions and to 2) select all strains that
hold potential to be included in the inoculums formulations for soybean pro-
duction under Ivorian agro-climatic conditions.

2. Materials and Methods

2.1. Biological Materials

Soybean \([\text{Glycine max} \, (L.) \, \text{Merrill}]\) cultivars Canarana, Doko and Piramama
provided by the National Agricultural Research Centre (CNRA) in Bouake, Côte
d’Ivoire, were used as plant material in this experiment.

Sixteen (16) indigenous authentic rhizobiaisolated from soybean nodules in
Côte d’Ivoire and one (01) reference strain (\textit{Bradyrhizobium japonicum} IRAT
FA3) were used in this work. The origins and the sources of these bacteria are
described in Table 1.

2.2. Treatments and Experiment Design for the Pot Experiment

A pot experiment was conducted under the controlled conditions at Jean Lo-
rougnon Guede University in Daloa (Cote d'Ivoire). The pot experiment was
statistically laid in Completely Randomized Design (CRD) on sterile sand with
nineteen (19) treatments including sixteen (16) indigenous rhizobial isolates,
one (01) reference strain and two uninoculated treatments (one (01) uninocu-
lated and unfertilized treatment as negative control (T0) and one (01) uninocu-
lated and fertilized treatment (TN) as positive control) in three replications [16].

2.3. Evaluation of Indigenous Rhizobial Isolates Infectivity and
Effectiveness

Seeds of each soybean cultivars were sterilized in Mercuric chloride (0.1%) for
two min and then thoroughly rinsing with six changes of sterile distilled water.
Thereafter, four seeds were sown on plastic pots containing sterile sand. Seven

Table 1. Origins and host plants of the indigenous rhizobial isolates and reference strain
used in this study.

| Reference strain and local isolates | Host plants (Soybean cultivars) | Origins | Sources |
|-----------------------------------|---------------------------------|---------|---------|
| Reference strain:                 |                                 |         |         |
| \textit{Bradyrhizobium japonicum} |                                 |         |         |
| IRAT FA3                          |                                 |         | [14]    |
| Local rhizobia isolates:          |                                 |         |         |
| RSC309; RSC310; RSC312; RSC323;   | Piramama                        | Daloa (Cote d’Ivoire) | [15]    |
| RSC324; RSC325                   |                                 |         |         |
| RSC114; RSC115; RSC119           |                                 |         |         |
| RSC207                           |                                 |         |         |
| RSC412; RSC413                   |                                 |         |         |
| RSC502; RSC504; RSC506; RSC508    |                                 |         |         |
|                                 |                                 |         |         |
|                                 |                                 |         | This study |

RSC: Rhizobia isolated from soybean nodules in cote d’Ivoire.
days after sowing, each plant of a pot except the controls was inoculated with 1 mL of broth culture of each isolate beforehand grown on YEM liquid medium to exponential phase. Plants were supplied with distilled water every two days and they were saturated once a week with a nitrogen-free nutrient solution. Furthermore, TN control received weekly 0.05% (w/v) KNO₃ as nitrogen source. For each treatment, plants were harvested 45 days after sowing (DAS) and nodule number, Plant height and matter weight were evaluated per treatment and per variety. Matter (root and shoot) of each treatment was dried three days at 70°C and was used to calculate the Relative effectiveness (RE) of isolates according to Maâtallah et al. [17] by the following equation:

$$RE = \frac{\text{Inoculated plant dry matter}}{\text{N-Fertilized plant dry matter}} \times 100$$

Nitrogen fixing effectiveness classified as: Ineffective (RE < 35%); Low-ly-effective (35% < RE < 50%); Effective 50% < RE < 80%; and highly effective (RE > 80%). Relationship between nodule number-plant height and nodule number-total matter were examined.

2.4. Statistical Analysis

The data of measured parameters recorded were pooled together and subjected to statistical analysis using the STATISTICA program (7.1). Plant growth and nodulation parameters were subjected by analysis of variance. The difference between the treatments means were evaluated at 5% level of significance using Fisher’s LSD test.

4. Results and Discussion

4.1. Impact of Indigenous rhizobia on Nodule Number and Nodule Dry Weight on Soybean Varieties

This study was performed in sterile sand to evaluate the infectivity of indigenous rhizobia isolates on three soybean varieties (Canarana, Doko and Piramama). The results revealed that inoculation was affected significantly (P < 0.05) nodule number and nodule dry mass on soybean varieties (Table 2).

All indigenous rhizobial isolates were able to form nodules on tested varieties. In the other hand, uninoculated control did not show any nodule, demonstrated that aseptic conditions were met in the experimental set up. Results revealed that all indigenous isolates were able to form nodules on three tested varieties of soybean. In the other hand, uninoculated control did not show any nodule, demonstrated that aseptic conditions were met in the experimental set up. The same report was made by Amani et al. [15] and Kumar and Reddy [18] during authentification of soybean and French bean rhizobial isolates respectively, in the same conditions. Therefore, analysis of variance showed that nodulation varied significantly (P < 0.05) between tested isolates and soybean varieties.

The nodule number per plant ranged from 0 for isolates RSC114 and RSC207 to 52 for isolate RSC504 with Doko, from 0 for isolate RSC114 to 28 for RSC119
Table 2. Impact of indigenous rhizobia, introduced strain and nitrogen fertilizer on nodule number and nodule dry weight on three soybean cultivars grown on sterile sand at flowering stage.

| Treatments | Nodule Number (plant⁻¹) | Nodule Dry Weight (mg plant⁻¹) |
|------------|--------------------------|-------------------------------|
|            | Doko    | Canarana | Piramama | Doko   | Canarana | Piramama |
| RSC114     | -       | -        | 11 ± 2.08hi | -     | -       | 80 ± 8.56f |
| RSC115     | 39 ± 2.00c | 10 ± 1.52ge | 58 ± 4.51b | 266.67 ± 8.77b | 46.67 ± 5.77d | 125 ± 8.23de |
| RSC119     | 45 ± 3.00b | 28 ± 4.00a | 42 ± 2.38d | 263.33 ± 6.70bc | 80 ± 10.00a | 253.33 ± 12.28a |
| RSC207     | -       | 10 ± 1.52ge | 13 ± 3.05h | -     | 15 ± 5.00e | 113.33 ± 8.79e |
| RSC309     | 40 ± 3.05c | 16 ± 2.00d | 42 ± 2.86d | 250 ± 10.00c | 53.33 ± 5.77d | 133.33 ± 5.77cde |
| RSC310     | 10 ± 1.52f | 13 ± 2.00ef | 38 ± 3.25de | 166.67 ± 4.86e | 45 ± 5.00de | 130 ± 10.00cde |
| RSC312     | 18 ± 2.65e | 8 ± 1.73e | 30 ± 2.08f | 183.33 ± 5.77d | 15 ± 2.50e | 116.67 ± 9.27e |
| RSC323     | 1 ± 0.58h | 10 ± 1.53ge | 36 ± 1.53e | 10 ± 2.86j | 15 ± 3.00e | 120 ± 9.87e |
| RSC324     | 35 ± 3.05d | 18 ± 2.00cd | 50 ± 2.08c | 110 ± 10.00f | 66.67 ± 6.25b | 213.33 ± 10.33b |
| RSC325     | 7 ± 1.53fg | 12 ± 2.00fg | 39 ± 3.57de | 73.33 ± 3.05h | 36.67 ± 7.63e | 146.67 ± 12.55cd |
| RSC412     | 8 ± 1.53f | 18 ± 2.00cd | 15 ± 0.58h | 77.67 ± 2.52h | 21.67 ± 2.89f | 78 ± 5.77f |
| RSC413     | 4 ± 1.53g | 13 ± 2.00ef | 9 ± 1.15i | 46.67 ± 2.89i | 21.67 ± 2.77f | 80 ± 10.00f |
| RSC502     | 46 ± 1.56b | 15 ± 2.00de | 64 ± 3.51a | 181.33 ± 10.26d | 56.67 ± 5.77cd | 246.67 ± 8.31a |
| RSC504     | 52 ± 2.00a | 21 ± 3.00c | 36 ± 1.53e | 321 ± 13.26a | 69 ± 8.89c | 153.33 ± 5.77c |
| RSC506     | 34 ± 1.73d | 12 ± 2.00fd | 26 ± 2.89g | 190 ± 10.00d | 36.67 ± 7.63e | 146.67 ± 12.55cd |
| RSC508     | 47 ± 2.52b | 24 ± 4.00b | 41 ± 3.05d | 273.33 ± 5.77b | 73.33 ± 7.73bc | 193.33 ± 11.54b |
| IRAT FA3   | 34 ± 1.73d | 27 ± 5.00a | 35 ± 1.53ef | 95 ± 3.84g | 89.67 ± 10.25a | 133.33 ± 10.27cde |
| TN         | -       | -        | -         | -     | -       | -         |
| T0         | -       | -        | -         | -     | -       | -         |
| LSD 5%     | 3.67    | 2.33     | 5         | 14.66 | 10      | 26.67     |

TN: uninoculated and fertilized control; T0: uninoculated and unfertilized control. Means in the same column followed by the same letter are not significantly different at the 5% probability level by LSD’s test.

With Canarana and from 9 for isolate RSC413 to 64 for isolate RSC502. Thus, across all tested treatments, isolate RSC502 produced the higher nodule number.
Considering the varieties, Piramama and Doko were more receptive to inoculation with local rhizobial isolates than Canarana. Indeed, RSC115, RSC119, RSC309, RSC324, RSC502, RSC504 and RSC508 induced the formation of high nodules relative to the introduced strain IRAT FA3 with varieties except Canarana. Tested isolates RSC508, RSC502 and RSC119 induced the largest number of nodules per plant respectively 52; 47; 46 and 45 against 34 nodules for the strain IRAT FA3 on Doko. On Piramama, tested rhizobia strains RSC502, RSC115 and RSC324 were revealed more infective with 64; 58 and 50 nodules respectively against 35 to the strain IRAT FA3 (Figure 1). Hungria et al. [19] also reported that inoculation of soybean significantly increased the nodule number over the control. Attempts realized in Mozambique were also found that some indigenous rhizobia were effective than five reference strains used in the country to improve soybean production [20]. Unlike Doko and Piramama varieties, local rhizobia were unable to increase the number of nodules on Canarana compared IRAT FA3 excepted RSC119, which induced nodules production like the latter. Based on nodulation indigenous rhizobia RSC115, RSC119, RSC309, RSC324, RSC502, RSC504 and RSC508 were more infective than the introduced strain IRAT FA3. When the analysis of variance was performed separately for each cultivar, it appeared that Piramama and Doko had best response to inoculation.

(Figure 1. Nodules induced on roots of the soybean cultivars by local rhizobia. (a) Canarana cultivar without inoculation; (b) Canarana cultivar inoculated with isolate RSC119; (c) Doko cultivar inoculated with isolate RSC508; (d) Piramama cultivar inoculated with isolate RSC502.)
comparing to Canarana. There were more nodules on these varieties than Canarana. The greater number of nodules due to inoculation suggested that there is better combining and symbiotic relationship between indigenous isolates and soybean cultivars Doko and Piramama. However, soybean cultivar Canarana with a fail response to inoculation may possess a particular $R_j$ gene that play a role in controlling the plant’s compatibility with specific rhizobial strains. In addition, indigenous tested rhizobia may show a preference for particular genotypes among the compatible genotypes [21] [22].

Such as nodule number, nodule weight of soybean varieties was significantly promoted by inoculation with local rhizobia (Table 2). However, it varied according to the treatment and the variety. In fact, with Doko variety, ten (10) local isolates increased the dry weight of the nodules compared to IRAT FA3. RSC504 induced the highest weight of nodules (32 mg·plant$^{-1}$) than IRAT FA3 (95 mg·plant$^{-1}$). Regarding Piramama variety, isolates RSC119, RSC324, RSC502 and RSC508 induced higher nodular dry weight compared to IRAT FA3 that was statistically similar to the weight produced by the isolates RSC115, RSC207, RSC309, RSC310, RSC312, RSC323, RSC325, RSC504 and RSC506. RSC119 promoted the highest nodule dry weight (253.33 mg·plant$^{-1}$) than IRAT FA3 strain (113.33 mg·plant$^{-1}$) and other isolates. Contrary to Doko and Piramama, all local tested isolates did not exhibited nodules dry weight higher than IRAT FA3 on Canarana variety except RSC 119 which favored identical nodule dry weight as this strain. Researchers such as Htwe et al. [23] were found the same results with other soybean cultivars. These authors exhibited that the local strains Bradyrhizobium spp. SHY6-1 and B. elkanii SAY3-4 isolated in Nyanmar have given the highest nodule dry weights than the exotic B. japonicum USDA110.

4.2. Indigenous Rhizobial Isolates Contribution on Plant Height and Matter Production

The results revealed that inoculation was affected significantly ($P < 0.05$) plant height and plant dry matter of soybean varieties (Table 3).

Inoculation of soybean varieties with native rhizobia and reference strain increased plant height and influenced total dry matter compared to the uninoculated negative control (Table 3). The table shows that local isolates RSC502 and RSC508 respectively provided the most plant height with Doko and Piramama followed to the positive control and other treatments. In example, the height of Doko plant inoculated with RSC502 was 84.47 cm against 64 cm for positive control (TN). The higher height with Piramama was recorded with the isolates RSC508 (55 cm) which was statistically similar to the isolates RSC119, RSC324, RSC325, RSC502 and RSC506. These isolates promoted plant height than the uninoculated and nitrogen fertilizer control (TN). None local rhizobia did not improve Canarana cultivar growth more than the positive control. Therefore, local isolate RSC324 and the reference strain IRAT FA3 were statistically identical.
Table 3. Impact of indigenous rhizobia, introduced strain and nitrogen fertilizer on plant height and plant matter production on three soybean cultivars at flowering stage.

| Treatments  | Doko Plant Height (cm) | Doko Plant Dry Matter (g∙Plant⁻¹) | Canarana Plant Height (cm) | Canarana Plant Dry Matter (g∙Plant⁻¹) | Piramama Plant Height (cm) | Piramama Plant Dry Matter (g∙Plant⁻¹) |
|-------------|------------------------|----------------------------------|-----------------------------|--------------------------------------|-----------------------------|--------------------------------------|
| RSC114      | 53 ± 3.61hi            | 29.97 ± 0.90fg                   | 46.10 ± 1.01de              | 1.60 ± 0.35j                         | 1.41 ± 0.02fg               | 2.57 ± 0.40cd                       |
| RSC115      | 73.13 ± 1.98bc         | 32.33 ± 2.52cdef                 | 48.43 ± 1.25cde             | 4 ± 0.56b                            | 1.17 ± 0.04gfh              | 2.6 ± 0.10cd                        |
| RSC119      | 76 ± 2.00b             | 33.93 ± 2.30cd                   | 51.93 ± 0.38ab              | 4.5 ± 0.5a                           | 2.18 ± 0.13bc               | 4.17 ± 0.17a                        |
| RSC207      | 55.10 ± 4.71h          | 29.80 ± 0.72fg                   | 44.10 ± 1.45ef              | 2.02 ± 0.27i                         | 1.77 ± 0.06de               | 1.83 ± 0.12ef                        |
| RSC309      | 67.40 ± 2.76cde        | 32.6 ± 0.53cdef                  | 45.67 ± 0.47def             | 2.98 ± 0.06ef                         | 1.98 ± 0.03cde              | 2.63 ± 0.21cd                       |
| RSC310      | 57.03 ± 0.95gh         | 31.67 ± 2.08cdef                 | 42.67 ± 1.52f               | 2.56 ± 0.16gh                         | 1.66 ± 0.25gfh              | 1.43 ± 0.12fg                        |
| RSC312      | 56.27 ± 1.55h          | 30.27 ± 0.25efg                  | 48.17 ± 2.75cde             | 2.69 ± 0.11fg                         | 1.13 ± 0.06gfh              | 2.4 ± 0.26d                         |
| RSC323      | 48.23 ± 1.32i          | 31.20 ± 1.93defg                 | 42.33 ± 2.31f               | 1.62 ± 0.06j                          | 1.31 ± 0.02gfh              | 2.4 ± 0.26d                         |
| RSC324      | 65.83 ± 0.96def        | 38.4 ± 0.66a                     | 52.87 ± 2.05ab              | 3.52 ± 0.33cd                         | 2.5 ± 0.43a                 | 2.77 ± 0.40bcd                      |
| RSC325      | 63.40 ± 2.95efg        | 30 ± 0.56efg                     | 53.50 ± 0.75ab              | 2.23 ± 0.25hi                         | 1.62 ± 0.08ef               | 2.38 ± 0.33d                        |
| RSC412      | 56.93 ± 4.15gh         | 30.26 ± 0.25efg                  | 44.10 ± 1.45ef              | 2.54 ± 0.17gh                         | 1.14 ± 0.03gh               | 1.47 ± 0.19efg                      |
| RSC413      | 59.17 ± 1.96fgh        | 29.06 ± 1.21g                    | 43.37 ± 2.51ef              | 2.13 ± 0.21i                          | 1.28 ± 0.03gh               | 1.8 ± 0.20ef                        |
| RSC502      | 84.47 ± 6.70a          | 33.17 ± 1.61cde                   | 54.23 ± 1.90ab              | 4.47 ± 0.30a                          | 1.71 ± 0.06df               | 2.63 ± 0.23cd                       |
| RSC504      | 76.17 ± 3.62b          | 34.57 ± 2.50b                    | 47.50 ± 0.50d               | 4.61 ± 0.14a                          | 2.3 ± 0.10ab                | 2.5 ± 0.10cd                        |
| RSC506      | 68 ± 4.00cde           | 30.06 ± 1.01f                    | 53.67 ± 1.67ab              | 3.62 ± 0.15c                          | 1.85 ± 0.13de               | 2.87 ± 0.23bc                       |
| RSC508      | 70.67 ± 2.57bcd        | 32.33 ± 2.52cdef                 | 55 ± 0.00a                  | 3.85 ± 0.04bc                         | 2.23 ± 0.06abc              | 4.16 ± 0.40a                        |
| IRAT FA3    | 64 ± 4.36def           | 38.83 ± 3.56a                    | 48 ± 4.33c                 | 3.33 ± 0.23d                          | 2.46 ± 0.19ab               | 3.13 ± 0.15b                        |
| TN          | 73.67 ± 3.78bc         | 37.53 ± 2.20ab                   | 51.20 ± 3.14bc              | 2.23 ± 0.06hi                         | 2.5 ± 0.43a                 | 3.07 ± 0.15b                        |
| T0          | 54.77 ± 3.68hi         | 29.03 ± 0.84g                    | 38.67 ± 2.08g              | 1.40 ± 0.20j                          | 1.02 ± 0.14h                | 1.12 ± 0.10g                        |
| LSD 5%      | 6.87                   | 3.23                             | 3.4                         | 0.41                                  | 0.31                       | 0.44                                 |

TN: uninoculated and fertilized control; T0: uninoculated and unfertilized control. Means in the same column followed by the same letter are not significantly different at the 5% probability level by LSD’s test.

The results revealed that plant height and total biomass were significantly affected by inoculation on tested soybean varieties compared to un inoculated and unfertilized control. These results were similar to the findings of Tahir et al. [24] who reported that inoculation increase soybean growth in Pakistan. According to Sobral et al. [25], soybean growth promotion may be due to the capacity of

with the positive control. Thus, soybean varieties inoculated with local rhizobia and the reference strain produced greater biomass compared to the negative control. Total biomass produced on Doko with isolates RSC504 (4.61 g∙plant⁻¹), RSC119 (4.5 g∙plant⁻¹) and RSC502 (4.47 g∙plant⁻¹) were greater than positive control (2.23 g∙plant⁻¹) and the reference strain IRAT FA3 (3.33 g∙plant⁻¹). Greater biomass increasing on Piramama were obtained with isolates RSC119 (4.17 g∙plant⁻¹) and RSC508 (4.16 g∙plant⁻¹) compared to TN (3.07 g∙plant⁻¹) and the reference strain IRAT FA3 (3.13 g∙plant⁻¹). Considering Canarana variety, local tested rhizobia did not produced greater biomass compared with positive control. Nevertheless, isolates RSC324 and RSC504 had stimulated similar effect with TN and IRAT FA3 (Table 3).

The Results revealed that plant height and total biomass were significantly affected by inoculation on tested soybean varieties compared to uninoculated and unfertilized control. These results were similar to the findings of Tahir et al. [24] who reported that inoculation increase soybean growth in Pakistan. According to Sobral et al. [25], soybean growth promotion may be due to the capacity of
some *Bradyrhizobium* isolates to produce Indole Acid Acetic (IAA), solubilize phosphate and fix nitrogen. However, plant height and matter yield promoted by inoculation varied according to soybean cultivars. In the present study, the local rhizobia RSC115, RSC119, RSC309, RSC324, RSC502, RSC504 and RSC508 induced the higher plant height and improved total biomass than another. In addition, in comparison with the positive control (TN) representing the 100% level of plant total dry matter (biomass), most indigenous rhizobia tested showed higher biomass benefit. However, their relative effectiveness (RE) varied among soybean cultivars (Figure 2). Local isolates may be classed on three groups: highly effective (RE > 80%), effective (80% > RE > 50%) and slowly effective (RE < 50%). More than 88% of the isolates were found to be highly effective and 11% were effective on Doko. The higher effectiveness was recorded with RSC119, RSC504 and RSC502 with 206.73%, 201.79% and 200.45% respectively compared to TN (100%). Considering Piramama variety, 58% of local rhizobia were highly effective, 29% were effective and 11% were slowly effective. In opposite to Doko and Piramama, only 11% of local isolates were highly effective, 58% were effective and 11% were slowly effective on Canarana cultivar. Beyond soybean cultivars, isolates RSC115, RSC119, RSC324, RSC502, RSC504 and RSC508 were found to provide the best plant matter benefit on sterile sand. These results corroborated the finding of Guei et al. [26] who reported that nine local strains had good relative effectiveness than reference strain on Bambara groundnut cultivated in the Center West of Côte d’Ivoire.

4.3. Relationship between Nodule Production-Plant Height and Nodule Production-Total Dry Matter on Tested Soybean Cultivars

Correlation between nodule number-plant height and nodule number-total biomass was recorded in Figure 3. The relationship between nodule number

![Figure 2](image-url). Relative effectiveness (RE) of indigenous rhizobia on soybean varieties on sand pot. RE = (Inoculated plant dry matter/N-fertilized plant dry matter) × 100.
Figure 3. Correlation between nodulation and plant growth parameters of soybean cultivars grown in sterile sand.

with plant height was positively and significantly ($P < 0.01$) correlated on Doko and Piramama ($r = 0.70$ and $r = 0.51$ respectively) whereas, these parameters were negatively correlated on Canarana. A positive correlation was also recorded between nodule number and plant biomass on Doko ($r = 0.87$ and $P < 0.001$) and Canarana ($r = 0.5$ and $P < 0.05$) but negatively correlated on Piramama ($r = 0.41$ and $P = 0.08$). From the correlation analysis, it is observed that, both plant
height and plant biomass had significant positive correlation nodule number. These results showed that nodule number is an important factor which affects soybean growth. That’s have been previously reported by over authors [24] [27] [28]. Nevertheless, these results were contrary to those of Maâtllah et al. [17] who revealed that local rhizobia isolated in Morocco had increased matter advantage less than fertilizer application.

5. Conclusion and Perspectives

The results of present investigation demonstrated that the Ivorian soil contains native rhizobia capable of effectively inducing nodulation and improving the productivity of soybean varieties grown in Côte d’Ivoire. The three soybean varieties did not nodulate freely even without inoculation. Doko and Piramama nodulated better with the native rhizobia than Canarana variety. Indigenous rhizobia RSC115, RSC119, RSC324, RSC502, RSC504 and RSC508 increased nodule number (42 to 64) and nodule dry weight (213.33 to 321 mg·plant⁻¹) than the reference strain IRAT FA3 (34 nodule per plant and 95 to 133.33 mg·plant⁻¹) on Doko and Piramama. The higher effectiveness was recorded with isolates RSC119, RSC504 and RSC502 with 206.73%, 201.79% and 200.45% respectively compared to TN (100%) in the sterile sand. Based on the good symbiotic and agronomic performances of native rhizobia, six local isolates RSC115, RSC119, RSC324, RSC502, RSC504 and RSC508 have been selected to examine their potential performances under reel field conditions in order to select the best indigenous isolates for local inoculum production.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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